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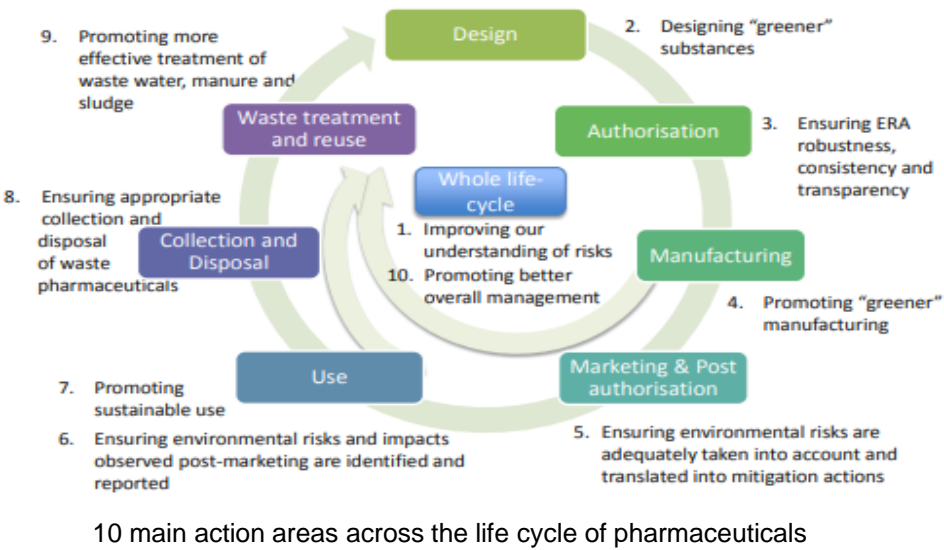
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INTRODUCTION

The Priority Substances Directive 2013/39/EU obliges the European Commission to develop a strategic approach to deal with the **water pollution due to pharmaceutical substances**. Among the emerging contaminants, pharmaceuticals are one of the most concerning group of substances in aquatic ecosystems because of their worldwide use, resistance to the wastewater treatments and effects on aquatic organisms. In 2017, a **European Strategy** on pharmaceuticals was established to define a series of actions for addressing the all EU member states to achieve the Directive's goals.



Climate changes can have relevant effects on the fate and pathways of the pharmaceuticals in the environment through different mechanisms:

- The increase of temperature and acidification in seawater can enhance the bioaccumulation of these substances
- Floodings can have an impact on wastewater treatment plants and deteriorate water, sediment and soil quality
- Water scarcity can enhance the concentration of pollutants in groundwaters and rivers.



Climate changes may cause some specific diseases and health effects and therefore influence the use of pharmaceuticals.

In 2015, has been established the first European **Watch List** of substances to be monitored into surface water bodies: Estrogens, Antibiotics and Diclofenac are included. In Italy, the use of pharmaceuticals is widely spread: the preliminary results of the WFD (Water Framework Directive) Watch List monitoring chaired by ISPRA Institute (see the Table below), and data collected in previous studies, highlight their presence in the Italian aquatic ecosystems.

PROPOSAL

It is necessary to elaborate and propose an efficient **monitoring strategy** for the detection of pharmaceuticals into Italian aquatic ecosystems which takes into account also climate changes; this strategy should be focused mainly on the three following aspects:

1) Substance selection/prioritization criteria

- Pharmaceutical use (e.g. see report AIFA-Italian Medicines Agency)
- Environmental Fate, Toxicity and Ecotoxicity properties (European and International Databases)
- Watch-List substances (in 2018 the EU decision 2018/840 has included also the antibiotics Amoxicillin and Ciprofloxacin for their ecotoxicological properties)

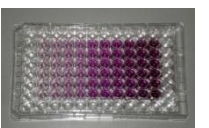
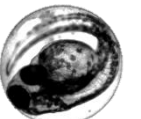
2) Site selection

- Presence of pollutant sources (hospitals, landfills, urban effluents, zootechnical activities)
- Monitoring programmes sites indicated in the Water Framework Directive (surveillance monitoring sites and watch list sites-see the map).



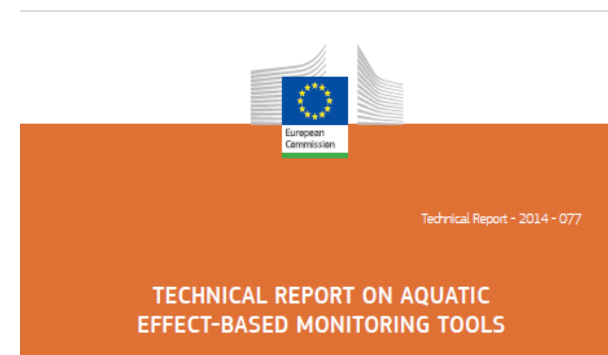
3) Use of Effect Based Tools to detect pharmaceuticals mixtures effects

- Groups of pharmaceuticals can have same mode of action (e.g. estrogens). It is therefore advantageous to match chemical analysis with the use of specific bioassays (**Effect-based tools**) when assessing the state of health of aquatic ecosystems.
- The use of **different bioassays** both on cell cultures and **different trophic level organisms** is strongly recommended. zebrafish (*Danio rerio*) early life stages, as well as cell lines *in vitro* test such as YES and YAS test, have been used in several studies in relation to different pharmaceutical effects; these assays are widely used to detect estrogenic and androgenic effects of pharmaceuticals.



Substances detected in Italy (watch list)	Recovery number > loq	Concentration Range (ng/l)
17-Alpha-ethinylestradiol (EE2)	0/35	-
17-Beta-estradiol (E2)	11/35	0.10 - 1.02
Estrone (E1)	28/35	0-10 - 4
Diclofenac	22/35	5 - 683
Erythromycin	1/36	20
Clarithromycin	3/36	54 - 200
Azithromycin	4/36	20 - 459

<i>In vitro</i> methods	<i>In vivo</i> methods
Ames test	Algal acute toxicity test
Comet Assay	Daphnia acute Immobilisation test
Yeast androgen screen	Fish Embryo Toxicity (FET) test
Yeast estrogen screen	



CONCLUSIONS

- Pharmaceuticals is a group of emerging compounds for which the collected data in ecosystems are **lacking** and **fragmented**.
- A monitoring program should be started in representative sampling stations of Italian river basins taking into account the most used pharmaceuticals and watch list substances. **This monitoring program should take into account the effects of climate changes on pharmaceuticals.**
- The use of **effect-based tools** can support the detection of the effects caused by pharmaceuticals, especially in areas where there are specific known emissions (e.g. pharmaceuticals industries).
- The monitoring plan should support the achievement of the objectives foreseen by the European Strategy on Pharmaceuticals.

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