Perfluorinated compounds Holistic Environmental Interinstitutional eXperience

Life Phoenix

WITH THE CONTRIBUTION OF THE LIFE FINANCIAL INSTRUMENT OF THE EUROPEAN COMMUNITY
LIFE16ENV/IT/000488-LIFE PHOENIX

Report of analysis to investigate the potential impacts of the new molecule cC6O4

[Deliverable n.4 of the Action C.1]

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REGIONE DEL VENETO

arpav
Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

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IRSA CNR

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Summary

Among the molecules of new interest, which have been designed and produced to replace PFOA, which is part of the long-chain PFAS, Solvay and Miteni have produced and used a new synthesis molecule, Difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate (CAS 1190931-27-1, ammonium salt), conventionally named cC6O4, which has already been found in groundwater and surface waters by ARPAV. This smaller molecule is a cyclic ether with only 5 fluorinated carbon atoms and therefore can be included into the class of short-chain PFAS.

The discovery of this molecule in the waters of Veneto Region, in which the LIFE experimentation is underway, made it necessary to carry out a preliminary risk assessment according to the model being developed as part of the LIFE PHOENIX activities. In the literature or in the dossiers of international institutions for this molecule, the values of the bioconcentration factors (BCF) or bioaccumulation (BAF) necessary for an assessment of accumulation and therefore of risk for the aquatic and terrestrial trophic chain are missing.

Water Research Institute (IRSA-CNR) carried out an additional activity with respect to what is written in the LIFE project, to obtain the missing BCF or BAF data and make an initial assessment of the accumulability of the substance in the context of activity B2 (Implementing an informative and statistic system) to support action B4 (Innovative and integrated forecast tools to support decision-making) in the field of transport modelling and distribution of short-chain PFAS in biological systems.

The subaction C1.3 results show that cC6O4 has physico-chemical characteristics similar to those of PFOA in terms of mobility and persistence.

The comparison between experimental and predicted BCF states that log BCF should be in a range under 2 and the molecule should not be classified as not bioaccumulative under REACH classification. But there is experimental evidence that this molecule can distribute in exposed biological systems. The affinity of cC6O4 with specific proteins, such as yolk proteins in eggs or albumin in human serum demonstrates the possibility of bird and human exposure and accumulation for this molecule. According to the weight of evidence (WoE) approach, bioaccumulation potency cannot be completely discarded.

Sommario (in Italian)

Tra le molecole di nuovo interesse, che sono state disegnate e prodotte per sostituire il PFOA, facente parte dei PFAS a lunga catena, Solvay e Miteni hanno prodotto ed utilizzato una nuova molecola di sintesi, Difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate (CAS 1190931-27-1, sale di ammonio) denominata convenzionalmente cC6O4 che è già stata riscontrata nelle acque di falda e superficiali da parte di ARPAV. Questa molecola più piccola è un etere ciclico con soli 5 atomi di carbonio fluorurati e quindi può essere assimilata alla classe dei PFAS a catena corta.

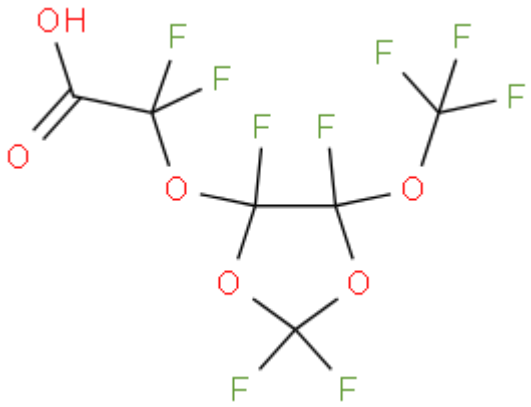
Il ritrovamento di questa molecola nelle acque venete, nelle quali è in corso la sperimentazione del LIFE, ha reso necessaria una valutazione preliminare del rischio secondo il modello in corso di sviluppo nell'ambito delle attività del LIFE PHOENIX. Nella letteratura o nei dossier delle istituzioni internazionali per questa molecola, sono mancanti i valori dei fattori di bioconcentrazione (BCF) o di bioaccumulo (BAF) necessari per una valutazione di accumulabilità e quindi di rischio per la catena trofica sia acquatica che terrestre.

IRSA-CNR ha svolto una attività integrativa rispetto a quanto scritto nel progetto LIFE, per ricavare il dato mancante di BCF o BAF e fare una prima valutazione dell'accumulabilità della sostanza nell'ambito dell'attività B2 (Implementing an informative and statistic system) per dare supporto all'azione B4 (Innovative and integrated forecast tools to support decision-making) nell'ambito della modellizzazione del trasporto e della ripartizione di PFAS a catena corta nei sistemi biologici.

I risultati della subazione C1.3 mostrano che cC6O4 ha caratteristiche fisico-chimiche simili a quelle del PFOA in termini di mobilità e persistenza.

Il confronto tra BCF sperimentale e previsto afferma che il log BCF dovrebbe essere in un intervallo inferiore a 2 e la molecola non dovrebbe essere classificata come non bioaccumulabile secondo la classificazione REACH. Ma ci sono prove sperimentali che questa molecola può distribuirsi nei sistemi biologici. L'affinità di cC6O4 con specifiche proteine, come le proteine del tuorlo nelle uova o l'albumina nel siero umano, dimostra la possibilità di esposizione e accumulo in uccelli e esseri umani per questa molecola. Secondo l'approccio Weight of Evidence (WoE), il potenziale di bioaccumulo di questa molecola non può essere completamente considerato trascurabile.

1. What is cC6O4?



REACH EC Number (pre-registration, by 2013)		REACH EC Number (registration, by Dec. 2019)	682-238-0
CAS Registry Number	1190931-41-9 (acid form) 1190931-27-1 (ammonium salt) 1190931-39-106 5 (potassium salt)	PubChem CID	
EU CLP Harmonized Classification*		DK-EPA / DTU QSAR-based CLP Advisory Classification	Aquatic Chronic 3
REACH registration cumulated minimum annual tonnage	10		
Molecular Formula	C6 H1 F9 O6	Molecular weight (g/mole)	340.06
Chemical Name	Difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate		
Synonymous	cC6O4; cyclic C6O4; F-Diox acid		

Following the industrial phase-out of PFOA, The diastereoisomeric mixture of four perfluoro([5-methoxy-1,3-dioxolan-4-yl]oxy) acetic acid (C6HF9O6), commercially known as cC6O4 or F-Diox acid, has been introduced as one of the alternative substances. cC6O4, a short-chain perfluoropolyether substance, has been registered and patented by **Solvay Specialty Polymers Italy S.p.A.** and **Miteni S.p.A** from 2011 to replace PFOA in accordance with the international EPA-PFOA Stewardship Program that proposed the abolition of PFOA and related chemicals by 2015 (ECHA, 2021; EFSA, 2014). Both ammonium difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate (CAS RN 1190931-27-1) and potassium difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate (CAS RN 1190931-39-5) are registered under REACH. The former was registered by Miteni in 2012 (which stopped production in 2019) and again in 2018 by Solvay (active registrant). The latter was registered by Solvay in 2011. MITENI S.p.A. (now inactive) produced over than 189 tons in the years 2010-2016 (Girardi et al., 2018). Wang et al. (2020) reported a cC6O4 ammonium salt production of 1-10 tons per year, while ECHA registered dossier reports a total tonnage band of 10-100 ton per year (ECHA, 2021). cC6O4 is used as surfactant and emulsifier in polymerization process of other substances (polymers, especially PTFE) and in the manufacture of cast film, fittings, valves, tapes and anti-stick coating (food contact materials) (Wang et al., 2020; EFSA 2014). Also a reduced form of

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cC6O4 was registered under REACH (Cas: 1190931-34-0: 2-[[2,2,4-trifluoro-5-(trifluoromethoxy)-1,3-dioxolan-4yl]oxy-ethanol) from 2010 by Solvay Specialty Polymers for intermediate use only.

All the chemical forms of cC6O4 (i.e. acid, ammonium and potassium salts) are highly soluble and completely dissociate in aqueous solutions, and, due to the strong C-F bond, they should be persistent and resistant to biodegradation in water (ECHA, 2021).

cC6O4 belongs to the class of perfluoroalkylether carboxylic acids (PFECA) introduced as alternative compounds to PFOA (Wang et al., 2020). These compounds contain oxygens in their carbon chain (ether group), but maintain similar chemical properties to those of older PFAS (Wang et al., 2015). They can be assimilated to short chain PFAS, and recent studies demonstrated that these chemicals are persistent and detected in both abiotic and biotic environments (Strynar et al., 2015; Washington et al. 2020, Pan et al. 2018).

2. What is known?

2.1 Environmental distribution

This molecule, at the best of our knowledge, has so far only been found in Italian waters.

The first detection of this molecule was claimed on April, 16th, 2019 with a press release by Environmental Protection Agency of Veneto (ARPAV) which found 42 ng/L in the Po river (Corbola station) and 65 ng/L in the drinking water of San Basilio Ariano (RO), close to the same river (https://www.arpa.veneto.it/arpavinforma/comunicati-stampa/archivio/comunicati-2019/arpav-segnala-la-presenza-del-composto-cc604-nel-po).

Further monitoring along the course of Po river on April, 2nd, 2019 gave the following results

Comune	cC6O4 (ng/L)
Castelmassa	85
Corbola	65
Villanova Marchesana	67
Taglio di Po	65

Recent monitoring carried out by the Regional Environmental Protection Agency of Veneto (Italy) detected relatively high levels of cC6O4, up to 3200 ng/L, in groundwater impacted by Miteni factory.

cC604 was found also in the river Bormida (a tributary of river Tanaro) downstream from the Solvay factory at Spinetta Marengo (AL) and in a 20 m-deep well used for drinking water production at Montecastello (AL) (località Pozzo Nuovo, fraz. Isorella, close to river Tanaro, a tributary of river Po) (cC6O4: 260 ng/L, Comunicato AMAG, Alessandria, del 13/06/2020).

Apart from the water bodies directly impacted by fluorochemical factories, cC6O4 was detected in groundwaters very close to landfills at Zevio (VR) and Sommacampagna (VR) (Regione Veneto; https://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-interne/pfas), and in Lombardia in some small streams (creeks Cosia, Terrò, and river Olona) which are highly impacted by discharges from industrial and urban wastewater treatment plants (ARPA Lombardia l monitoraggio delle sostanze perfluoroalchiliche (PFAS) in Lombardia. Audizioni della Commissione Ecomafie; 6 ottobre 2020; http://documenti.camera.it/leg18/resoconti/commissioni/stenografici/html/39/audiz2/audizione/2020/10/06/indice_stenografico.0083.html#)

2.2 Ecotoxicological and toxicological data

No environmental limits have been established yet, probably also due to a crucial lack of information about its ecotoxicological effects. To date, the acute toxicity of cC6O4 has been assessed only in the dossier of the European Chemicals Agency for REACH regulation, which determined the EC50 in *Daphnia magna* and *Danio rerio* respectively exposed to cC6O4 for 48 and 96 hours, via ecotoxicological tests (ECHA, 2021).

C.1.3 - Analysis to investigate the potential impacts of the new molecule cC6O4

The acute toxicity of the test item cC6O4 ammonium salt to zebra fish (*Danio rerio*) was determined in a 96-h static test according to the EU Commission Directive 92/69/, Part C.1 and Commission Regulation (EC) No 440/2008, Part C.1, as well as according to the OECD Guideline for Testing of Chemicals No. 203 (1992). A limit test was performed in accordance with the guidelines to demonstrate that the test item has no toxic effect on the test organisms at a nominal concentration of 625 mg cC6O4 ammonium salt/L corresponding to 100 mg dry salt cC6O4 ammonium salt/L (test item contains 84% water). Thus, a single nominal concentration of the test item was tested. Additionally, a control group was tested in parallel. In the control and at the test concentration of 100 mg dry salt/L, no mortality or other visible abnormalities were determined at the test fish during the test period of 96 hours.

In conclusion, the test item had no acute toxic effects on zebra fish up to the nominal concentration of 100 mg dry salt cC6O4 ammonium salt/L (corresponding to 625 mg test item cC6O4/L) under the present test conditions.

The acute toxicity of the test item cC6O4 ammonium salt to *Daphnia magna* was determined in a 48-hour static test according to the EU Commission Directive 92/69/EEC, Part C.2, Commission Regulation (EC) No 440/2008, Part C.2 and the OECD Guideline for Testing of Chemicals, No. 202 (2004). A limit test was performed in accordance with the guidelines to demonstrate that the test item cC6O4 ammonium salt has no toxic effect on the test organisms at a nominal concentration of 625 mg test item cC6O4 ammonium salt/L corresponding to 100 mg dry salt cC6O4 ammonium salt/L (test item cC6O4 ammonium salt contains 84% water). Thus, a single nominal concentration of the test item cC6O4 ammonium salt was tested. In the control and at the test item cC6O4 ammonium salt concentration of 100 mg dry salt cC6O4/L, no immobilized test organisms were observed during the test period of 48 hours. The summary of the biological results (based on nominal test item concentration of cC6O4 ammonium salt expressed as content of dry salt cC6O4 ammonium salt) is as follows:

– 48 -hour EC50: >100 mg/L
– 48 -hour EC0 and 48 -hour NOEC: ≥100 mg/L

In conclusion, the test item cC6O4 ammonium salt had no acute toxic effects on *Daphnia magna* up to the nominal concentration of 100 mg dry salt cC6O4 ammonium salt/L (corresponding to 625 mg test item cC6O4 ammonium salt/L) under the present test conditions.

Also for freshwater algae, no effects have been measured up to 100 mg/L, and the following data are registered in ECHA (2021):

EC50 (72h) for freshwater algae: 100 mg/L
EC10 or NOEC (72h) for freshwater algae: 100 mg/L

A recent study evaluated for the first time the effects of cC6O4 on cellular and biochemical parameters of the clam *Ruditapes philippinarum*, a bivalve species widely used in ecotoxicological investigations (Bernardini et al., 2021). The hypotheses tested were i) whether a short-term (7 days) or a long-term (21 days) exposure to

environmental realistic concentrations of cC6O4 (0.1 µg/L and 1 µg/L) could pose a potential risk to marine bivalves and ii) whether the effects induced by cC6O4 are comparable to those caused by PFOA.

Results indicate that cC6O4 may cause significant perturbations to the digestive gland microbiota, likely determining the impairment of host physiological homeostasis. Transcriptional analyses revealed several alterations of the gene expression profile: a large part of the altered pathways, including immune response, apoptosis regulation, nervous system development, lipid metabolism and cell membrane metabolism is the same in cC6O4 and PFOA exposed clams. In addition, clams exposed to cC6O4 showed dose-dependent responses as well as possible narcotic or neurotoxic effects and reduced activation of genes involved in xenobiotic metabolism.

In the same exposition experiment numerous biomarkers were measured in haemocytes/haemolymph, as well as in gills and digestive gland. The two-way ANOVA analysis performed for each biomarker response indicated that the two compounds (PFOA and cC6O4) affect most of the cellular and tissue parameters measured. Despite preliminary, the results obtained suggested that cC6O4 – similarly to PFOA - can affect both cellular and biochemical parameters of clams (Fabrello et al., submitted).

About human toxicology, Coperchini et al. (2020) presented an *in vitro* study which is the first evaluation of the potential adverse effects of the new emerging PFAS cC6O4 in cultured rat and human thyroid cells, compared with PFOA and PFOS.

cC6O4 exposure did not modify FRTL5 rat-thyroid cell lines and normal human thyroid (NHT) cell viability at any concentration and/or time points with no induction of necrosis/apoptosis. At difference, PFOS exposure reduced cell viability of FRTL5 while and NHT, while PFOA only in FRTL5. FRTL5 and NHT cell proliferation was reduced by incubation with by PFOA and PFOS, but not with cC6O4. ROS production by NHT and FRTL5 cells was not modified after cC6O4 exposure, at any time/concentration tested, suggesting its safety for thyroid cells in vitro.

In addition, a scientific opinion published by EFSA reported that cC6O4 does not induce gene mutations in mouse lymphoma cells and in bacteria, nor produces chromosome aberrations in rat bone marrow cells, as assessed by three *in vitro* and, more importantly, by one *in vivo* genotoxicity tests (EFSA, 2014).

cC6O4 has been measured in the serum of MITENI workers, with very variable concentrations and very high maximum concentrations, as reported in the following table from (Girardi et al., 2018)

Tabella 7.3. Valori di C6O4 rilevati nelle determinazioni sieriche nel periodo 2013-2017

	2013	2014	2015	2016	2017
N° campioni	79	87	87	123	116
Media	16,67	30,83	39,01	20,60	15,19
Mediana	0,5	2,7	6,7	2,4	1,2
Range	0,5 - 864,9	0,5 - 850,8	0,5 - 932,1	0,5 - 354,3	0,5 – 244,4

The highest median value (60.77 ng/mL) was measured for the workers of the pilot plant for cC6O4 recovery.

Although potentially reassuring, these previous and present findings do not allow drawing firm general conclusions as to the safety of cC6O4 exposure (Coperchini et al., 2020).

3. The LIFE PHOENIX results: Water occurrence of cC6O4 in 2019-2021.

3.1 Water sampling and analysis by CNR-IRSA

Under LIFE PHOENIX project some monitoring campaigns for water sampling have been carried out in Veneto, in Piemonte and along the course of river Po, in order to understand the distribution and to assess the potential risks for drinking waters.

The following campaigns have been carried out:

- 11/02/2020. Bormida river upstream the Solvay discharge; the Solvay discharge; drinking water at Spinetta Marengo (AL).
- 20/02/2020: sampling Po river from Piacenza to Ferrara; sampling groundwater at Miteni industrial site, at Creazzo (VI) and at Lonigo (VI); drinking water at Ferrara.
- 27/02/2020 Sampling of Po river upstream and downstream of the Tanaro confluence; Tanaro river downstream of the Bormida river confluence; Bormida river upstream and downstream of the Solvay discharge; the Solvay discharge; groundwaters at Spinetta Marengo (AL).
- 19/01/2021 Spinetta Marengo, Sampling of Po river downstream of the Tanaro confluence; Tanaro river downstream of the Bormida river confluence; Bormida river upstream and downstream of the Solvay discharge; the Solvay discharge
- 17/02/2021 Spinetta Marengo, Sampling of Bormida river upstream of the Solvay discharge; the Solvay discharge
- 04/03/2021 Spinetta Marengo, Sampling of Po river upstream and downstream of the Tanaro confluence; Tanaro river downstream of the Bormida river confluence; Bormida river upstream and downstream of the Solvay discharge; the Solvay discharge fish sampling in river Bormida, downstream of the discharge

The results are presented in the following table 3.1:

Table 3.1. *Concentrations measured in the IRSA monitoring campaigns*

Site	Sample type	Date	PFBA (ng/L)	PFBS (ng/L)	PFPeA (ng/L)	PFHxA (ng/L)	PFHpA (ng/L)	PFOA (ng/L)	PFNA (ng/L)	PFDA (ng/L)	PFHxS (ng/L)	PFOS (ng/L)	FOSA (ng/L)	4:2FTS (ng/L)	6:2FTS (ng/L)	cC604 (ng/L)
Spinetta Marengo	Drinking water	11/02/20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Valenza Po	River Po	27/02/20	<LOD	25	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2
Spinetta	River Bormida upstream Solvay	11/02/20	<LOD	14	<LOD	<LOD	<LOD	8	<LOD	<LOD	<LOD	12	<LOD	<LOD	<LOD	25
Spinetta	River Bormida upstream Solvay	27/02/20	<LOD	13	<LOD	<LOD	<LOD	5	<LOD	<LOD	<LOD	11	<LOD	<LOD	<LOD	12
Spinetta	Solvay discharge	11/02/20	<LOD	<LOD	<LOD	<LOD	376	2938	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	141 842
Spinetta	Solvay discharge	27/02/20	<LOD	<LOD	<LOD	<LOD	331	314	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17 354
Spinetta	groundwater	27/02/20	<LOD	<LOD	<LOD	<LOD	36	1110	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	354
Spinetta	River Bormida downstream Solvay	27/02/20	<LOD	<LOD	<LOD	<LOD	69	439	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	806
Rivarone (AL)	River Tanaro downstream of the Bormida	27/02/20	<LOD	22	<LOD	<LOD	4	30	<LOD	<LOD	<LOD	11	<LOD	<LOD	<LOD	233
Casei Gerola (PV)	River Po	27/02/20	<LOD	22	<LOD	<LOD	2	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	88
Piacenza	River Po	20/02/20	<LOD	20	<LOD	<LOD	3	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	35
Isola pescaroli (CR)	River Po	20/02/20	<LOD	24	<LOD	<LOD	3	5	<LOD	<LOD	<LOD	13	<LOD	<LOD	<LOD	54
Pontelagoscuro (FE)	River Po	20/02/20	<LOD	22	<LOD	<LOD	4	2	<LOD	<LOD	<LOD	13	<LOD	<LOD	<LOD	28
Ferrara	Drinking water	20/02/20	<LOD	8	<LOD	<LOD	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	37
Trissino (VI)	groundwater at Miteni industrial site	20/02/20	2878	4126	<LOD	<LOD	612	12525	<LOD	<LOD	540	6785	<LOD	<LOD	<LOD	1 583
Creazzo (VI)	groundwater from resurgence	20/02/20	279	301	90	116	58	1173	<LOD	8	22	289	<LOD	<LOD	<LOD	299
Lonigo (VI)	groundwater from well	20/02/20	1484	798	961	903	298	3833	<LOD	<LOD	93	149	<LOD	<LOD	<LOD	<LOD

3.2 Water monitoring activity by ARPAV

ARPAV during its institutional monitoring campaign added cC6O4 as a supplemental monitoring variable in some selected areas, both in groundwaters, discharges and surface waters.

The area under consideration includes two river basins (the Po and the Brenta-Bacchiglione catchments) contributing to the north-Adriatic Sea basin. Both river basins are characterized by the presence of significant industrial plants producing fluoro-chemicals and fluoro-polymers, seriously impacting the quality of ground and surface waters and related ecosystems.

Po river is the Italian longest watercourse (652 km) with the greatest discharge (average 1,470 m^3/s) which flows across the so-called Padana plain where more than 16 million people lives, generating nearly 40% of the Italian Gross Domestic Product by intensive industry, agriculture, livestock breeding and other economic activities. The sub-basin under consideration belongs to the lowest tract of the river from Regione Lombardia to the basin closure (Occhiobello) and its delta, whose waters are used to supply drinking water and to aquaculture (mainly mollusc). The quality of the water is severely impacted by PFAS discharges from a PTFE plant at Spinetta Marengo, Piemonte (located about 350 km upstream) and from the highly industrialised and urbanized area around Milano (Valsecchi et al., 2015).

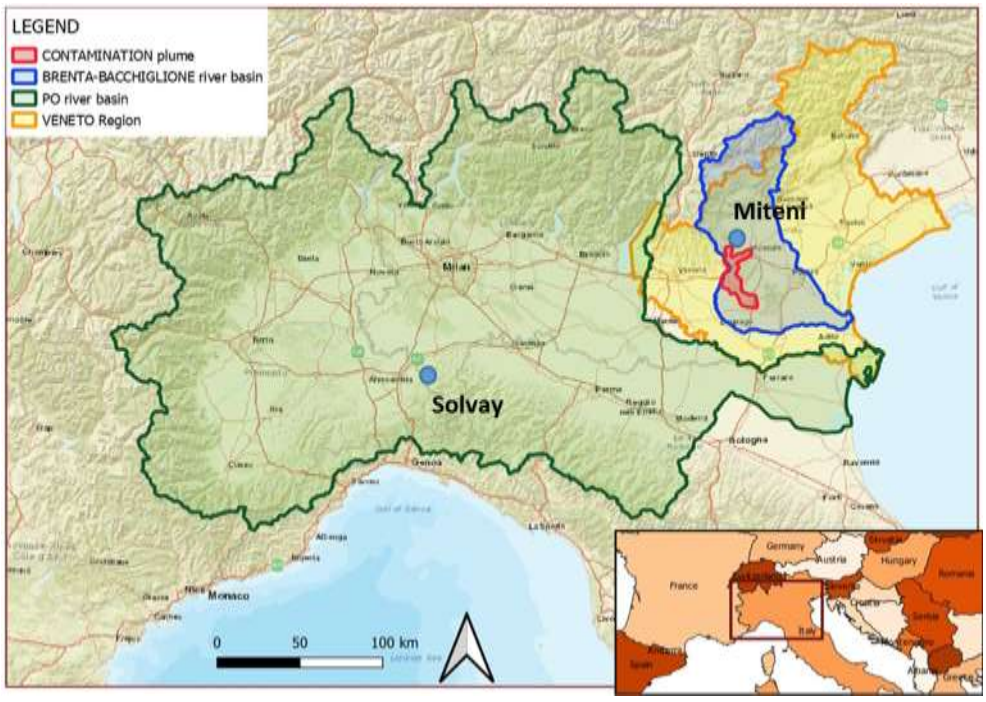

Figure 3.1: *Map of Po and Brenta-Bacchiglione river basins catchments and plume of PFAS contamination* (credit Paolo Ronco - RIVE).

The Brenta-Bacchiglione river system flows into the northern Adriatic Sea between the lagoon of Venezia and the delta of the Po. The area is deeply industrialized (mainly SMEs, in particular upstream) and also characterize by intensive agriculture and breeding activities (downstream). In particular, one of its tributaries, the Fratta-Gorzone river, receives treated wastewaters from a large textile-tannery district where a fluoro-

chemical plant, owned by Miteni in Trissino (V), sited in the recharge area of a very large aquifer, produced PFAS compounds since the early '60. This plant has been identified in 2013 by CNR-IRSA and ARPAV, as the main pollution source of both surface and ground-waters in an area of 600 km^2, where 140.000 people have been directly exposed to PFAS mainly by drinking waters at concentrations above 70 µg/l as ∑ PFAS.

Complete dataset for PFAS concentration of groundwaters of Veneto region is available at:

https://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-interne/pfas

The total number of cC6O4 measurements is 2307 with a percentage of detection of 26%. The distribution of positive samples is the following, with more than 150 samples with concentrations above 100 ng/L, which is considered a threshold level for drinking water protection for the sum of PFAS, according to Directive 2020/2184.

Table 3.2 . *Distribution of cC6O4 concentrations in groundwater of Veneto (2019-2020)*

N	2307
N>LOD (40 ng/L)	592
40 ≤ N<100 ng/L	30
100 ≤ N<500 ng/L	149
500 ≤ N<1000 ng/L	7
1000 ng/L ≤ N	11

The following Table presents the statistical distribution of cC6O4 data in the sites where there were positive findings.

Table 3.3 *Statistical distribution of groundwater cC6O4 concentration in the sites with positive cC6O4 findings*

	Zevio (VR)	Sommacampagna (VR)	Castelgomberto (VI)	Montecchio Maggiore (VI)	Trissino (VI)	Sovizzo (VI)	Creazzo (VI)	Vicenza (VI)
N	122	53	7	150	63	10	41	80
N><LOD	25	2	1	53	32	10	28	2
MIN	<LOD	<LOD	<LOD	<LOD	<LOD	72	<LOD	<LOD
MAX	295	7250	183	400	3265	320	345	153
MEAN	21.0	95.1	26.1	69.8	285.1	168.8	168.3	2.9
MEDIAN	<LOD	<LOD	<LOD	<LOD	127	155.5	208	<LOD
% Detection	20.5	3.8	14.3	35.3	50.8	100.0	68.3	2.5

Positive data of the two locations in province of Verona (Zevio and Sommacampagna) have been obtained in groundwaters very close to some industrial landfills, suggesting an infiltration of leachates into groundwaters.

The other sites in province of Vicenza are located along the groundwater path coming from Miteni industrial site, which feeds also some resurgences in the Creazzo area.

This monitoring action will be complemented by the further development and implementation of a 3D fate&transport model of PFAS diffusion in groundwater, developed by ARPAV under LIFE PHOENIX project. This model, particularly addressing the different mobility patterns of the various PFAS according to their adsorption properties, has been validated during the project by a dedicated monitoring campaign by ARPAV, using the site-specific geological physico-chemical variables of the area (Action B4). The model has been tested also with new emerging PFAS compounds, such as Gen-X and cC6O4, which have been already monitored in the groundwater.

The following figure, presented at the third International Webinar on 3rd March 2021 (Title: Modelling and monitoring legacy and emerging PFAS pollution at a catchment scale) by M. Mazzola (ARPAV): *3D modelling for assessing and forecasting PFAS distribution and evolution in a groundwater at a catchment scale,* shows the comparison between the measured cC6O4 data in the groundwater and simulated cC6O4 distribution.

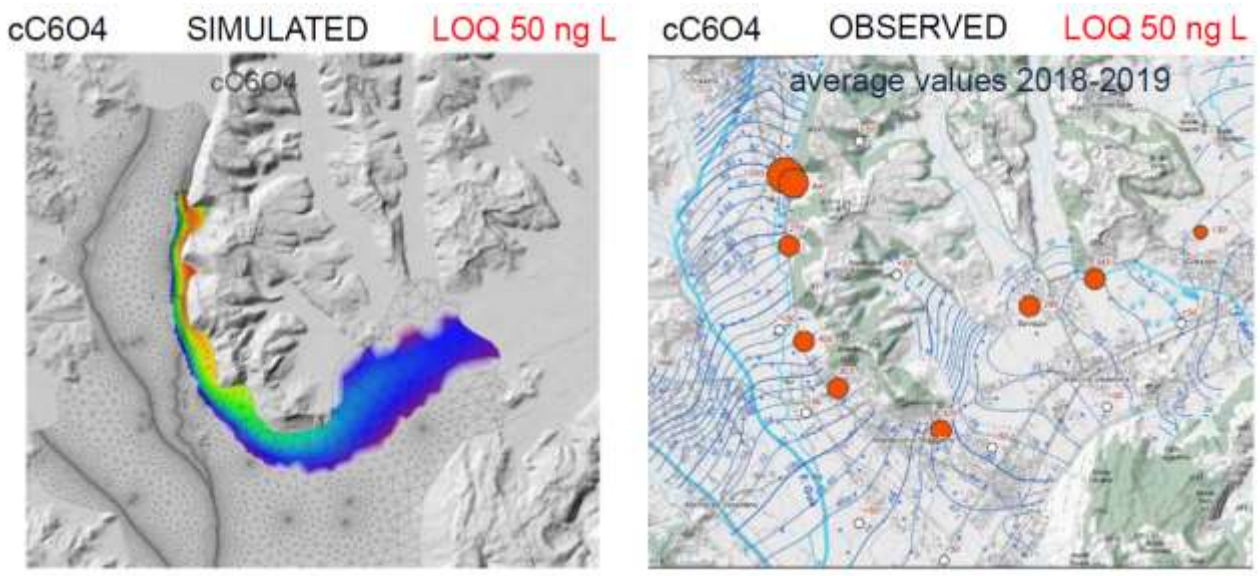

Figure 3.2:*Comparison between the modelled (left) and the observed (right) concentrations of cC6O4 in the groundwater from the Trissino site* (credit Massimo Mazzola – ARPAV).

Complete dataset for surface waters is available at:

https://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-interne/pfas

The total number of cC6O4 measurements is 443 with a percentage of detection of 16%. The distribution of positive samples is the following, with more only 14 samples with concentrations above 100 ng/L.

Table 3.4. *Distribution of cC6O4 concentrations in surface waters of Veneto (2019-2020)*

N	443
N>LOD (40 ng/L)	72
40 ≤ N<100 ng/L	58
100 ≤ N<500 ng/L	13
500 ≤ N<1000 ng/L	0

1000 ng/L ≤ N	1

The following Table presents the statistical distribution of cC6O4 data in the sites where there were positive findings.

Table 3.5 *Statistical distribution of surface water cC6O4 concentration in the sites with positive cC6O4 findings*

	Cologna Veneta	Vicenza	Melara	Castelmassa	Occhiobello	Villanova Marchesana	Corbola	Taglio di Po	Porto Tolle	Ariano Polesine	Canaro
River	**Fratta**	**Retrone**	**Po**	**Po**	**Po**	**Po**	**Po**	**Po**	**Po**	**Po di Goro**	**Scolo Poazzo**
N	115		6	51	1	32	27	28	18	7	11
N>LOD	2	1	2	19	1	20	6	8	8	2	1
MIN	<LOD		<LOD	<LOD		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
MAX	407	60	290	261	40	226	1190	107	70	60	60
MEAN	2.9		60.0	32.0		56.4	54.7	22.2	22.2	15.7	5.5
MEDIAN	<LOD		<LOD	<LOD		52	<LOD	<LOD	<LOD	<LOD	<LOD
% Detection	1.7		33.3	37.3		62.5	22.2	28.6	44.4	28.6	9.1

The sampling site in Cologna Veneta is sited on Fratta channel which collects the discharges from ARICA collectors which drains 5 WWTPs including the one of Trissino, where Miteni plant has discharged until the closure in 2019. There were only 2 positive findings on Fratta channel, while in 53 samples of ARICA collectors from 06/09/2018 to 10/12/2020 no positive findings have been claimed (but the LOD in the discharge samples was 200 ng/L). There was only one finding in Retrone river which has origin from resurgences in the impacted Creazzo area. The other findings were along the last tract of the Po River, impacted by the discharges of Solvay in Spinetta Marengo (AL). This suggests that the compound cC6O4 is persistent and mobile in natural waters and can reach easily the fragile ecosystems of the river Po Delta. The maximum concentration (1190 ng/L) was measured at Corbola, while the average concentrations in every Po sites range from 20 to 60 ng/L.

Figure 3.3 : *ARPAV sampling points along the last tract of the Po river*

4. cC6O4: Bioaccumulation

4.1 Field bioaccumulation

4.1.1. Clam bioaccumulation in Venice Lagoon

Manila clams (*R. philippinarum*) (3.64 ± 0.32 cm shell length) were collected within the Venice lagoon in February 2020 for the lab bioaccumulation experiments (Bernardini et al., 2021). Before exposition, IRSA-CNR analyzed the specimens to find the background concentrations of PFOA and its substitute cC6O4. The analysed clams show measurable concentrations of the PFAS uptake in their environment of origin, the Lagoon of Venice, which should represent a low impacted area because it is not directly influenced by Po and Brenta rivers. In fact, the PFOA concentration (0.9±0.5 µg/kg ww) is lower than those measured in the Goro Lagoon (1.6-5.8 µg/kg ww) (Mazzoni et al. 2016), which receives Po river water contaminated by fluoropolymer plants discharges (Valsecchi, 2015; Rusconi et al., 2015). The PFOA level observed in the Manila clams of the present study is similar to PFOA concentration reported in clams from the Aichi and Kumamoto area, a populated and industrial inland sea region of Japan (0.3-1.5 µg/kg ww) (Fujii et al., 2020), whereas it is higher than that reported in clams from Nunavut, a sparsely populated area of Canada with no industrial activity (<0.4 µg/kg ww) (Ostertag et al., 2009). These findings suggest that the PFAS level in the Manila clams collected in the lagoon of Venice could be considered an indicator of the background concentration of areas impacted by anthropic activities. Similarly, average cC6O4 concentration (0.19±0.01 µg/kg) ww can be considered an indicative level of the cC6O4 bioaccumulation in the clams of the Lagoon of Venice suggesting that cC6O4 is already diffused in the aquatic environment, even in areas not directly impacted by industrial sources.

4.1.2. Egg monitoring in a perfluoropolymer factory site (Spinetta Marengo) in the Po river valley

The main monitoring actions implemented by CNR-IRSA was the development of an innovative passive sampling and biomonitoring tool, based on the PFAS monitoring in bird eggs integrated by the environmental monitoring campaign of waters. This combined approach has given an overview of the quality status of the area and, building on already available data about PFAS distribution, has supported the characterization of the different pathways and patterns of exposure of humans and ecosystems both by terrestrial and aquatic trophic chain. Bird eggs have been collected by artificial nest-boxes, targeting insectivorous, resident, common birds that breed in nest-boxes (e.g. *Sturnus vulgaris/unicolor, Parus major*). Nest-boxes have been spread across impacted and un-impacted areas, where data on PFAS in water, soil and invertebrates are available.

Figure 4.1. *Map of the sampling site.* Legenda: Yellow pointe: the nest boxes for birds; The pink triangles: 1. The outlet of the plant discharge; 2. The treatment plant; 3. the perfluoropolymer Factory Site PFS (including polytetrafluoroethylene).

In February 2020, a series of wooden nest-boxes were distributed within a 3 km buffer in the environment surrounding a fluoropolymer plant (FPS), sited in Spinetta Marengo, located in the Western sector of the Po River valley. Since the 1980s, the target industrial plant produced several fluorinated monomers and fluids, in addition to fluorocarbon-based polymers (fluorinated plastomers and elastomers) including PTFE. PFOA has been used for many years as an emulsifier in the PTFE polymerization and it was present at high concentration in the industrial wastewaters (Rusconi et al. 2015), which contaminated the receiving rivers (Loos et al. 2008; Valsecchi et al. 2015). Since 2015, PFOA concentration in the fluoropolymer factory's discharge decreased because it was replaced by cC6O4 as processing aids in the production of fluoropolymers (EFSA 2014). In 2020 monitoring of the industrial wastewaters determined concentrations of ca. 50-100 µg/L of cC6O4 and <10 µg/L of PFOA.

In April 2020, a total of 14 nest-boxes were hosting complete clutches: 1 of great tit (*Parus major*), 1 of blue tits (*Cyanistes caeruleus*) and 11 of starling (*Sturnus vulgaris*). We collected one egg per brood in the case of starling and two eggs per brood for tits (eggs are much smaller) for PFAS analysis. Eggs were randomly chosen and in all the cases when the clutch was completed and the adults incubating. Additionally, we collected 5 eggs of starling from 5 different clutches laid in a rural site (RS) in northern Italy, far from any known point source of PFAS release. Sample treatment and analytical methods are described in Mazzoni et al. (2016).

Distribution patterns of PFAS in wild bird eggs

PFAS concentrations (Table 4.1) and distribution patterns in whole eggs by bird species and sampling sites are shown in Figures 4.2 and 4.3. PFAS concentrations in starling eggs collected around the fluoropolymer factory were at least one order of magnitude higher than those collected in the rural site. PFAS levels in tit eggs were also very high and comparable with those of starlings collected in the polluted site, but, at the moment, we lack any reference values for unimpacted tit eggs. For all perfluorinated compounds except PFOA

and cC6O4, concentrations in the sampled eggs were independent of the species. In contrast, PFOA concentrations in starling eggs collected around the perfluopolymer factory were two orders of magnitude higher than those measured in the tit samples (Fig. 4.2 left plot). Comparing eggs collected close to other PTFE factories, PFAS concentrations in Italian site were higher than those determined in free-ranging home-reared chicken eggs collected within 2 km of a fluorochemical industrial park that has been producing PTFE for 15 years in northern China (Su et al. 2017). The PFAS distribution patterns of starling eggs in Italy and chicken eggs around the Chinese fluorochemical plants are similar, apart from PFBA which was not included in the Italian monitoring. For both species feeding on soil invertebrates, PFOA was the predominant compound in whole eggs. However, the PFAS concentrations measured in the eggs from great tits and starling collected close to the Italian fluoropolymer plant were lower than those recorded in the eggs of great tits breeding near the Belgian fluorochemical plant (Groffen et al., 2017, 2019).

If we consider the egg fingerprint of all PFAS except PFOA and cC6O4, which are the compounds specifically used in the PTFE production site, (Fig. 4.3 on the left), both tit species were similar to the starling and PFOS did not predominate over the other PFAS, unlike the starling collected in the in the unimpacted (RS) area. On the contrary, considering all the PFAS (Fig. 4.3 on the right), PFOA dominated the PFAS composition in the starling, with an average percentage of 87%, whereas cC6O4, its recent substitute, had the highest concentrations in the tit eggs, but not exceeding 40%.

These differences may be explained by the different diets of these species. Starlings feed mostly on ground insects and invertebrates (e.g earthworms, Fink et al. (2020)), whereas tits feed mainly on insects and spiders that they capture by foliage gleaning (Cramp and Perrins 1993). It is possible that PFOA used for many years in the local PTFE production had heavily contaminated the soil due to atmospheric deposition (Davis et al. 2007). For this reason, the terrestrial diet of the starling is likely to be enriched with PFOA, compared to the diet of tits. We hypothesize that cC6O4 has not yet heavily contaminated the soil because it was only recently introduced; it might be present in air or aerosol emissions too, resulting in surface deposition on local vegetation and the contamination of insects feeding on that vegetation.

Most of the results have been published in:

Morganti M., Polesello S., Pascariello S., Ferrario C., Rubolini D., Valsecchi S. and Parolini M., Exposure assessment of PFAS-contaminated sites using avian eggs as a bio-monitoring tool: a frame of reference and a case study in the Po River valley (Northern Italy), *Integrated Environmental Assessment and Management*, 2021, on line published. https://doi.org/10.1002/ieam.4417

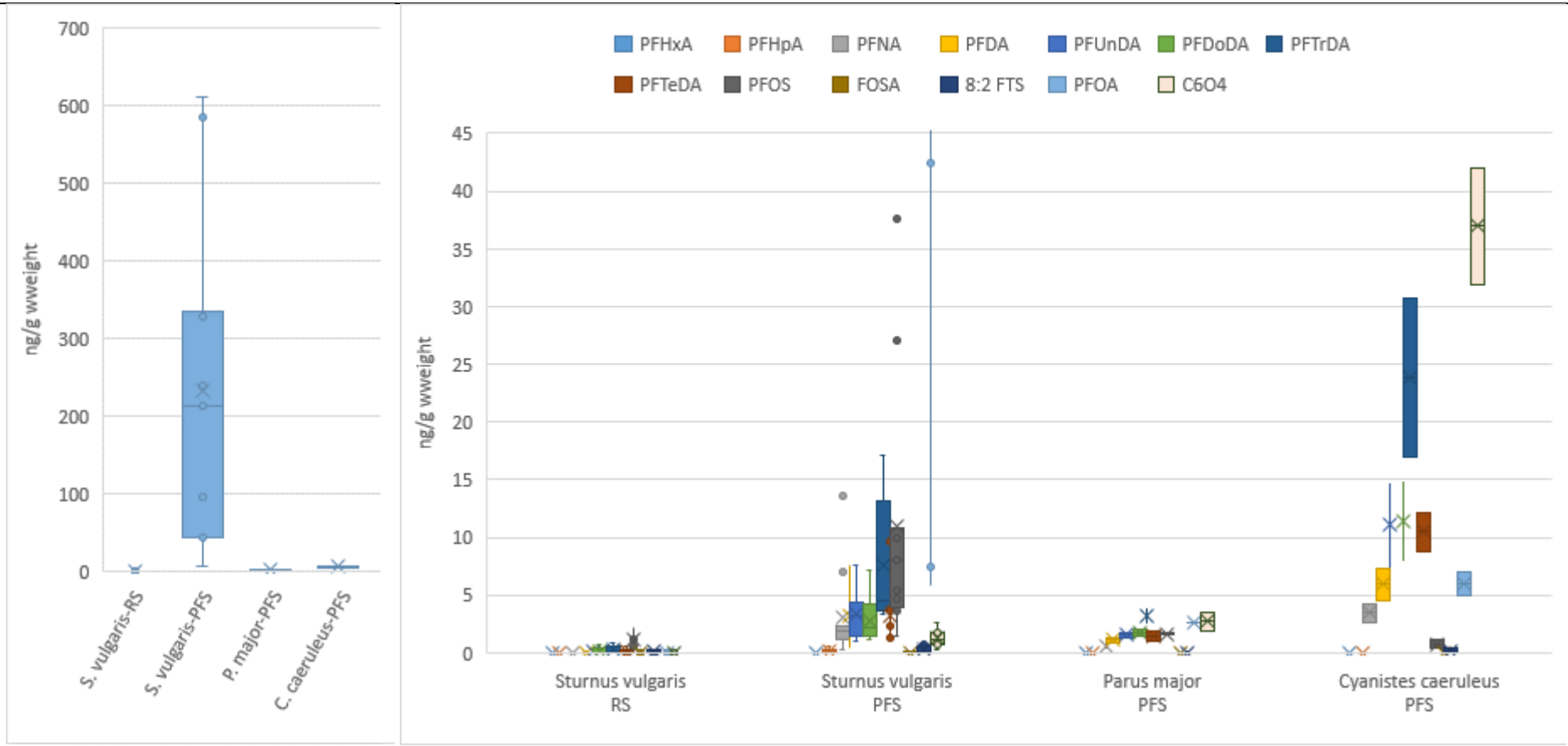

Figure 4.2. *PFAS concentration in the whole eggs according to the bird species and sampling sites*. PFS, perfluoropolymer factory site; RS, rural site. Left plot only PFOA. Right plot all the measured PFAS with PFOA of *Sturnus vulgaris*-PFS out of y axis scale. *Sturnus vulgaris*- RS (N= 5 nests with 1 egg each); *Sturnus vulgaris*-PFS (N=11 nests with 1 egg each); *Parus major*-PFS (N= 1 nest with 2 eggs); *Cyanistes caeruleus*-PFS (N= 1 nest with 2 eggs).

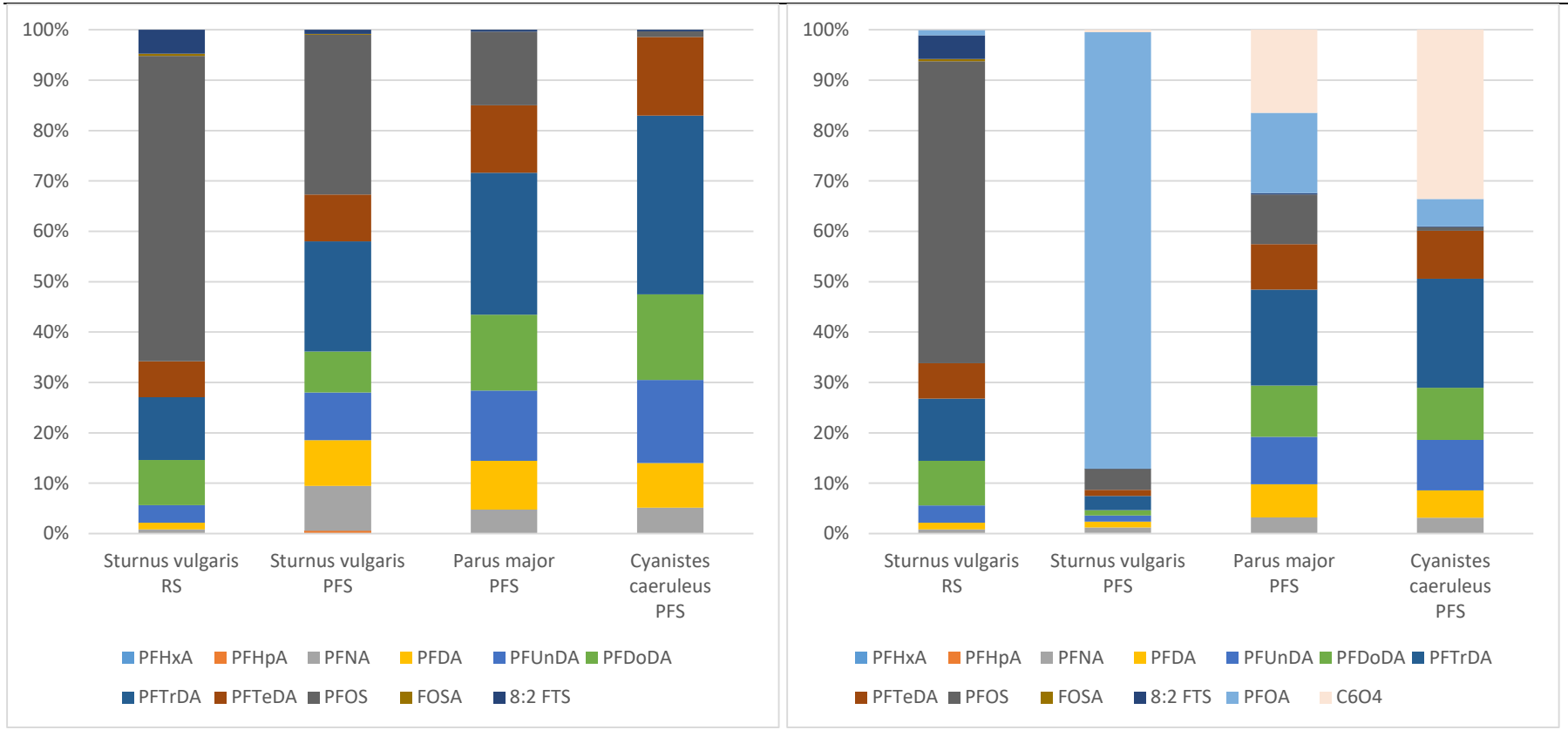

Figure 4.3 *Percentage distribution pattern of the PFAS in the whole eggs according to the bird species and sampling sites.* Left plot: all the measured PFAS except PFOA and cC6O4. Right plot: all the measured PFAS including PFOA and cC6O4. PFS, perfluoropolymer factory site; RS, rural site. *Sturnus vulgaris*- RS (N= 5 nests with 1 egg each); *Sturnus vulgaris*-PFS (N=11 nests with 1 egg each); *Parus major*-PFS (N= 1 nest with 2 eggs); *Cyanistes caeruleus*-PFS (N= 1 nest with 2 eggs).

Table 4.1. *Average PFAS concentration in the whole eggs according to the bird species and sampling sites.* PFS, perfluoropolymer factory site; RS, rural site. Left plot only PFOA. *Sturnus vulgaris*- RS (N= 5 nests with 1 egg each); *Sturnus vulgaris*-PFS (N=11 nests with 1 egg each); *Parus major*-PFS (N= 1 nest with 2 eggs); *Cyanistes caeruleus*-PFS (N= 1 nest with 2 eggs).

Site	Sample type	year	PFHxA (ng/g ww)	PFHpA (ng/g ww)	PFOA (ng/g ww)	PFNA (ng/g ww)	PFDA (ng/g ww)	PFUnDA (ng/g ww)	PFDoDA (ng/g ww)	PFTrDA (ng/g ww)	PFTrDA (ng/g ww)	PFOS (ng/g ww)	FOSA (ng/g ww)	8:2FTS (ng/g ww)	cC604 (ng/g ww)
Rural	*Sturnus vulgari*	2020	<LOD	<LOD	<LOD	0.02	0.03	0.07	0.18	0.24	0.14	1.2	0.01	0.09	<LOD
PFS Spinetta	*Sturnus vulgari*	2020	<LOD	<LOD	233	3.1	3.1	3.3	2.8	7.6	3.2	11.0	0.05	0.28	1.3
PFS Spinetta	*Parus major*	2020	<LOD	<LOD	2.7	0.53	1.1	1.6	1.7	3.2	1.5	1.6	<LOD	0.04	2.7
PFS Spinetta	*Cyanistes caeruleus*	2020	<LOD	<LOD	5.9	3.5	5.9	11	11	24	11	0.8	<LOD	0.20	37

4.2 Laboratory bioaccumulation

CNR-IRSA, in collaboration with the University of Padova (Bernardini et al., 2021), determined the for the first time on the bioaccumulation of cC6O4 on a marine invertebrate, the Manila clam (*Ruditapes philippinarum*), the most important bivalve species cultured in the Venice Lagoon, exposed to environmentally realistic concentrations of cC6O4 and PFOA (0.1 µg/L and 1 µg/L) for 21 days.

Analyses of cC6O4 and PFOA bioaccumulation was carried out on soft tissue samples (10 specimens per concentration), haemolymph (10 specimens per concentration) and waters of the exposition tanks.

The concentrations of cC6O4 and PFOA measured in the experimental tanks and in exposed clams are reported in Table 4.2. cC6O4 and PFOA concentrations in water have been sampled after water change, each sampled thrice: at time 0, 24 h and 48 h before water renewal. During the exposure period, no loss of spiked chemicals was observed. To calculate bioaccumulation factors (BAF) in clams, the mean concentration of each chemical measured in the water was used.

Clams accumulated both cC6O4 and PFOA, but at different proportion depending on the tissue: in soft tissues PFOA is accumulated more than five times than cC6O4 (BAF=119 and 21 in PFOA_H and cC6O4_H, respectively), while in the haemolymph the accumulation of PFOA is only 0.6 times the accumulation of cC6O4 (5.4 and 9.5 µg/kg ww for PFOA and cC6O4 respectively, Table 4.2).

Table 4.2. *PFOA e cC6O4 concentrations in exposure water and in the soft tissue and haemolymph of Manila clams after 21 days of exposition*.

Sample	Water nominal concentration (µg/L)	Water measured concentration (µg/L)	Sample (g ww)	Sample (mL)	PFOA (µg/kg ww)	cC6O4 (µg/kg ww)	BAF (L/kg)	LogBAF
Clam soft tissue								
CTRL	0	<LOD	15.51		**1.5**	**0.22**		
cC6O4_L	0.1	0.11±0.02	24.41		**0.73**	**2.3**	20	1.3
cC6O4_H	1	1.01±0.07	19.41		**0.58**	**21.1**	21	1.3
PFOA_H	1	0.93±0.31	24.84		**110**	**0.18**	119	2.1
Clam haemolymph								
CTRL	0	<LOD	3.51	3	**0.30**	**<0.14**		
cC6O4_L	0.1	0.11±0.02	3.74	3.6	**<0.07**	**<0.14**	-	
cC6O4_H	1	1.01±0.07	4.18	3.6	**<0.07**	**9.5**	9.4	1.0

PFOA_H	1	0.93±0.31	2.91	2.5	**5.2**	**<0.14**	5.6	0.8

The PFOA bioaccumulation factor (BAF) is in line with literature data for aquatic organisms (Valsecchi et al. 2017; RIVM, 2017). Our data suggest a 5 times lower accumulation potential of C604 as compared to PFOA in clam soft tissues. However, considering that some PFAS, including PFOA, have an immunotoxic potential in the marine bivalve *Perna viridis* - probably due to direct and indirect interactions with the haemocyte membrane (Liu et al. 2018) - we also measured PFOA and cC6O4 concentrations in the haemolymph. Contrarily to what observed in soft tissues, concentration in haemolymph (at 1 µg/L of contaminants) showed values higher for cC6O4 than for PFOA (Table 4.2), suggesting that cC6O4 might reach high levels in specific tissues, potentially triggering similar toxicity to PFOA even if bioaccumulates less in clam whole body.

The predicted anionic permeability of cC604 (based on the correlation of its hexadecane/water partition coefficient and COSMO calculations, as described in Ebert et al. 2020) is close to the value of PFOA. By running the COSMOmic calculations to predict the membrane/water partition coefficients for cC604, the sorption to membrane of cC604 is in the same range as for PFOA, similar to the results for the anionic permeability (F. Allendorf, personal communication). This indicates that cC604 has similar physico-chemical properties and behaves similarly as PFOA.

4.3 Modelling the bioaccumulation:

E. Benfenati: ***The prediction of the properties of the substance cC604.***

This part has been carried out by Emilio Benfenati (Istituto Mario Negri, Milano) in the framework of the collaboration between LIFE PHOENIX and LIFE VERMEER projects.

Istituto Mario Negri predicted the log Kow (named logP), BCF, BAF, Koc and kM properties of the substance of interest (cC6O4) using *in silico* models.

To be predicted the following modified SMILES for the EPISuite with the ammonium salt were used, the neutralised one for all the other tools (included EPISuite neutralized)

Modified for EPISuite: C(=O)(C(OC1(C(OC(O1)(F)F)(OC(F)(F)F)F)F)(F)F)ON

Neutralized: C(=O)(C(OC1(C(OC(O1)(F)F)(OC(F)(F)F)F)F)(F)F)O

These *in silico* models have been used:

EPISuite, of the US EPA, for the prediction of logP, BCF, BAF, half-life in fish, and Koc.

EPISuite provides predictions both for the ammonium salt and for the neutral form. This software platform is the only one offering this possibility. This is quite useful, since the target compound is the ammonium salt. Thus, two predicted values are obtained in most cases.

EPISuite does not provide a simplified measure to evaluate the reliability of the predictions.

OPERA, of the NIH. The model has been developed by Kamel Mansouri, previously at US EPA.

OPERA does not provide a measure to evaluate the reliability of the predictions.

T.E.S.T., of the US EPA.

This software has a battery of models, which are then integrated into a single prediction (average value) named consensus. This software shows the most similar compounds too. Thus, this software can be used for read-across as well.

T.E.S.T. does not report the prediction if the value of the reliability is below a certain threshold, therefore only predictions inside the applicability domain are reported.

VEGA, mainly developed by Mario Negri, but with the contributions of many other laboratories.

VEGA includes some of the models previously mentioned. In these cases, the results were not reported, unless there were differences.

VEGA provides a measure to evaluate the reliability of the predictions. In all cases, the predictions are indicated with high uncertainty (low applicability domain index). Thus, the results should be considered very carefully, and the predictions may be not reliable.

This software shows the most similar compounds too. Thus, this software can be used for read-across as well.

C.1.3 - Analysis to investigate the potential impacts of the new molecule cC6O4

It is known that the predictions of perfluorinated substances is a very difficult task. This has been verified within a study done by Istituto Mario Negri for the Italian Ministero dell'Ambiente (Benfenati et al.) (https://www.minambiente.it/sites/default/files/archivio/allegati/reach/relazionefinalestudiopfas.pdf).

The problem starts from the difficulty to predict simple physico-chemical properties, such as logP, and is reflected in the more difficult endpoints, such as BCF, aquatic toxicity, etc. Indeed these endpoints are closely related to logP. One of the main causes of these the lack of experimental values for this kind of substances. Due to the lack of experimental values for these representatives, the model is not able to learn specific features, and thus in most of the cases the model applies general descriptors which refer to the global population of substances. Unfortunately, the partitioning of these substances, even in the simple case of logP, is not driven by the classical features applicable to the rest of the substances.

In these conditions, it is very important to look very carefully to the predictions, and consider the presence of experimental data on related substances, which may be indicated by some software. This strategy is called read-across, and the similar substances are assumed to have experimental values similar to the target substance. Table 4.3 reports all the predictions.

Table 4.3. *The predictions using the in silico models for cC6O4. In red data for PFOA*

	logKow	**logBCF**	**log half-life (days)**	**log BAF**	**Koc 1 (L/kg)**	**Koc 2 (L/kg)**
EPISuite NH4	0.92	0.5	0.0774	0.19	10	6.955
EPISuite neutral	2.36	0.5	0.563	1.35	10	12.55
OPERA	3.323	0.695				2.665
T.E.S.T. consensus		1.43				
T.E.S.T. hierarchical		*1.92*				
T.E.S.T. single		*2.88*				
T.E.S.T. group		*-1.23*				
T.E.S.T. fda		*0.09*				
T.E.S.T. nn		*3.38*				
T.E.S.T. PFOA exp		*3.12*				
T.E.S.T. PFOA predicted		*2.27*				
CAESAR		0.06				
CAESAR PFOA exp		3.12				
CAESAR PFOA predicted		2.534				
VEGA Arnot		2.66				
VEGA kM			0.2			
VEGA Meylan	4.16					
VEGA MLogP	0.45					
VEGA ALogP	6.2					

logP: The different models indicate a very large spread of values, from 0.45 to 6.2. The different models probably give importance to the two apparently conflicting features, on one hand the polar moieties, with the carboxylic acid and the presence of O atoms, and on the other hand the presence of the aliphatic part, with

the F atoms. Unfortunately, the similar compounds (offered only by VEGA) do not help, because they are quite different. It is not possible to assess logP based on these predictions.

BCF: BCF is typically affected by logP, and most models use logP to get the predicted value. We have seen that logP has a large spread of values. A further difficulty is that perfluorinated compounds tend to link to proteins, and this increases the complexity and the uncertainty.

The predictions of the different QSAR model show very high spread of values. If we consider for T.E.S.T. the consensus value, and not the individual values of the different models, the range goes from 0.06 to 3.12 log units. Even if in all cases the substances should not be classified as bioaccumulative, the values from the different models are quite diverse. The picture would be worst considering the spread of values of the individual models in T.E.S.T., which go from -1.23 to 3.38 log units. In this case the models indicate some similar compounds, which can be used for read-across.

Read-across for BCF:

PFOA has an experimental value of 3.12 log units, in T.E.S.T. and in CAESAR. However, in the VEGA Arnot model its value is much lower: 0.977. In the same data set, the analogue with 10 carbons has an experimental value of 3.04 log units. This fact may show that the experimental value of PFOA may be affected by uncertainty too. The predictions of PFOA with CEASAR and T.E.S.T. are lower, of almost one log unit.

There are other similar substances given by VEGA, as in Figures 4.4 and 4.5.

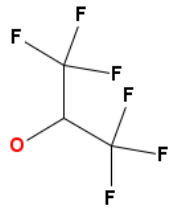

Figure 4.4. *Similar number 4 by CAESAR. Similarity 0,614. CAS: 920-66-1. Experimental log BCF 0.3; Predicted logBCF 0.601.*

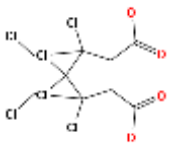

Figure 4.5. *Similar number 5 by CAESAR. Similarity 0,614. CAS: 115-28-6. Experimental log BCF 0.32; predicted log BCF0.261.*

The same substance in the KNN model in VEGA has an experimental value of -0.168 log units.

The line of reasoning which can be used is to compare the different molecules, the target compound and these three similar substances.

PFOA contains a carboxylic group, as cC604. However, PFOA has more carbon atoms: 8 versus 6. The number of F atoms is 15 versus 9. Conversely, cC604 has 3 oxygen atoms (emiacetal without O-H residues). Thus, there are three elements which indicate a lower BCF: less carbon atoms, less F atoms, three O.

The experimental PFOA value can be considered as an upper limit. Thus, 3.12 log units is the worst case, and the upper limit.

Other pieces of information may be obtained from the similar as in Figures 4.4 and 4.5.

The substance in Figure 4.4 has a much lower number of carbon atoms, 3 versus 6. Thus, in this sense this target is something in between the PFOA and this similar, which can be considered the lower limit. The number of F atoms is 6. Also in this case the target compound is supposed to be between this similar and PFOA. Conversely, this similar does not has the acid group, and there is only one oxygen, as O-H, instead of the three O atoms. Thus, considering the polarity of cC604 compared to both PFOA and similar as in Figure 4.4, a reduction of the log BCF value is expected compared to the simple average value between the log BCF of PFOA (3.12 in the worst case, since there is also a value much lower) and of CAS 920-66-1 (0.3). The average may be around 1.7, and a lower value is expected.

We can also consider the similar as in figure 4.5. This substance does not contain fluorine, but it is chlorinated. The substance is surely complex, and has only very limited features in common to the target compound: one carboxylic group, similar number of carbons and a number of chlorine atoms instead of fluorine. Regarding the polarity, the similar compound has 2 carboxylic groups, but it misses the 3 oxygens. Nevertheless, its BCF value is quite low, the same as similar 2.

Thus, the realistic log BCF value of the cC604 is something lower than 1.7, which may be close to 0.3.

This data range is very similar to that experimentally obtained for clam (see section 4.2): PFOA LogBCF 2.1; cC6O4 LogBCF 1.3, while LogBCF of ammonium salt, reported in ECHA (2021), based on experimental test, is 2.8.

These considerations regarding read-across have the limit that they refer to the neutral form, not to the salt. Since the substance of interest is the salt, the real value of the salt is expected to be lower than what discussed above.

BAF: The BAF value of the target compound should be close to 0.2. Here we have only EPISuite. However, this value is consistent with the considerations done on BCF using read-across.

Half-life in fish: There is a spread of values regarding this property, but in any case it should be 0.5 day or, quite probably, lower.

Koc: The Koc value of the target, as salt, should be something between 7 and 10.

Koc of ammonium salt, reported in ECHA (2021), based on an experimental test, is 11 L/kg at 22 °C and 5.96 % organic carbon, while Kd is 0.653 L/kg under the same conditions.

5. Conclusions

The work carried out under the sub-action C1.3 allowed to a first classification of the risks connected with the newly discovered molecule cC6O4.

This molecule has been introduced mainly as a substitute of PFOA in the polymerization process od tetrafluoroethylene and for that reason it should have physico-chemical characteristics similar to those of PFOA itself.

This fact has been confirmed by both the experimental and modelling work carried out under LIFE PHOENIX project.

Persistence, mobility and bioaccumulation are similar, and it is also probable that both molecules have similar toxicological mode of action, particularly because they share the same chemical moiety.

With the data collected in the present deliverable we can propose a first classification of the molecule cC6O4 according to the criteria used or proposed under REACH.

Mobility

Mobility of this molecule has been verified in real environments, because they have been found in surface and groundwaters, even at a certain distance from the pollution sources. Furthermore, the low adsorption on soil is demonstrated by the findings in grundwaters close tn the rivers after bank filtration at Montecastello AL), close to river Tanaro, and San Basilio Ariano (RO), close to river Po.

The proposed criteria for this new classification under REACH is: the lowest log Koc in the pH range of 4-9 should be lower than 4.0 (M) 3.0 (vM)

For cC6O4 the log Koc of ammonium salt, reported in ECHA (2021), based on an experimental test, is 1.04, confirmed also by QSAR modelling.

cC6O4 can be classified as vM

Persistency

Experimentally, low biodegradation of cC6O4 is demonstrated by the detection of significant concentrations of this molecule at the delta of river Po, after some hundreds km from the pollution source.

According to ECHA dossier (ECHA, 2021) cC6O4 ammonium salt was investigated for its ready biodegradability in a "28-Day DOC Die- Away Test" according to the Commission Regulation (EC) No 440/2008, C.4-A and the OECD Guideline for Testing of Chemicals, No. 301 A (1992). cC6O4 ammonium salt was not biodegradable under the test conditions.

The few data available suggest that cC6O4 is very persistent as other perfluoroalkyl and perfluoroalkylether carboxylic acids.

cC6O4 might be classified as P or even vP

Bioaccumulation

C.1.3 - Analysis to investigate the potential impacts of the new molecule cC6O4

According to the criteria developed for legacy POPs, a substance fulfils the bioaccumulation criterion when the bioconcentration factor in aquatic species is higher than 2000 (logBCF=3.3). Experimental and predicted BCFs are much lower and the molecule should not be classified as not bioaccumulative.

Nevertheless, as in the case of PFOA, this criteria could be not effective, when we are dealing with molecules characterised by other accumulation mechanisms.

According to EACH, bioaccumulation studies in aquatic species, preferably fish, do not need to be conducted if: the substance has a low potential for bioaccumulation (for instance a log Kow≤ 3) and/or a low potential to cross biological membranes, or direct and indirect exposure of the aquatic compartment is unlikely.

Predicted logKow has a very large spread of values, from 0.45 to 6.2 and the potential to cross biological membrane cannot be considered negligible. The affinity of cC6O4 with specific proteins, such as yolk proteins in eggs (Morganti et al., 2021) or albumin in human serum (Girardi et al, 2018) demonstrates the possibility of bird and human exposure and accumulation for this molecule. According to the WoE approach, bioaccumulation potency cannot be completely discarded.

Toxicity

No sufficient data are available for this molecule both for humans and ecosystems. But the few data available suggest a mode of action similar to that of PFOA

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7. Dissemination of results

Experimental data produced within LIFE PHOENIX project have been published in the following international refereed articles:

Bernardini I., Matozzo V., Valsecchi S., Peruzza L., Dalla Rovere G., Polesello S., Iori S., MarinM.G., Fabrello J., Ciscato M., Masiero L., Bonato M., Santovito G., Boffo L., Bargelloni L., Milan M., Patarnello T., The new PFAS C6O4 and its effects on marine invertebrates: First evidence of transcriptional and microbiota changes in the Manila clam *Ruditapes philippinarum*, Environment International 152 (2021) 106484

Morganti M., Polesello S., Pascariello S., Ferrario C., Rubolini D., Valsecchi S. and Parolini M., 2021. Exposure assessment of PFAS-contaminated sites using avian eggs as a bio-monitoring tool: a frame of reference and a case study in the Po River valley (Northern Italy), Integrated Environmental Assessment and Management, on-line published: 25 March 2021, https://doi.org/10.1002/ieam.4417

Most of the results of the Sub-action C1.3 have been presented at the second International Webinar on 3rd March 2021 (Title: *Modelling and monitoring legacy and emerging PFAS pollution at a catchment scale*).

At the webinar more than 100 people attended.

The whole recording of the webinar is available in the Youtube channel of the LIFE PHOENIX project:

https://www.youtube.com/watch?v=mrsO7oeSEt8 (57 views until 09/04/21)

and all the presentations are available on the LIFE PHOENIX website:

https://www.lifephoenix.eu/en/-/attivit%C3%A0-di-networking-ciclo-di-webinar-internazionali-sui-nuovi-strumenti-per-una-governance-integrata-dell-inquinamento-da-pfas

See the program of the webinar in the following picture.

Perfluorinated compounds
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Webinar - 3 March 2021 (10:00-12:15 a.m.)

Connect at: https://global.gotomeeting.com/join/763096733

Modelling and monitoring legacy and emerging PFAS pollution at a catchment scale

Application of 3D modelling of the groundwater diffusion of PFAS from an industrial contaminated site will be presented, showing also the possibility of deriving fore-casting scenarios. The diffusion in water bodies and ecosystems of emerging PFAS from recent production, also used as an alternative to already restricted legacy PFAS, will be also presented, showing the implementation of advanced monitoring and biomonitoring approaches.

Chair	Massimo Mazzola, Veneto Regional Agency for Environment Protection (ARPAV), Italy Sara Valsecchi, Institute for Water Research (IRSA-CNR), Italy
Speakers	Emilio Benfenati - Istituto Mario Negri, Italy
	Vanessa Groppi - Veneto Region, Italy
	Massimo Mazzola - ARPAV, Italy
	Sara Valsecchi - Institute for Water Research (IRSA-CNR), Italy
	Håkon Austad Langberg - Norwegian Geotechnical Institute (NGI), Norway

Scientific program

10:00	Brief introduction on the LIFE PHOENIX Project	Vanessa Groppi
10:15	3D modelling for assessing and forecasting PFAS distribution and evolution in a groundwater at a catchment scale	Massimo Mazzola
10:45	Environmental distribution and monitoring of new alternative PFAS in contaminated sites	Sara Valsecchi
11:05	Substituting harmful chemicals. The challenge of perfluorinated compounds	Emilio Benfenati
11:35	The paper industry as a source of precursors to Perfluorinated Alkyl Acids (PFAA) in a Norwegian lake	Håkon Austad Langberg
11:55	Final remarks and discussion	

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