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CHEMICAL SAFETY REPORT

Public version - consolidated

Legal name of applicant(s):	DiaSorin S.p.A. (legal entity) The application is for one legal entity: DiaSorin S.p.A. and it involves 2 manufacturing sites: • Saluggia (VC) – Italy (site A) • Dartford – United Kingdom (UK Branch of DiaSorin S.p.A.) (site B)
Submitted by:	DiaSorin S.p.A.
Date:	09/09/2020
Substance:	4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated [covering well-defined substances and UVCB substances, polymers and homologues] (4-tert-OPnEO), EC: -, CAS: -
Use title:	industrial use, as nonionic surfactant, employed in purification processes in IVDs industry
Use number:	1

7. Exposure assessment

7.1. Introduction

The family of 4-tert-OPnEO substances are widely used under the trade mark of Triton X-100 and Igepal CA-630; Triton X-100 is one of the most commonly used by the *In Vitro* Diagnostics (IVD) industry.
Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) is a nonionic surfactant that has a hydrophilic polyethylene oxide chain (on average it has 9.5 ethylene oxide units) and an aromatic hydrocarbon lipophilic (i.e. 4-(1,1,3,3-tetramethylbutyl)-phenyl group) or hydrophobic group. Igepal CA-630 is a nonionic, octylphenoxy poly(ethyleneoxy)ethanol, branched.
Igepal CA-630 differs from Triton X-100 by having slightly shorter ethylene oxide chains. These two detergents may not be considered to be functionally interchangeable for most applications.

Triton X-100 and Igepal CA-630 uses in IVD production

IVDs recognize the formation of immunoassay links between the biological product manufactured in the laboratory and the element to be diagnosed in the patient's biological sample. IVDs have an important role in population blood bank screening and virus safety, including HBV, HIV and HCV viruses. They range from blood donor screening, to hospital use IVDs, immunodiagnostic IVDs, self-testing devices and laboratory tests. There are a considerable number of assays involved across the healthcare spectrum and the IVD and laboratory reagents industry.

Triton X-100 and Igepal CA-630 are critical surfactants in the production of *In Vitro* Diagnostics reagents: Triton X-100 and Igepal CA-630 are used as surfactants during the purification processes for the preparation of antigens which are subsequently included in IVDs. They are not present in the final product. **This use is subject to REACH authorisation**.

7.2. Applicant Sites

The applicant DiaSorin is an Italian multinational Group and the application for authorisation is requested for DiaSorin Italia S.p.A. (including sites located in Italy and United Kingdom, respectively), which are the two company sites using Triton X-100 and Igepal CA-630.
Both sites apply similar processes, technical equipment and Risk Management Measures. Therefore, the technical description detailed below applies to both production sites even if different equipment may be used (e.g. storage).

Table 15. Sites details

Site name	**Details**
Saluggia (VC) – Italy (site A) Headquartered, industrial and R&D site in Saluggia (VC), Italy	The DiaSorin plant is located in Saluggia industrial area, named and known as Sorin site. The industrial area is closed and bounded by fences; in the area companies are present other than DiaSorin, which are operating in the biomedical sector. About 1800 people are employed as active workers on the site. In the immediate surroundings of the Sorin area, there are the EUREX-SOGIN site and the Avogadro depot, where activities related to the storage of materials deriving from the production of nuclear energy are carried out, no longer performed in Italy for several decades. The town of Saluggia is about 3 km away, the inhabitants of the municipality are just over 4200.
Dartford – United Kingdom (UK Branch of DiaSorin) (site B) industrial site in Dartford, United Kingdom	Dartford lies within the area known as the London Basin. The low-lying marsh to the north of the town consists of London Clay and the alluvium brought down by the two rivers—the Darent and the Cray—whose confluence is in this area. The higher land on which the town stands and through which the narrow Darent valley runs, consists of chalk surmounted by the Blackheath Beds of sand and gravel. Within the town boundaries there are several distinct areas: the town centre and two important areas of open space and several industrial estates.

7.3. Overview of uses and Exposure Scenarios

7.3.1. A backwards overview: from the final product to the Triton X-100/Igepal CA-630 use

The diagnostic tests are aimed at both private and hospital analysis laboratories worldwide, in immunodiagnostics and molecular diagnostics markets.

The immunodiagnostics technology is based on the detection of antibodies and/or antigens to highlight the presence of diseases in a sample of human fluid. In fact, antibodies specific for an antigen of interest are useful tools in diagnostics.
Molecular Diagnostics technology allows diagnosis of a pathology by detecting specific RNA or DNA sequences (nucleic acids) in patients' biological fluids or in their abnormal cells.
Both the technologies are based on testing kits (reagents and consumables) which are specific to the individual technological platforms dependent upon the type of technology used.
Triton X-100 and Igepal CA-630 are involved in antigen purification processes to prepare IVD reagents. The final product consists in a kit that can be used for diagnosis of several pathologies, like hepatitis, borreliosis, autoimmune disease and HIV etc.

Diagnostic kits The DiaSorin diagnostic tests are biological components aimed at determining the presence of specific elements (virus, hormones, etc.) in the patient's blood sample. These cutting-edge diagnostic products can identify the presence of the desired element even in small quantities and with a high degree of specificity in the patient's sample.	

IVD kits are loaded into specific technological platforms (analyzers) used in professional laboratories. Mainly patented by DiaSorin the platforms are able to recognize the presence of the immunoassay link between the biological product manufactured in laboratory and the element in the patient's sample, using different technologies i.e. CLIA (Chemiluminescence) and ELISA (Colorimetry).

Technological platforms Analysis of the biological sample is carried out by instruments based on specific technologies: in immunodiagnostics, DiaSorin offers the market proprietary-based platforms on the CLIA (Chemiluminescence) and ELISA (Colorimetry) technologies.	  

The platforms are able to run multiple different assays at a time, even with a small number of samples and contain all assay-specific reagents. The Immunodiagnostics technology is based on CLIA (Chemiluminescence) and ELISA (Colorimetry) methods.

The chemiluminescence immunoassay (CLIA) technology is an assay that combines the chemiluminescence method with immunochemical reactions. CLIA utilizes chemical substances that generate light emission, through chemical reactions, to label the antibody.

The ELISA (enzyme-linked immunosorbent assay) is a well-established antibody-based ready-to-use tool for detecting and quantifying antigens of interest. ELISA is a "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

7.3.2. Uses subject to REACH authorisation - description

Triton X-100 and Igepal CA-630 are used as surfactants during the purification processes for the preparation of antigens, which will be in turn included into IVDs. They are not present any more in the final product.

Purifications, performed in biochemical/biotechnological and biological laboratories, can be described as the same as the procedures involved are also similar. Purification can be distinguished into 2 phases: the buffer preparation, including weighing and dilution and the buffer application.

The quantity of Triton X-100 and Igepal CA 630 used by DiaSorin is less than 3 kg/year in total, less than 2,5 kg/year at Dartford and 0,5 kg/year at Saluggia. These amounts are based on 2018 consumption but it can be assumed as an annual average consumption for the last 3 years.

Table 16. Formulation

Formulation
ES-1: Buffer preparation Contributing activity/technique for the environment: **- ERC 2:** formulation into mixture Contributing activity/technique for the workers: **- PROC 5:** mixing or blending in batch processes **- PROC 9:** transfer of substance or mixture into small containers (dedicated filling line, including weighing) **- PROC 15**: use as laboratory reagent **Product Category formulated:** PC 0: other: buffer **Technical function of the substance:** surfactant

Table 17. Uses at industrial sites

ES-2: Uses at industrial sites
Buffer application Contributing activity/technique for the environment: **- ERC 4**: use of non-reactive processing aid at industrial site (no inclusion into or onto article) Contributing activity/technique for the workers: **- PROC 5:** mixing or blending in batch processes **- PROC 9:** transfer of substance or mixture into small containers (dedicated filling line, including weighing) **- PROC 15**: use as laboratory reagent **Product Category formulated:** PC 0: other: buffer **Sector of end use:** SU 0: other: preparation of antigens, which will be in turn included into IVDs **Technical function of the substance:** surfactant

Substance supplied to that use: in a mixture Subsequent service life relevant for that use: no

Notes: although the actual use of Triton X-100 and Igepal CA-630 includes the preparation of small quantities and involves small laboratories, PROC5 and PROC9 are used to describe the life cycle, as the substance is used in industrial processes, accounting for a possible increase in the amount of substance used.

As stated above, both Triton X-100 and Igepal CA-630 are used in purification processes of antigens in preparation for the production of IVD reagents. They are particularly useful for enabling lysis of cell membranes, dissolving lipids from membranes and dissolving proteins within their native form so that they remain immunologically active.

Currently, Triton X-100 is involved in the antigen purification processes to prepare an important number of IVD-kits used for the detection of [redacted]. Igepal CA-630 is used for the purification of a molecular product for a [redacted].

In both Saluggia and Dartford sites, Triton X-100 and Igepal CA-630 are used in biochemistry/biotechnology and biology laboratories for antigen purification processes. In particular Triton X-100 and Igepal CA-630 are used for the preparation of buffers used during purification procedures. The purification processes of interest can be divided into 4 types on the basis of the buffer function:

- lysis of cell material – the buffer acts to disrupt the bacterial wall cell and allow the extraction of the cell proteins
- washing – the buffer is used to remove possible contaminants bound to the protein of interest
- storage – the buffer helps to maintain, in solution, the purified proteins, avoiding aggregation and/or precipitation phenomena
- cell lysis – the buffer is used for lysis of the eukaryote cell membranes to extract proteins from the cell nucleus

7.3.2.1 Activity in biochemistry/biotechnology laboratories

Triton X-100 and Igepal CA-630 are used for the preparation of buffers used in antigen purification processes. As mentioned above, the products can be applied for lysis of cell material, washing, storage and cell lysis.

[redacted] At the Saluggia site, over a 1-year period, [redacted] of Triton X-100, each one of [redacted], were expected to be used for around [redacted] preparations. However, [redacted] of the product was actually used due to it expiring; the remaining [redacted] was lost as special waste and disposed of in accordance to specific procedures. Igepal CA-630 was also used for the preparation of a buffer. Over a 1-year period a maximum of [redacted] was expected to be used for [redacted] preparations/year. However, [redacted] % of the product was actually used due to it expiring; the remaining [redacted] was waste and disposed in accordance to specific procedures in compliance with applicable regulations.

For both Triton X-100 and Igepal CA-630, DiaSorin is considering to purchase smaller sizes, in order to avoid expiring of a large part of the product and reducing waste as much as possible.

Once opened, the Triton X-100 and Igepal CA-630 [redacted] is stored in a compartment under laboratory bench within a reagent cabinet.

At Dartford site (B), the quantity of Triton X-100 used for these processes is delivered in [redacted] from the supplier. About [redacted] of the content of the bottles is used to prepare the buffer; [redacted] is unused. Approximately [redacted] containing Triton X-100 are produced a year. Of the whole buffer solutions prepared, approx. [redacted] of the volume is used in the manufacturing process directly. The remaining [redacted] is overage to ensure sufficient stock is available at the time of use.

Igepal CA-630 is not used.

Table 18. Annual amounts (2018)

	Saluggia - site A		Dartford - site B
	Triton X100	**Igepal CA-630**	**Triton X-100**
Box / year	[redacted]	[redacted]	[redacted]
N. preparations / year	[redacted]	[redacted]	[redacted]
Product actually used*	[redacted]	[redacted]	[redacted]
Product lost as waste*	[redacted]	[redacted]	[redacted]

*Amount respect to the open box

**Calculated taken into consideration the use of [redacted] of [redacted]

The antigens purification processes involved the following steps:

- Weight - in order to prepare the buffer, the necessary amount of product is dosed using graduated automatic disposable pipettes and transferred to a beaker;
- Buffer preparation - Triton X-100/Igepal CA-630 can be dissolved in purified water or added to aqueous buffers. The beakers used during the preparation of Triton X-100 based buffers have a volume in the range of [redacted], whilst the beakers used during the preparation of Igepal CA-630-based buffer have a volume in the range of [redacted].

Table 19. Buffers preparation and use – biochemistry lab.

Details	**Saluggia - site A**		**Dartford - site B**
	Triton X-100	**Igepal CA-630**	**Triton X-100**
N. of buffers prepared / year	[redacted]	[redacted]	[redacted]
Beakers volume	[redacted]	[redacted]	[redacted]
Amount in prepared buffers	[redacted]	[redacted]	[redacted]
Volume of buffer prepared	[redacted]	[redacted]	[redacted]
N. of involved workers for each activity	[redacted]	[redacted]	[redacted]
Time of exposure for workers	[redacted]	[redacted]	[redacted]

- Finished buffers are used in the manufacturing process, for either:
  - treatment of cell cultures and / or fermentation products - suspending biological material
  - extraction / separation using high pressure homogenization, sonication and / or centrifugation (which take place in closed containers) and dialysis

  Buffers are applied to dissolve the biological matter in bottles, sample tubes, beakers or conical flasks (containers are cleaned by hand washing after usage).

The indicated annual quantities are referred to 2018 and they can be considered as conservative representative values. Based on market development analysis, the Applicant actually expect the used quantity of 4-tert-OPnEO to progressively decrease in the future. In fact, most of the IVD products involved in these purification processes will have a decrease of manufactured quantities (estimated 10 %/year decreases).

Table 20. Substance application and function

Use type	Function	Example of application	Possible concentrations
Lysis of eukaryotic cells	Dissolve membrane or added at the last stage of lysis, as a stabiliser	Applied before incubation or for pellet resuspension. The suspension can undergo to stirring, aliquots division and/or centrifugation.	[redacted]
Lysis of cell material			
Washing buffer	Remove contaminants from the pellet		[redacted]
Storage buffer	Help to solubilise aggregates	Applied before incubation. The suspension can undergo to stirring, aliquots division and/or centrifugation.	[redacted]

- The Triton X-100/Igepal CA-630 buffers are removed by centrifugation.
  The centrifugation process is done at laboratory scale using closed vials. Once centrifugation is completed, vials are opened and resulting liquid is poured out and collected in a tank for disposal as hazardous waste by licensed company. Both incineration and physical chemical/biological liquid treatment are used (see § 7.3.3).

  With reference to physical/chemical treatment performed by the disposal company, the following treatments steps occurs.
- pH control, as necessary acid and alkaline dosage and anionic polyelectrolyte for flocculation at pH 9.5 - 10. Sedimentation and filtration.
- Liquid containing organic substances and surfactant is treated with Fenton process using hydrogen peroxide, ferrous chloride and calcium hydroxide. Stoichiometric reagents dosage after execution of Jar – test.
  (Extract from License of disposal company [redacted] who is performing chemical and physical treatment, complete License document attached – Annex 8)

- Final purification - the final antigen purification occurs in a chromatography column. In the column the Triton X-100/Igepal CA-630 based buffer is not present, except for possible trace amounts due to the preceding steps. Residues of Triton X-100/Igepal CA-630 would negatively impact this purification step.
  Possible traces of Triton X-100/Igepal CA-630 from the column are not released to any sewage system; they are collected in place and classified as special waste and

a licensed company manages its disposal.

- This waste is included into the categories 'liquid waste-production scraps' for Saluggia and 'manufacturing process effluent waste' for Dartford. See § 7.3.3. 'Residues and wastes'

The liquid wastes produced in these processes, where necessary, are treated with hypochlorite for disinfection purposes; liquid and solid special wastes are collected in dedicated containers, which are taken away and treated by licensed companies (details in Annex 7). After usage the laboratory glassware is cleaned up by hand washing.
Glassware after the first hand washing is sent either to laundry machine or hand washed depending on the protein content of the solutions.
Glassware resulting from the use of solutions without proteins are normally hand washed while those resulting from the use of solutions with proteins content are sent to laundry machine where higher temperatures allows for better removal of proteins.

7.3.2.2 Activity in biology laboratory

Triton X-100 is also used for the production of one antigen. This use is [redacted].
The buffer is used for the [redacted], depending on the method: Triton X-100 acts by dissolving the phospholipidic membrane, forming a [redacted] channel. The process is obtained treating the cell cultures with Triton X-100 buffer. The buffer for this usage is directly prepared in the biology laboratory, following the same procedures described for the biochemistry laboratory.

The [redacted] is performed using 3 different buffers, one of which prepared using Triton X-100. The buffer volumes are [redacted]. Triton X-100 is diluted to [redacted] when used for [redacted], respectively.
The cells treated with Triton X-100 buffer can be stirred and then the suspension can be filtered.
After centrifugation and supernatant elimination, the pellet is treated one more time with a buffer; this does not include Triton X-100.

Table 21. Buffers preparation and use – biology lab.

Details	**Saluggia site - A**
	Triton X-100
N. of buffers prepared / year	[redacted]

Details	**Saluggia site - A**
	Triton X-100
Bakers volume	[redacted]
Amount in prepared buffers	[redacted]
Volume of buffer prepared	[redacted]
N. of involved workers for each activity	[redacted]
Time of exposure for workers	[redacted]

Process temperature of all the different production steps in both laboratories is at controlled Room Temperature in the range 18-24 °C

7.3.2.3 Storage

Saluggia - site A

When goods arrive into the industrial site, they undergo document acceptance. This includes the number and entirety of goods, as well as the temperature and conditions of storage (on the basis of the supplier indications provided by the transport documents).

[redacted]

Goods are moved to a dedicated area, where they are pulled out from bulk transport boxes. Triton X-100 and Igepal CA-630 are contained in boxes and are labelled with a specific code and lot number. Goods are retained in quarantine, until the end of the documentation check. After this period, they are prepared for storage (at room temperature) in dedicated buildings, which used an automatic elevator mechanism for placing material on and off the shelves.

Boxes are directly placed on a shelf at the counter level and then the shelf is moved to the storage level by a computerized system.

Possible expired batches are stored in another area and disposed as special waste.

Batches are moved from warehouse storage to laboratory storage (both accesses controlled), by technicians, using a trolley.

Dartford - site B

[redacted]

When goods arrive to the Dartford site, they undergo document acceptance: number and entirety of goods, as well as the temperature, conditions of storage and COSHH hazards associated. Goods are moved to a dedicated inspection area (chemical cabinet), where they are unpacked from bulk transport boxes awaiting inspection.

The goods are held in an inspection location (chemical cabinet) up to the end of the documentation check. After this period, they are moved to an unrestricted status (chemical cabinet) until requisitioned by the user department. All locations are temperature controlled (18 - 25 °C).

Note: expired batches are stored in another area and disposed of as special waste.

Batches are moved from warehouse storage to laboratory storage (warehouse is access controlled during working hours only via the site perimeter fence, the laboratory building is access controlled), by technicians, using a trolley, bottles of Triton X-100 are double contained during this transport.
The movement requires the material to leave the warehouse building and travel across the rear yard of the building, adjacent to a tidal river and into the main manufacturing building.

Once opened, the Triton X-100 bottles are stored in corrosive chemical cabinets within the laboratory.

[redacted]

7.3.3. Residues and wastes

Saluggia - site A

Purified antigens do not contain residues of Triton X-100/Igepal CA-630, that are completely removed during the purification processes as residues of Triton X-100/Igepal CA-630 would negatively impact the subsequent purification steps.

Considering the potential uses, the possible ways Triton X-100 and Igepal CA-630 could be released into the environment are:

- solid waste - disposable matter come into contact with Triton X-100/Igepal CA-630. Solid wastes are classified as special waste 18.01.03* (hazardous waste), collected in dedicated bags and disposal is managed by a licensed company;

  "Disposable matter" refers to:
  - graduated disposable plastic pipettes (1-100 ml)
  - plastic bottles (100 ml – 3 l)
  - disposable dialysis tubes,
  - centrifugation tubes
  - and PPEs like protective gloves, lab coats for bio-hazard, face masks

  Waste definition and management is in accordance with:
  - Directive CE 2008/98
  - Decision CE 2000/532
  - Italian Decree D.Lgs. 152/2006

  Hazardous wastes are defined by Directive CE 2008/98 art. 3(2)
  'hazardous waste' means waste which displays one or more of the hazardous properties listed in Annex III.
  Management of hazardous wastes is described in the same Directive art.17
  Control of hazardous waste
  Member States shall take the necessary action to ensure that the production, collection and transportation of hazardous waste, as well as its storage and treatment, are carried out in conditions providing protection for the environment and human health in order to meet the provisions of Article 13, including action to ensure traceability from production to final destination and control of hazardous waste in order to meet the requirements of Articles 35 and 36.

  EWC codes (*) are defined in accordance with - Decision CE 2000/532 establishing a list of wastes

- solid waste - empty bottles are classified as special waste 15.01.10* and disposal is managed by a licensed company; unused raw material remaining in the original bottles is not separated from their bottles and it is sent for disposal, together with the bottle as waste of chemical laboratory reagent 16.05.06*, using licensed disposal company.
- liquid waste - production scraps (including the residues from cleaning procedure: the first hand washing of the glassware). This is classified as special waste 07.05.01*, collected by tanker truck and disposal is managed by a licensed company.
- residual traces remaining in the glassware after the hand-wash; the effluent is discharged into the site collecting system.

Details on the companies currently in charge for waste management, e.g. waste transfer station and incinerator, are available in Annex 7.

Summarizing, at both sites solid and liquid wastes generated during all the different processes which potentially contain 4-tert-OPnEO are collected for treatment as wastes.

a. Aqueous diluted wastes are collected in tanks properly identified for disposal as hazardous waste.

b. All other wastes, unused raw material and solid wastes potentially containing 4-tert-OPnEO are segregated and labelled (see pictures below, with example of waste box). The boxes have symbols indicating that they are dedicated to special solid waste and the bag also is a yellow special bag, which identifies this kind of waste

These wastes are then collected and temporary stored in a hazardous waste storage area before disposal using licensed company. Storage area is covered, paved and provided with containment system to collect potential spills (see picture example of the waste storage area in Saluggia).

Standard operating procedures are in place describing the steps for waste collection and disposal:

SOP 21.0014 'Waste management procedure Saluggia site. (Annex 3)

DEHS6.2 Waste management procedure Dartford site. (Annex 4)

Here below an extract of the adopted instruction for waste management is provided.

Hazardous waste management

After use, hazardous materials still need to be stored and properly disposed to prevent pollution.

The following actions are carried out:

- *Wastes generated in labs are stored in dedicated containers (see example of container for solid hazardous wastes 18.01.03*) properly labelled and then transferred to the temporary storage area (see picture of the waste storage area).*
- *the waste and waste containers are completely isolated from surface water drains or from direct discharge to the environment. The area is bounded to contain spillages.*
- *Personnel as part of the general Environmental Health and Safety training is trained on hazards and how to operate according to procedures.*
- Wastes are transferred from the site where they are generated to the place where the treatment takes place using licensed carriers; this is in accordance with the existing regulation on wastes and dangerous goods transportation.

As per the current European Regulation requirement, we receive copies of the document/waste card confirming the waste has been received by the treatment facility. Moreover, declaration to confirm the kind of treatment performed has been released by the disposal companies (Annex 7). Here below a translation of such declaration:

[redacted]

We hereunder declare that waste generated by DiaSorin in Saluggia, EWC 150110 and 160506* are temporary stored at our facility* [redacted] *and then transferred at incineration treatment plants.*

[redacted]

Authorization waste treatment

We hereunder declare that the treatment plant for hazardous wastes for incineration [redacted]*, located* [redacted] *is authorized to temporary store wastes and to treat wastes by incineration according to Authorization granted.*

[redacted]

We confirm that your industrial waste named aqueous rinsing solutions EWC 070501 disposed at our treatment facility are treated using Chemical-Physical and Biological treatment.*

In order to reduce the substance releases, the glassware used for the buffers preparation/application containing 4-tert-OPnEO is firstly hand-washed and the used water is collected and disposed as liquid waste (operative instructions are given in Annex 1). Thus, only the potential substance residuals, after this washing stage, are collected through the wastewater net system of the whole industrial site.

The industrial site sewer tunnel flows into the Dora Baltea, which is a river in northern Italy. It is a left-hand tributary of the Po River (of which flow is ~171.1 m^3/s at Crescentino (VC), just beyond the Dora Baltea immission [Pascale et al, 2005]); Po originates by Monviso, is about 650 kilometres long and it ends at a delta projecting into the Adriatic Sea.

In the proximity of sewer tunnel discharge, Dora Baltea flow can be estimated around 70 m^3/s[1]; the data is provided by the Piedmont Regional Environmental Protection Agency (i.e. ARPA Piemonte).

Map and satellite view of Saluggia site

Dartford - site B

Purified antigens do not contain any residue of Triton X-100, since it is almost completely removed during the purification processes; residues of Triton X-100 would negatively impact any subsequent purification steps.

Considering the usages subject to authorisation, the possible ways for environmental release of Triton X-100 are:

- solid waste - disposable material come into contact with Triton X-100 e.g. filters. Solid waste is collected and disposed of as general waste for incineration by a

[1] Data published on the ARPA Piemonte website.

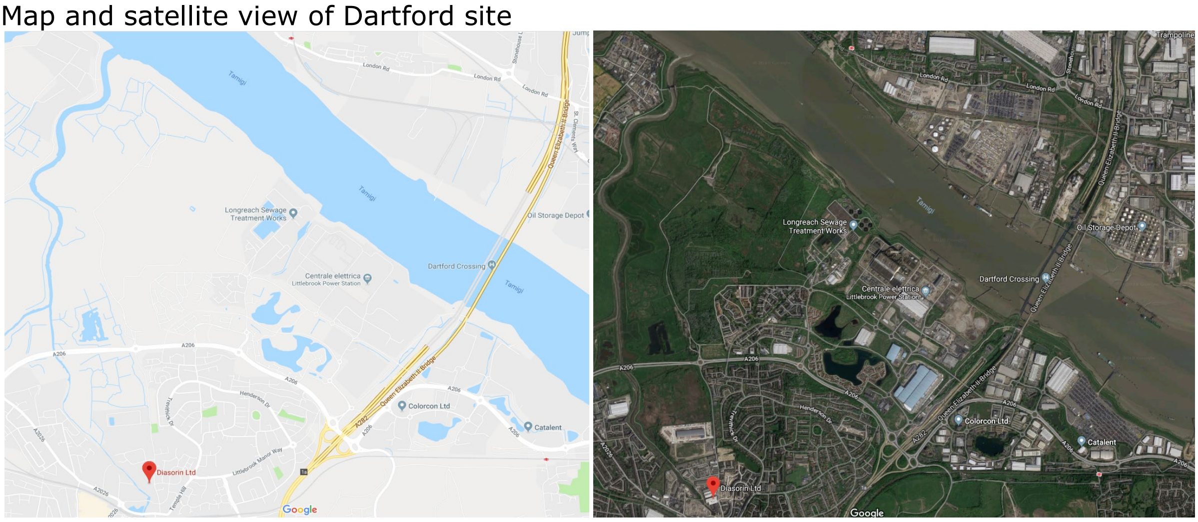
licensed company (EWC 16.03.05*)

- solid waste - empty raw material bottles - classified as special waste and disposal is managed by a licensed company (15.01.10*)
- liquid waste - unused raw material - classified as special waste and disposal is managed by a licensed company (16.03.05*)
- liquid waste – remaining buffer solution [redacted] is collected and classified as special waste and disposal is managed by a licensed company (16.03.05*)
- liquid waste - manufacturing process effluent waste is collected and classified as special waste and disposal is managed by a licensed company (16.03.05*)
- liquid waste – glassware cleaning process – the first washing is collected, classified as special waste and disposal is managed by a licensed company (16.03.05*);
- transportation of the raw material across the rear yard adjacent to a tidal river is double contained to prevent spillages.

The only source of direct release into the environment is the glassware washing after the first collection of hand washing. Details on the companies currently in charge for waste management, e.g. waste transfer station and incinerator, are available in Annex 3.

For purification processes at Dartford site (B), glassware is rinsed by hand in the sink and the plant's discharges are directed into to the municipal wastewater net, that is managed by the Thames Water company. After several steps of water treatment, the municipal sewer waters are discharged, into the Thames river, which is characterized by a flow rate approx. 65 m^3/s.

Map and satellite view of Dartford site



8. Environmental Releases and Measures of risk Management

8.1. Emission assessment

As described in the distribution summary (i.e. 4.1.2), once released to the environment via wastewater effluent, it can be expected that 4-tert-OP and short chain 4-tert-OPnEO will distribute into sediment, while longer 4-tert-OPnEO and 4-tert-OPnEC remain in the water phase.
In the water column, long chain 4-tert-OPnEO are expected to further degrade to short chain 4-tert-OPnEO or 4-tert-OPnEC depending on the environmental conditions.
As the short chain 4-tert-OPnEO are expected to distribute into sediment, they may contribute to the overall sediment load. Degradation half-lives are low and 4-tert-OP is a very stable product in sediment (no mineralization after 83 days under anaerobic conditions). Thus, once released to surface water and distributed to sediment, degradation of 4-tert-OPnEO will remain a long-lasting source for 4-tert-OP.
Release to soil via sewage sludge may be an additional relevant source of 4-tert-OP and short chain ethoxylates due to the high adsorption of 4-tert-OPnEO by sludge.
Results for NPnEO (used in the evaluation) indicate that short chain ethoxylates may degrade to 4-tert-OP in soil but slowly, thus once released to soil, short chain 4-tert-OPnEO may contribute to the overall concentration of 4-tert-OP in soil. Because conversion is slow, it can be expected that these ethoxylates are a constant source of 4-tert-OP in soil.
4-tert-OP itself is a stable metabolite which strongly adsorbs to soil, sludge and sediment. In sediment no elimination was observed under anaerobic conditions after 83 days (European Chemicals Agency, 2011).

The environmental distribution of 4-tert-OPnEO in the environment is complex, particularly due to the UVCB nature of the substance, which includes components having different degrees of ethoxylation.
Since a description of partitioning and distribution of the substance within the environment is not definitive, the main focus of the environmental analysis has been on the estimation of the releases.

As described in the previous paragraphs most of the residual substance is collected and disposed as a special waste, using the appropriate procedures by licensed companies, either intermediate or final disposers. The only source of direct release into the environment is the laboratory glassware washing.

Due to the release pathway, water compartment can be considered the main concern.
For dynamic systems like rivers, the substance is expected to be non homogeneously and discontinuously distributed, due to the fact that it is likely to be transported over a wide area. Thus, possible accumulation in sediment has not been estimated, as well as potential for long-transport has not been considered due to the quantities involved and the dilution potential along the river course (in the case of Saluggia, ca 5.5 km south, Dora Baltea flows into the Po river, which ends in the Adriatic Sea; in the case of Dartford, Thames flows into the North Sea ca 20 km east).

There is no risk of direct soil release due to the usage method for the substance. While air compartment can be considered as negligible due to the physicochemical characteristics of the substance.

8.2. Environmental releases

8.2.1. Fate of special waste

As already described for both Saluggia and Dartford sites (i.e. A and B, respectively) the disposal of solid and liquid, classified as general or special waste, is managed by the licensed company either intermediate or final disposers; the most of waste is usually treated as biohazardous matter and, usually, the final step is incineration for solid wastes and unused raw material and physical-chemical/biological water treatment for aqueous diluted liquid waste.
In accordance to the European Directive 2008/98/EC *any original waste producer or other holder carries out the treatment of waste himself or has the treatment handled by a dealer or an establishment or undertaking which carries out waste treatment operations or arranged by a private or public waste collector* is responsible for waste management.

The waste producer has the responsibility to identify adequate company(ies) for the substance transport and disposal. The possible intermediate(s) and the final disposer(s) should be able to manage and treat with adequate technologies for the specific waste type as well as the specific substance.
Thus, DiaSorin designates only licensed companies, which can guarantee the correct disposal of the substance and carry out the waste disposal activities complying with the applicable regulations, including the preparation of the necessary documentation.
In addition, in order to face with the responsibility delegation among the actors of the waste management chain, the presence of possible intermediate steps before the final disposal and the possibility of changing the companies in charge (either intermediate or final disposers), applicant informs the next actor in supply chain about the hazard profile of the waste. Moreover, the applicant carries out inspections of the waste disposal companies to verify their waste management practices.

In any case, the disposal and any possible environmental release of Triton X-100 and Igepal CA-630 can be considered as almost completely traceable.

At Dartford site, waste management involves a waste transfer station, that has to complete the Part E of the hazardous waste consignment note (HWCN), to show that the waste has been accepted into a licensed facility. Such facility is capable of storing and bulking waste streams. Once accepted, the waste is sorted and shipped to end disposal locations. In Annex 4, the permit number of the transfer station and the EWC codes accepted by its permit are listed.
Once Part E is signed on the HWCN, the licensed company legally takes ownership of the

waste and declares it is being handled correctly. Most prolifically the waste by DiaSorin is treated by incineration; the specific permit number for the company currently in charge of incineration is available in Annex 4.
As for water used by hand washing the glassware, it enters the sewage water, that is treated by the Thames Water company before release into the Thames river.

As for the Saluggia site, the actual waste management involves different companies, depending on the type of waste and the treatment needed. Declaration by disposers, quality certification and official documents by public authorities are available in Annex 7 and 8. As for water used to complete the washing phase, after the first rinse, residual quantities of Triton X-100 and Igepal CA-630 enter the sewage water and then are released to Dora Baltea. As the waste producer, such as DiaSorin, has a Duty of Care to ensure the waste is disposed of in the correct manner after being received by a disposal company, audits at the intermediate facility up to the various end disposal points are implemented.
Intermediate(s) and the final disposer(s) can change in the course of the authorisation iter; however, the applicant archive all the related documents.

Diluted liquid waste resulting from production processes is treated in physical chemical/biological water treatment plant: the chemical treatment is using Fenton oxidation reaction.
Chemical waste water treatment based on Fenton reaction (oxidation) it's a well-recognized technology effective in the treatment of surfactants and their degradation products. The attached document from Krzysztof Barbusinki concerning the chemistry of the 'FENTON reaction' (Annex 10) describes possible reaction mechanisms of the Fenton reactions and refers to its ability to be effective in the treatment of various industrial wastewater components including surfactants. Here below an excerpt of this document:

"Fenton reagent is effective in treating various industrial wastewater components including aromatic amines [4], a wide variety of dyes [5-7], pesticides [8-10], surfactants [11-13], explosives [14] as well as many other substances."

Moreover, according to specific studies performed on 4-nonylphenol ethoxylates (NPnEO), Fenton reaction is effective in the degradation of NPnEO and its degradation products.

4-nonylphenol ethoxylates are considered close analogues to octylphenol ethoxylates as even ECHA is reporting in the 'Support document for the identification of 4-(1,1,3,3-tetramethylbutyl) phenol, ethoxylated as substances of very high concern' of 12 December 2012.

Attached a document from the US 'National Center for Biotechnology Information' (NCBI) reporting data concerning the effectiveness of the Fenton reaction in the treatment of NPnEO and its degradation products. Please find below the summary of this study (Annex 11).

"Fenton oxidation was a feasible method that was used to degrade NPEOs in aqueous solution. NPEO removal could reach 81% within 2 min under the following optimal condition: pH 3.0, temperature 25–30°C, [NPEO]=3.25×10^{-5} M, [H_2O_2]=9.74×10^{-3} M, and [Fe^{2+}]=3.25×10^{-3} M. The degradation of NPEOs followed pseudo-first-order kinetics. [...] The HPLC and GC-MS analytical results indicated that a stepwise EO unit shortening process took place. They also showed that NP, short-chain NPEOs, and NPECs were the primary intermediates, most of which degraded at the end of the reaction."

Based on the above consideration, the waste water treatment of 4-tert-OPnEO diluted solution, involving a step of chemical treatment using Fenton reaction coupled with a second step of biological treatment is considered effective in the degradation of 4-tert-OPnEO and its degradation products.

8.2.2. Estimation of environmental releases

The applicant intends to focus the risk assessment on the release into the wastewater drainage systems, due to the fact that the disposal, as special waste, is ruled by a specific law.

The following table reports the quantities used site-by-site, over a period of 1 year.

The supplied bottle volume of Triton X-100 and Igepal CA-630 are:

Saluggia
Triton X100 – bottle volume of [redacted]
IGEPAL CA630 – bottle of either [redacted] or [redacted] bottles

Dartford
Triton X100 – bottle of [redacted]

Table 22. Data on Triton X-100 / Igepal CA-630 used over 1 year

	Saluggia site (A)		Dartfors site (B)
	Triton X-100	**Igepal CA-630**	**Triton X-100**
Box / year	[redacted]	[redacted]	[redacted]
Product actually used*	[redacted]	[redacted]	[redacted]
Product lost as waste*	[redacted]	[redacted]	[redacted]

*Amount respect to the open box
**Calculated taken into consideration the use of [redacted]

These are average numbers.
For Saluggia the total number of buffers prepared is the result of those prepared in biochemistry and biology laboratories.

§ 7.3.2: activity in biochemistry/biotechnology laboratories
Table 18: annual amounts
no. preparations/year (Saluggia): [redacted] (Triton X-100) + [redacted] (Igepal CA-630)
no. preparations/year (Dartford): [redacted] (Triton X-100)

Table 19: buffers preparation and use – biochemistry lab. and biotechnology lab.
no. preparations/year (Saluggia): [redacted] (Triton X-100) + [redacted] (Igepal CA-630)
no. preparations/year (Dartford): [redacted] (Triton X-100)

§ 7.3.2: activity in biology laboratory
Table 21:
no. preparations/year (Saluggia): [redacted] (Triton X-100)

§ 8.2.2: estimation of environmental releases
Table 23:
Saluggia site – biochemistry + biology labs
no. buffers prepared/year: [redacted] (Triton X-100) + [redacted] (Igepal CA-630)
Table 24:
Dartford site – biotechnology lab
no. of preparation is not explicitly reported, but can be derived from no. of beckers used per year and no. of beckers used per preparation, i.e. [redacted]
Accordingly, it is [redacted], as reported in table 18-19.

Following, data related to the Triton X-100 and Igepal CA-630 usage is reported. The residual parts not collected and not managed as special waste, has been considered in order to estimate the potential environmental release.
Relative density is reported by suppliers as 1.06 g/ml for both reagents, in all calculations 1 g/ml value has been used.

<u>Residues from buffer preparations</u>

Saluggia site (A)

The *Total volume of buffer solution*, over 1 year, indicated below, takes into consideration all the preparations, in which Triton X-100/Igepal CA-630 are used.

Regarding the residues onto glassware, it has been assumed that [redacted] beakers/laboratory glassware are washed per lot process and it has been assumed that residual buffer for each beaker is [redacted] in the case of Triton X-100 and [redacted] in the case of Igepal CA-630. The residual liquid of [redacted] has been estimated from the drips deposited after the beaker was drained, using the biggest container that could be used among all the preparations.

The *Residue of substance per beaker* has been calculated considering the *Estimated residue of the buffer remaining in the beaker* and the highest concentration at which substance can be used, among all the preparations (i.e. Triton X-100 at [redacted] and Igepal CA-630 at [redacted]). It should be underlined that, since the volume of the buffer preparation as well as the percentage of the substance used varies preparation-by-preparation, the *Residue of substance per beaker* value can be considered as an overestimate.

Table 23. Data representative for the buffer preparation in both biochemistry and biology labs

	Triton X-100	**Igepal CA-630**
N. of buffers prepared / year	[redacted]	[redacted]
Total volume of buffer solution	[redacted]	[redacted]
Number of beakers per each buffer usage	[redacted]	[redacted]
Amount of substance in the prepared buffers**	[redacted]	[redacted]
Estimated residue of buffer per beaker*	[redacted]	[redacted]
Residue of substance per beaker	[redacted]	[redacted]
Total residues from buffer usage across a year	[redacted]	[redacted]

* Residue liquid of [redacted] for Triton X-100 has been estimated from the drips deposited after the beaker drain, using the biggest container that can be used.
**The highest concentration at which substance can be used, among all the preparations.

It should be noted that the glassware used for the buffers preparation/application containing 4-tert-OPnEO is firstly hand-washed and the used water is collected and disposed as liquid waste; this practice is already implemented, in order to guarantee the correct management of the substance during the glassware rinsing; washing operative instructions is available in Annex 1.

The potential substance residuals after this washing stage, estimated in traces, are collected through the wastewater net system of the whole industrial site.

Dartford site (B)

The estimated quantity of Triton X-100 entering the drainage system over a year through this cleaning process is 13.8 g based on the calculations in the table below.

Table 24. Data representative for the buffer preparation in thebiotechnology lab

	% Triton X-100 within Buffer		
	[redacted]	[redacted]	[redacted]
Residue within the beaker (ml)*	[redacted]	[redacted]	[redacted]
Number of beakers per each buffer usage	[redacted]	[redacted]	[redacted]
Number of beakers per year	[redacted]	[redacted]	[redacted]
Total volume of residual buffer in beakers (ml)	[redacted]	[redacted]	[redacted]
Weight Triton X-100 (g)	[redacted]	[redacted]	[redacted]
Total weight released to drain across a year	[redacted]		

* Residue liquid of [redacted] for Triton X-100 has been estimated from the drips deposited after the beaker drain, using the biggest container that can be used.

The used amount of Triton X-100 at the Saluggia site ([redacted]) is more than [redacted] times lower than the used amount at Dartford site ([redacted]), while the calculated "Total residue from buffer usage across a year" at Saluggia site ([redacted]; table 23) is around [redacted] higher than the "Total weight released to drain across a year" at Dartford site ([redacted]; table 25) This is because the total residues depend on the no. of beckers and Triton concentration used in the buffers:

Saluggia: [redacted] preparations with Triton X-100, [redacted] beckers per preparation = [redacted] beckers
Buffers concentration assumed to be the highest in all cases, i.e [redacted]; residue of buffer per becker [redacted] ml
[redacted]

[redacted] preparations with Igepal CA-630, [redacted] beckers per preparation = [redacted] beckers
Buffer concentration [redacted] residue of buffer per becker [redacted] ml
[redacted]

Dartford: [redacted] beckers used per preparation; residue of buffer per becker [redacted] ml
[redacted] beckers with triton at [redacted] % ([redacted] preparation)
[redacted] beckers with Triton at [redacted] % ([redacted] preparations)
[redacted] beckers with triton at [redacted] % ([redacted] preparations)
[redacted]
Total = [redacted]

The beakers volume used for the buffer preparation ranges from 0,5 litre up to 10 litres

<u>Measurements for estimation of 4-tert-OPnEO in both sites</u>

There are no representative data and/or measurements regarding the actual environmental releases of Triton X-100 and Igepal CA-630. Currently, a validated standard method to specifically measure Triton X-100 and Igepal CA-630 is not available.

A substance in the class of 4-tert-OPnEO was used for an analytical investigation, in cooperation with an external laboratory, aimed to verify and estimate the entity of the possible buffer residue remaining in the beaker.

With reference to the development of an analytical experimental investigation to estimate the residue remaining in the beaker an analytical calibration curve has been prepared. To build the calibration curve for 4-tert-OPnEO, the chemical laboratory requires to use a raw material at known concentration.

Since the exact concentration of 4-tert-OPnEO for Triton X100 is not known, the decision was to use another commercially available Triton solution for which the concentration of 4-tert-OPnEO is declared. [redacted] has been used, for which the Certificate of Analysis is attached (Annex 12)

LOQ was not determined, but the calibration curve has been prepared using as a lower limit the value of 20 ppb. Please see analytical method attached where the lowest Calibration Curve concentration is reported. However, we are already working with an external laboratory in order to validate a method to detect 4-tert-OPnEO in effluents and to measure its degradation products

Two buffers were prepared in the biggest containers and at the highest concentrations used in purification (i.e. [redacted]); buffers were shaken to guarantee the maximum contact with the container's wall, before discharge. The residues of three beakers per buffer concentration were collected in a well-defined volume of water and concentrations were measured. The mean of the three samples was indicative of a buffer residue per beaker of [redacted]

Overall, such values gave an approximate confirmation of the [redacted] residue, used for the estimation of the maximum possible discharges per buffer preparation, taken into consideration that the glassware involved in the actual purification processes has different sizes and that the beaker for buffer preparation and simulation is expected to be the one with the highest amount of residues. In fact, it is likely that residues deriving from actual applications could be characterized by a lower concentration and amount of Triton X-100/Igepal CA-630 and due to the fact that the analysis was done on the biggest beaker.

The above method cannot be used because it has been developed only at laboratory scale in controlled environment using purified water and a substance of the family 4-tert-OPnEO.

The method has not been validated and could not be used for estimating the release of 4-tert-OPnEO in waste water where other substances are present and may interfere in the analysis precluding the accuracy of the method.
(see Annex 9 – 10 - 11)
Since we are not currently aware of a recognized standard analytical method to detect the substance of concern, we are already working with an external independent laboratory in order to validate a method to detect the substance of concern in effluents and to measure its degradation products. Once a method is validated, we will implement the periodic monitoring and report information as required

Residues pathway

As previously described, the residues from buffer preparations follow the hereunder described path:

- at Saluggia site (A), both in biochemistry and biology laboratories, the glassware used for the buffer preparation/application containing 4-tert-OPnEO is firstly hand-washed and the used water is collected and disposed as liquid waste (operative instructions are given in Annex 1). It may be assumed that only the potential substance residuals, after this washing stage, are collected through the wastewater net system of the whole industrial site that discharges into the Dora Baltea river. The overall water flow of industrial site sewer tunnel is estimated to be about [redacted]. Annex 6 reports specific data for 2018 referred to the wastewater by DiaSorin, i.e. [redacted], and the relative percentage with respect to the wastewater by the whole industrial site, i.e. 24.8 %.

- at Dartford site (B), laboratory glassware is rinsed by hand and the radula collected; the second washing water is discharged in the sink, which is connected to the municipal wastewater net, which discharges into the Thames river, after treatment. Since the actual water consumption during the rinsing procedures can considerably vary use-by-use and cannot be precisely estimated, no dilution has been considered; the actual municipal drain flow, collecting the plant's discharges, is unknown, thus no dilution has been considered, following a worst-case approach.

In both Saluggia and Dartford sites, the possible release events are limited [redacted] per year, respectively) and precisely identifiable. The release pathway is traced and the environment discharge point(s) identified.

Dilution discharges

In the following table, data related to the dilution of the discharges is presented.

In both sites the worst-case approach is considered. In fact, considering the river flow, it is assumed that all the discharge is diluted at the same time in the same aqueous column.

Table 25. Data related to the discharges fate

Saluggia site (A)	
	Triton X-100 and Igepal CA-630
Industrial site sewer tunnel	[redacted]*
River flow	Dora Baltea flow: 70 m³/s
Dartfors site (B)	
	Triton X-100
Plant flow discharged in sewer	[redacted]**
Municipal drain flow	n.a.
River flow	Thames flow: 65 m³/s

*Estimated: [redacted]

**Estimated: [redacted]

Residues and possible environmental releases

For a complete overview, two scenarios can be considered: the maximum possible discharge per buffer preparation (considering amount of substance per beaker) and the total discharges over 1 year (considering the total volume of buffer solutions).

Table 26. Estimate of 4-tert-OPnEO residues

	Saluggia site (A)	Dartfors site (B)
RESIDUES OF THE SUBSTANCE PER SINGLE PREPARATION		
Residues of substance per preparation in glassware*	[redacted] of Triton X-100 x [redacted] beakers [redacted]	[redacted] of Triton X-100 x [redacted] beakers [redacted]
Possible releases per preparation	traces	[redacted]
RELEASES OF THE SUBSTANCE OVER 1 YEAR		
Total residues	traces / year	[redacted]

* Calculated from the maximum residue for the buffer preparation beaker, which is expected the beaker with the most residues among the five used per use. In addition, the residue of buffer per beaker is calculate for the highest concentration [redacted] at which substance can be used, among all the preparations.

As anticipated in the previous paragraph, actual maximum possible residues per buffer preparation are expected to be lower than the estimated value (i.e. [redacted]) due to the fact that the residues per beakers used during the preparation have been estimated to be the same for all the glassware used; in addition, residues have been calculated from the buffer at the highest concentration at which substance can be used, among all the preparations.

Residual per becker is [redacted] (equivalent to [redacted] of substance in a buffer at [redacted]). Assuming that first hand-wash is done with an average of [redacted] of water (taking into account the different beckers volumes), dilution [redacted] occurs. Estimated residual after first hand wash is [redacted] per becker. [1]a total of ca. [redacted] is released. Analytical evidences will be provided as soon as the validated analytical method will be finalised and inserted in the quarterly report

First hand wash is described in the Triton operating instruction, provided in Annex 1, for which a translation has been reported at point 4 'Operating Instruction for Triton X100 and IGEPAL CA-630'.

With reference to Dartford, having implemented the same procedure to collect first rinsing as for Saluggia (see Annex 2), the total release, assuming same residue/becker as for Saluggia based on an average concentration of buffer at [redacted] is:
[redacted] beckers/year * [redacted] residue/becker = [redacted] g/year

For Saluggia site, since the glassware used for the buffer preparation/application containing 4-tert-OPnEO is firstly hand-washed (see operative instruction document in Annex 1) and the used water is collected and disposed as liquid waste, almost all the residues of substance per preparation is treated as special waste. Therefore, the only source of direct release into the environment are possible traces of 4-tert-OPnEO entering the wastewater net system of the whole industrial site that discharges into the Dora Baltea river. Considering the subsequent diluting conditions (flow of [redacted] for wastewater net system and 70 m^3/s for Dora Baltea river), the maximum possible discharges per buffer preparation for Saluggia site are reasonably estimated to be lower than the PNEC values proposed for 4-tert-OP and nonylphenol (see section 6.2).

Regarding the Dartford site, an estimation of the maximum possible discharges per buffer preparation cannot be likely performed due to fact that data on the municipal drain flow is not available. An rough estimation of the municipal sewer flow could be made considering the Dartford population of ca 100000 (numbered 97365 at the time of the 2011 census and Dartford's residents in 2015 were 103900[2]) and considering a pro capite water consumption of ca 150-200 l/day; if a water consumption of ca 3-5 l for the hand washing of all the [redacted] beakers is conservatively assumed, the release results to be in the order of 10^{-2} µg/l in Thames, per buffer preparation.
The estimation of discharges has been performed considering the dilution in the Thames river flow, thus, further dilution capacity among the course of river and the emission in other waters (river and sea) have not been taken into consideration. In addition, timing

[2] Authority Monitoring Report (AMR)2015-2016. Dartford Borough Council September 2016. Available on: http://www.dartford.gov.uk/__data/assets/pdf_file/0010/241498/AMR-2015-16-FINAL.pdf

and kinetic of degradation as well as possible mechanism of substance reduction of availability/removal are not taken into consideration.

In conclusion, values for releases to the environment are:

- Saluggia, less than 0,6 g/year
- Dartford, less than 0,3 g/year

Based on market development analysis, we actually expect the used quantity of 4-tert-OPnEO to progressively decrease in the future. In fact most of the IVD products involved in these purification processes will have a decrease of manufactured quantities (estimated 10%/year decreases) it seems reasonable to come to a release even below in some years.

8.2.3. Sources of environmental releases and surroundings

As anticipated, the source of DiaSorin environmental releases, in Europe, are limited to the sewer tunnel serving Saluggia plant and discharging in Dora Baltea river and to the drain flow serving the Dartford plant and discharging into the Thames river.

Considering the UVCB nature of the substance, which can determine a different distribution and partitioning, as well as the limited quantities involved, the diluting capability of water and the renewal due to the river flow, it is very difficult to identify and circumscribe a particular area of concern.

Saluggia site (A)

Regarding the Italian site in Saluggia (Vercelli, Piedmont), the Regional Environmental Protection Agency (ARPA Piemonte), in collaboration with the Regione Piemonte (the administration of Italian Piedmont division) and the University of the Piedmont, created a web portal (http://webgis.arpa.piemonte.it), which represents the information heritage on the theme of water in Piedmont; here, information about the health status and quality of waters derived from both historical studies and monitoring initiated for several years and still ongoing is published.

Regarding the Dora Baltea (monitoring point Dora Baltea - Saluggia - 06GH4F168PI – 039025), the following status is referred to the triennium 2014-2016, including the water body from Saluggia, up to the inflow into the Po river (ca 5.5 km south).

good	overall status The overall state of a body of water is obtained by taking into account the worst result between the Ecological State and the Chemical State.
good	chemical status Evaluation of the chemical state defined at Community level on the basis of a list of 33 + 8 priority hazardous or hazardous substances, for which the European Environmental Quality Standards, established by Directive 2008/105/EC[3] implemented in Italian Legislative Decree 219/10.
good	ecological status Sum of different indices, as Macrobenthos, Diatoms, Macrophytes, fisheries fauna, basic parameters to support biological components, exceeding the threshold values for specific pollutants (Table 1/B of Decree 260/2010), morphology, hydrology and hydromorphology.
elevated	ICMi diatom index Bioindicators for the evaluation of the biological quality of watercourses. The index is multimeter, obtained from the combination of the Pollutant Sensitivity Index (IPS) and the Trofico Index (TI).
elevated	LIMeco (pollution level by Macrodescribers for ecological status)

[3] The list of substances include also Octylphenol ((4-(1,1′,3,3′-tetramethylbutyl)-phenol), CAS 140-66-9).

	Synthetic index, describing the quality of running waters as regards nutrients and oxygen.
good	specific pollutants Environmental Quality Standards defined at National level.

Further details are given at http://webgis.arpa.piemonte.it/monitoraggio_qualita_acque/indexp_i.php?numcodice=039025

Data above mentioned is monitored by Regional Environmental Protection Agency on tri-annual basis. In the context of the river chemical status monitoring, the levels of octylphenol ((4-(1,1',3,3'-tetramethylbutyl)-phenol), CAS 140-66-9) are also recorded. For the triennium 2017-2019 year chosen for the monitoring is 2019, thus analysis are ongoing. Data will be published at the end of the monitoring period and data can be requested to the Regional Environmental Protection Agency.

In addition, the same information is also available for the Po river (in which Dora Baltea flows into); at the monitoring point of Trino (VC)[4] (n. 06SS4T385PI – 001230), which is the nearest monitoring point after Dore Baltea flows into Po river (ca 20 km east), the levels of octylphenol resulted to be lower than 0.05 µg/l for the triennium 2012-2014 and 2014-2016.

Dartford site (B)

Regarding the Britain site in Dartford (in the Kent county), the government Department for Environment Food & Rural Affairs (DEFRA), created a web portal (https://environment.data.gov.uk/catchment-planning/), which helps you explore and download information about the water environment.

There are various monitoring points along the course of Thames river; the one in the Dartford nearby is the Thames Middle, National Grid Reference TQ3295080508.

The overall classification for the water quality status was moderate in 2016.

moderate	overall status The overall state of a body of water is obtained by taking into account the worst result between the Ecological State and the Chemical State.
fail	chemical status Evaluation of the chemical state includes the evaluation of priority substances, priority hazardous substances and other pollutants.
moderate	ecological status Sum of different indices, as supporting elements (surface water), biological quality elements, physico-chemical quality elements and specific pollutants.
good	priority substances

	Monitoring the levels of 1,2-dichloroethane, Atrazine, Benzene, Chlorpyrifos, Chlorfenvinphos, Dichloromethane, Diuron, Fluoranthene, Isoproturon, Lead and Its Compounds, Napthalene, Nickel and Its Compounds, Pentachlorophenol, Simazine, Trichloromethane
fail due to the tributyltin compounds levels; the levels of all the other chemicals are classified as good	priority hazardous substances Monitoring the levels of Anthracene, Benzo (b) and (k) fluoranthene, Benzo (ghi) perelyene and indeno (123-cd) pyrene, Benzo(a)pyrene, Cadmium and Its Compounds, Di(2-ethylhexyl)phthalate (Priority hazardous), Endosulfan, Hexachlorocyclohexane, Mercury and Its Compounds, Nonylphenol, Tributyltin Compounds, Trifluralin
good	other pollutants Monitoring the levels of Carbon Tetrachloride, DDT Total, para - para DDT, Tetrachloroethylene, Trichloroethylene
Moderate	Supporting elements (surface water) Mitigating Measures Assessment
Moderate due to the status of angiosperms; the status of fish, invertebrates, macroalgae and phytoplankton is classified as good	Biological quality elements Taken into consideration the status of angiosperms, fish, invertebrates, macroalgae, phytoplankton
moderate	Physico-chemical quality elements Taken into consideration Dissolved Inorganic Carbon, Dissolved Oxygen
Moderate due to the zinc levels; the levels of all the other chemicals are classified as high	Specific pollutants Monitoring the levels of Triclosan, 2,4-dichlorophenol, 2,4-dichlorophenoxyacetic acid, Arsenic, Copper, Diazinon, Dimethoate, Iron, Linuron , Mecoprop, Permethrin, Phenol, Toluene, Un-ionised ammonia, Zinc.

8.3. Risk Management Measures and Operational Conditions

Since a 'Predicted No Effect Concentration' (PNEC) cannot be adequately established, appropriate and effective RMMs and OCs should be implemented to prevent or minimise release into environmental areas as far as technically and practically possible.

As previously described, from the arrival of the lots, to storage, transport to laboratories and opening up of the substance boxes, there is no possibility of substance release.

Currently, most of Triton X-100 and Igepal CA-630 used is collected and disposed by licensed companies. Almost all of the instruments, which can come in contact with the substance, are single-use, collected and disposed. Organic matter deriving from the purification processes are collected and disposed by licensed companies.
The only direct environmental releases are the residues from washing of laboratory glassware. In both Saluggia and Dartford sites the release events are limited in terms of number of events per year.
The release pathway is completely traceable and the environment discharge point(s) is(are) well identified: the point of possible environmental release is limited to the sewer tunnel serving Saluggia plant and to the drain flow at which the Dartford plant is connected.

The substance is only used indoor, in a limited number of laboratories, by trained operators, who are informed about the substance, its hazardous profile and the risk that can entail its environmental release.
The procedures for preparation of buffers, both using Triton X-100 and Igepal CA-630, are detailed in work sheets, which should be followed by technicians trained in the process for quality reasons. However, the work sheets also prevent loss of material during preparation.
Possible transfer of the substance onto the operator, from the opening of the bottle, can be considered as minimal due to the fact that the operators wear single-use gloves, which are removed and disposed. As already reported in the section dedicated to the description of the uses and exposure scenario, during the buffer preparation, the necessary amount of product is collected, weighed and transferred to the lab glassware using graduated automatic disposable pipettes in order to control the substances use and to prevent any loss. Also during this phase, possible risk of transfer of the substance onto the operator and/or lab counter can be considered as negligible.

Regarding the buffer application almost all the objects that can come in contact with the substance are single-use. Residues from the antigen treatment (chemical and biological matter, etc) are collected and disposed off. Licensed companies are charged for waste disposal in accordance with local applicable waste legislation and the necessary documentation retained.

Currently, applicant informs the intermediate and/or final disposer about type of the waste and where appropriate, chemical analyses are performed as well for the characterization of wastes.

At Saluggia site (A), in order to reduce the substance releases, the glassware used for the buffers preparation/application containing 4-tert-OPnEO is firstly hand-washed and the used water is collected and disposed as liquid waste; operating instruction are under development, to guarantee the correct management of the substance (see the operative instruction document in Annex 1).

The substance is managed by trained workers, informed about the substance hazardous profile and the suitable risk management and that any release should be limited.

In addition to training for the personnel, good practices to avoid the accidental release of substances are adopted, secondary containment for storage and transportation.

- No emission in air is expected since the substance is low volatile.
- All residues are collected and managed as waste.

(see Annex 1, Operating Instruction for which a translated abstract is provided here below):

Operating Instruction for Triton X-100 and IGEPAL CA-630

Instruction and protective measure

- *Wear protective gloves and goggles.*
- *Avoid release to the environment.*
- *Do not spray, do not eat, drink, smoke when using the substance. Rinse your hand after using the substance.*
- *Operate using disposable graduated pipettes.*
- *Residual of dilute solutions shall be collected in dedicated tank located in building 10 and 9z for further disposal as hazardous waste.*
- *All reusable material shall be rinsed accurately and resulting waters collected in the identified tank for further disposal as a waste. (as a general rule use a volume of water of 1/5 of the total volume of the container)*
- *Unused raw materials shall be transferred to the waste temporary storage area as hazardous wastes to be disposed as unused laboratory reagents*

Both sites have emergency procedures and teams trained to respond to accidental spills, absorber material is used, resulting wastes are properly identified, segregated and disposed as hazardous wastes using licensed companies.

For Saluggia, the activities are described in the local procedure SOP 21.0007 *'Attività in caso di emergenza sito Saluggia* (Annex 5) i.e.' Emergency response activities at Saluggia site for which a translated abstract of § 4.3 of the SOP is reported here below:

Emergency in case of spills of flammable liquid or any hazardous chemicals

In case of accidental hazardous material spill emergency response team have to be activated; they will take care of:

- *at least two persons of the emergency team have to reach the safety cupboard where the emergency devices and material are stored (booths, dressing material, face mask with filters, gloves) and bring absorber material;*
- *make sure that all the personnel have been evacuated;*
- *reach the place of the emergency, identify type of hazardous substances, pressure devices, gas bottles, other;*
- *use absorber material to contain the spill;*
- *collect the absorber material in a dedicate container and clean up the area.*
- *container with absorber material have to be stored at the hazardous waste storage area for disposal.*

Once the issue is resolved, equipment and material in the safety cupboard need to be restored.

For Dartford emergency measures are included in general operating instruction for waste management - DEHS6.2 (Annex 4) *Waste Disposal Procedure* for which the extract is provided here below:

Spillages

All items used to clean up spills must be disposed of as per the recommendations within the MSDS (see Table 1).

A dedicated spill team and Breathing Apparatus Team are available for large spills (see 'Emergency Posters). All large spills must be reported to the EHS manager.

The RMMs and OCs implemented at Saluggia and Dartford sites are aimed to limit the substance releases, as much as possible.

Saluggia

All material potentially contaminated with 4-tert-OPnEO is collected and managed as hazardous wastes, apart from residual after first washing of glassware. Therefore, emissions are negligible (less than 0,6 g/year).

Dartford

All material potentially contaminated with 4-tert-OPnEO is collected and managed as hazardous wastes. At the time of the preparation of the authorization dossier, only the residual of washing of glassware was sent to the Municipal sewage net. Soon after the submission of the request for authorization, in June 2019, as additional control measure, in Dartford a similar procedure (Annex 2) as in Saluggia has been implemented, requiring to collect the first glassware rinsing and to dispose it as hazardous waste using licensed companies. As a result of this, emissions even for Dartford are negligible (less than 0,3 g/year).

Moreover, it is important to note that since the distributed volume for the ELISA technology products, mainly manufactured in Dartford, is progressively reducing with a rate of about 10%/year, it seems reasonable to come to a release even below 0,1 g/year, in some years.

The operative conditions and procedure actually applied are minimising emissions as much as possible according to what is technically and practically possible.

9. References

Ahel M, Giger W, Koch M. (1994a). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - I. Occurrence and transformation in sewage treatment. Water Research 28(5):1131-1142.

Ashfield LA, Pottinger TG, Sumpter JP. (1998). Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modification to growth and ovosomatic index. Environ Toxicol Chem 17(3):679-686.

Balch G, Metcalfe C. (2006). Developmental effects in Japanese medaka (Oryzias latipes) exposed to nonylphenol ethoxylates and their degradation products. Chemosphere 62(8):1214-1223.

BAuA, Federal Office for Chemicals, Germany. Annex XV dossier. Proposal for identification of a substance as a CMR 1A or 1B, PBT, vPvB or a substance of an equivalent level of concern. Substance Name(s): 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated - covering well-defined substances and UVCB substances, polymers and homologues.

Committee for Risk Assessment (2014). Opinion on an Annex XV dossier proposing restrictions on Nonylphenol and Nonylphenol ethoxylates ECHA/RAC/RES-O-0000005317-74-01/F. Adopted 3 June 2014

Dow surfactants – alternatives to alkyl phenol ethoxylate (APE, APEO) surfactants, Form No. 119-02307-0414MM

Dussault EB, Sherry JP, Lee HB, Burnison BK, Bennie DT, Servos MR. (2005). In vivo estrogenicity of nonylphenol and its ethoxylates in the Canadian environment. Human and Ecological Risk Assessment 11(2):353-364.

European Chemicals Agency (2011). SVHC Support Document -4-(1,1,3,3-tetramethylbutyl)phenol, 4-tert-octylphenol.

European Commission (2016). Report from the commission to the European Parliament, the Council and the European Economic and Social Commitee in accordance with Article 138(7) of REACH to review if the scope of Article 60(3) should be extended to substances identified under Article 57(f) as having endocrine disrupting properties with an equivalent level of concern to other substances listed as substances of very high concern. COM(2016) 814 final. Brussel, 20.12.2016.

Environment Agency UK (2005). Environmental Risk Evaluation Report: 4-tert-Octylphenol. April 2005

Hamada et al. (2009). Effect of additives on protein aggregation, Current Pharmaceutical Biotechnology, 10, pp. 400-407.

Health & Safety Executive Chemicals Regulation Division (2018). Substance evaluation conclusion as required by REACH Article 48 and evaluation report for Nonylphenol, branched, ethoxylated EC No 500-209-1 CAS No 68412-54-4. Evaluating Member State(s): United Kingdom. Dated: February 2018.

Isidori M, Lavorgna M, Nardelli A, Parrella A. (2006). Toxicity on crustaceans and endocrine disrupting activity on Saccharomyces cerevisiae of eight alkylphenols. Chemosphere 64(1):135-143.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. (1996). Inhibition of testicular growth in rainbow trout (Oncorhynchus mykiss) exposed to estrogenic alkylphnolic chemicals. Environomental Toxicology and Chemistry 15(2):194-202.

Jobling S, Sumpter JP. (1993). Detergent components in sewage effluent are weaklyoestrogenic to fish: An *in vitro* study using rainbow trout (Oncorhynchus mykiss) hepatocytes. Aquatic Toxicol 27(3-4):361-372.

Johnson AC, White C, Bhardwaj L, Jürgens MD. (2000). Potential for octylphenol to biodegrade in some english rivers. etc 19(10):2486-2492.

Johnson M. (2013). Detergents: Triton X-100, Tween-20 and more, Mater methods 3:163.

Jonkers N, Knepper TP, de Voogt P. (2001). Aerobic biodegration studies of nonylphenol ethoxylates in river water using liquid chromatography - Electrospray tandem mass spectrometry. Environmental Science and Technology 35(2):335-340.

Madigou T, Le Goff P, Salbert G, Cravedi JP, Segner H, Pakdel F, Valotaire Y. (2001). Effects of nonylphenol on estrogen receptor conformation, transcriptional activity and sexual reversion in rainbow trout (Oncorhynchus mykiss). Aquatic Toxicol 53(3-4):173-186.

McAdam EJ, Bagnall JP, Soares A, Koh YKK, Chiu TY, Scrimshaw MD, Lester JN, Cartmell E. 2011. Fate of Alkylphenolic Compounds during Activated Sludge Treatment: Impact of Loading and Organic Composition. Environ Sci Technol 45(1):248-254.

Member State Committee (2012). Support document for identification of 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated as substances of very high concern because, due to their degradation to a substance of very high concern (4-(1,1,3,3-tetramethylbutyl)phenol) with endocrine disrupting properties, they cause probable serious effects to the environment which give rise to an equivalent level of concern to those of CMRs and PBTs/vPvBs. Adopted on 12 December 2012.

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environ Toxicol Chem 20(2):297-308.

Miles-Richardson SR, Pierens SL, Nichols KM, Kramer VJ, Snyder EM, Snyder SA, Render JA, Fitzgerald SD, Giesy JP. (1999). Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristics and gonads of fathead minnows (Pimephales promelas). Environmental Research 80(2 II):S122-S137.

Nichols KM, Snyder EM, Snyder SA, Pierens SL, Miles-Richardson SR, Giesy JP. (2001). Effects of nonylphenol ethoxylate exposure on reproductive output and bioindicators of environmental estrogen exposure in fathead minnows Pimephales promelas. Environ Toxicol Chem 20(3):510-522.

Pascale M., Perosino G.C. e Zaccara P. (2005). Parco fluviale III A 2000 – 2006 progetto Aqua. Regione Piemonte. May, 2005

Pessala P, Keranen J, Schultz E, Nakari T, Karhu M, Ahkola H, Knuutinen J, Herve S, Paasivirta J, Ahtiainen J. (2009). Evaluation of biodegradation of nonylphenol ethoxylate and lignin by combining toxicity assessment and chemical characterization. Chemosphere 75(11):1506-1511.

Petit F, Le Goff P, Cravedi JP, Valotaire Y, Pakdel F. (1997). Two complementary bioassays for screening the

estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. J Mol Endocrinol 19(3):321-335.
Risk Assessment Commettee. Risk-related considerations in applications for authorisation for endocrine disrupting substances for the environment, specifically OPnEO and NpnEO. Agreed at RAC-43.
Sjöström AE, Collins CD, Smith SR, Shaw G. (2008). Degradation and plant uptake of nonylphenol (NP) and nonylphenol-12-ethoxylate (NP12EO) in four contrasting agricultural soils. Environmental Pollution 156(3):1284-1289.
Swedish Chemicals Agency (2013). Annex XV restriction report proposal for a restriction nonylphenol and nonylphenolethoxylates in textiles. Version 3. Date 29 July 2013.
United Nations. Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria.
Varineau PT, Williams JB, Yunick RPCC. (1996). The biodegradation of 14C ringlabelled nonylphenol ethoxylate in a semi-continous activated sludge system. Unpublished report.
Vlaardingen PLA, Posthumus, R, Traas, TP. (2003): Environmental risk limits for alkylphenols and alkylphenol ethoxylates. RIMV report 601501019/2003. http://www.rivm.nl/bibliotheek/rapporten/601501019.pdf
White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. (1994). Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135(1):175-182.
Yu Y, Zhai H, Hou S, Sun H. (2009). Nonylphenol ethoxylates and their metabolites in sewage treatment plants and rivers of Tianjin, China. Chemosphere 77(1):1-7.

Other data sources

Safety Data Sheet - IGEPAL® CA-630. [redacted]
Safety Data Sheet - Triton™ X-100. [redacted]
Safety Data Sheet - Triton X-100. [redacted]
Safety Data Sheet - Triton™ X-100. [redacted]
Technical sheet - IGEPAL® CA-630. [redacted]
Technical sheet - Triton™ X-100. [redacted]

Legislation

Commission Regulation (EU) 2017/999 of 13 June 2017 amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC and amending Regulation (EC) No 1907/2006.

ECHA Databases of registered substances

REACH Registration dossier Nonylphenol, branched, ethoxylated (EC: 500-209-1/ CAS: 37205-87-1, 68412-54-4). Available on: https://echa.europa.eu/it/registration-dossier/-/registered-dossier/2032/1
REACH Registration dossier 2-phenoxyethanol; phosphoric acid (EC: 609-691-9 / CAS: 39464-70-5). Available on: https://echa.europa.eu/it/registration-dossier/-/registered-dossier/25499
REACH Registration dossier Phenol, ethoxylated, esters with acrylic acid (EC: 500-133-9 / CAS: 56641-05-5). Available on: https://echa.europa.eu/it/registration-dossier/-/registered-dossier/21709
REACH Registration dossier 4-Nonylphenol, ethoxylated (EC: 500-045-0 / CAS: 26027-38-3). Available on: https://echa.europa.eu/it/registration-dossier/-/registered-dossier/17700