CHEMICAL SAFETY REPORT

Public Version

Legal name of applicant(s):	*Abbott Laboratories Limited*
Submitted by:	*Abbott Laboratories Limited*
Substance:	*4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated*
Use title:	*1*
Use number:	*Professional use as a surfactant, in Wash Buffer components used in conjunction with Fluorescence In Situ Hybridisation (FISH) test kits and/or their Laboratory Developed Test (LDT) equivalents, in clinical diagnostic use for medical analysis of human tissue and blood samples to identify characteristic genetic abnormalities related to specific disease conditions.*

2022

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LIST OF ABBREVIATIONS

4-tert-OPnEO	4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated
4-tert-OP	4-(1,1,3,3-tetramethylbutyl)phenol
ALK	Anaplastic Lymphoma Kinase
AoA	Analysis of Alternatives
BCF	Bioconcentration Factor
CDx	Companion Diagnostics
CHESAR	Chemical Safety Assessment and Reporting tool
CLP	Classification, Labelling and Packaging
CSA	Chemical Safety Assessment
CSR	Chemical Safety Report
DAPI	4′,6-diamidino-2-phenylindole
DU	Downstream Users
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EEA	European Economic Area
EO	Ethylene Oxide
EQS	Environmental Quality Standards
ERC	Environmental Release Category
ES	Exposure Scenarios
FISH	Fluorescence In Situ Hybridisation
GHS	Globally Harmonised System
GPR	General Purpose Reagent
IVD	*In-Vitro* Diagnostic Devices
LDT	Laboratory Developed Test
NPnEO	Nonylphenol ethoxylates
PCR	Polymerase Chain Reaction
PEC	Predicted Exposure Concentration
PNEC	Predicted No-Effect Concentration
R&D	Research and Development
REACH	Registration, Evaluation, Authorisation and Restrictions of Chemicals
RMM	Risk Management Measures
RUO	Research Use Only
OECD	Organisation for Economic Co-operation and Development
NOEC	No observed effect concentration
LOEC	Lowest Observed Effect Concentration
MSC	Mixed Sex Character
SEA	Socio-Economic Analysis
SME	Small Medium Enterprises
SSC	Salium-Sodium citrate
STP	Sewage Treatment Plant
SVHC	Substances of Very High Concern

DECLARATION

Abbott Laboratories Limited is aware of the fact that evidence may be requested to support the information provided in this document.

We, Abbott Laboratories Limited, request that the information blanked out in the "public version" of the Chemical Safety Report is not disclosed. We hereby declare that, to the best of our knowledge as of today, **16th June 2022**, the information is not publicly available, and in accordance with the due measures of protection that we have implemented, a member of the public should not be able to obtain access to this information without our consent or that of the third party whose commercial interests are at stake.

Signature

Date, Place

16 June 2022

Tim Zurow
Director, Global Technical Operations
Abbott Laboratories Limited

9. EXPOSURE ASSESSMENT (and related risk characterisation)

9.0 Introduction

Abbott Laboratories ("Abbott"), is a worldwide Healthcare Company. Abbott has a broad range of branded generic pharmaceuticals, medical devices, diagnostics, and nutrition products. Abbott's diagnostics business provides in-vitro diagnostics (IVD) including immunoassays (e.g., blood screening products), clinical chemistry tests and molecular diagnostic products. Its medical tests and diagnostic instrument systems are used worldwide by hospitals, laboratories and blood banks for clinical diagnosis and monitoring diseases. Abbott's broad range of diagnostic tests includes immunoassays (HIV, hepatitis, thyroid function, fertility and pregnancy, cardiology, renal and metabolic markers, therapeutic drug monitoring, detection of drugs of abuse); as well as molecular diagnostic assays based on Polymerase Chain Reaction (PCR) and Fluorescence in situ Hybridisation (FISH).

Abbott FISH component and kit manufacturing occurs exclusively in the USA (Des Plaines, IL). Abbott Laboratories Limited ("the Applicant"), Abbott Diagnostics GmbH (the "Applicant") operates one FISH product distribution facility located in the EU (Wiesbaden-Delkenheim, Germany); this site acts as a distributer of Abbott FISH products that contain a wash buffer containing 4-tert-OPnEO. The products from there are imported into GB by Abbott Laboratories Limited, the Applicant. The Applicant distributes the tests in GB, where they are used by professionals in laboratories, hospitals, academic centres and cancer care facilities that test and treat cancer patients.

4-tert-OPnEO is contained in the post hybridisation wash buffer only (herein referred to as Wash Buffer) used to support Fluorescence In Situ Hybridisation (FISH) testing in approximately 400 of the Applicant assays, of which more than 100 ([redacted]) are classified as IVDs. FISH kits are used by medical laboratories and clinics in Great Britian for diagnosing cancer and determining the type of cancer of a patient. Some of the FISH products are Companion Diagnostics to certain targeted therapies for various solid tumour cancer and leukaemia types. Companion Diagnostics (CDx) are approved diagnostic tests that can be used by doctors to prescribe targeted treatment to cancer and leukaemia patients. The Applicant offers FISH products for two main types of analysis, namely genetics and oncology.

The exposure assessment presented here is done in the way that is conventional for a CSA/CSR. However, in the context of an authorisation, an exposure assessment that considers the amount of substance residing in the environment and the contribution that the continued use has on the environment is evaluated over the requested period (until January 2030).

An application for authorisation (ECHA reference number 11-2120816695-47-0000) of the continued use of the substance under EU REACH was submitted on 20 May 2019 by the EU distributor of the products of interest here. A positive opinion was adopted on the application on 19 May 2020, and the European Commission has approved the EU authorisation (EU AfA number: REACH/21/10/0) after the United Kingdom's exit date from the EU. Special transitional provisions (Article 127 GA of UK REACH) apply in such cases, whereby GB downstream users can continue to use the substance under the EU authorisation application but must submit their own authorisation for continued use under UK REACH within 18 months of the end of transition period, i.e. by 1 July 2022. That is the purpose of this application. This is an upstream application with the intention to cover all GB downstream users. The application for authorisation (AfA) is submitted by Abbott Laboratories Limited, owned by subsidiaries of Abbott Laboratories Inc, and a UK legal entity. In the context of this assessment the Applicant includes customers based in Scotland, Wales and England (herein referred to as Great Britain or GB). As a result of the Northern Ireland (NI) Protocol, NI customers are not included as part of this application.

9.0.1 Overview of uses and Exposure Scenarios

Finished FISH assay kits containing wash buffers are imported into Great Britain by the Applicant to its GB customers from a dedicated distribution centre in Wiesbaden, Germany. The tests are then used by professionals in laboratories, hospitals, academic centres and cancer care facilities that test and treat cancer patients.

Overview of FISH Assay Kits

The main components of a FISH assay supplied by the Applicant are:

- A set of fluorescent DNA probes, which bind on the desired section of the targeted chromosome(s) and can be detected by fluorescence microscopy on glass microscope slides.

- Wash buffers (2), which contain 4-tert-OPnEO. Alternatively, the end user lab prepares wash buffers from the supplied 4-tert-OPnEO (neat liquid) and 20X SSC (salts) kit components. These wash buffers are used to repeatedly to wash the slides with the hybridised DNA specimen to remove the unbound probe before examination of the slides using a fluorescence microscope.
- Other reagents and chemicals, which do not contain 4-tert-OPnEO, are used to facilitate the hybridisation and the other reactions in the assay.

4-tert-OPnEO is only present as a surfactant in the wash buffers, of FISH Assay kits, to wash unbound probe DNA and other unbound biological components originating from the specimen, including proteins from microscope slide on which the samples are prepared.

The 4-tert-OPnEO acts as an effective surfactant and wetting agent that aids in the gentle removal of coverslips from hybridised microscope slides (ensuring the target's unique cellular morphology remains unperturbed), promotes solubility of unhybridised (free) FISH probe, reduces nonspecific interactions between FISH probe and the cellular components present in human specimens, and minimises self-aggregation of the FISH probe as well as FISH probe coaggregation with proteins or other specimen components; all of which are necessary to support sensitive visualisation of specifically hybridised FISH probe and it's chromosomal target. As a result of the removal of these critical non-specific signals it ensures the precision, accuracy and specificity of the test. The use of sufficiently high concentration of an effective surfactant is considered essential to the correct functioning of the post hybridisation process and the generation of accurate results.

The following are the main steps in a typical FISH assay:

Step 1: Pre-treatment – this includes any pre-treatment of the specimen, if applicable for the specimen type. The specimen and the FISH probe DNA are then denatured at high temperatures in order to separate their two sets of complementary DNA strands. Note: pre-treatment is not needed for all sample types and not illustrated in Figure 1 below.

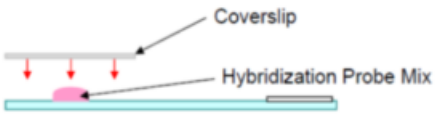

Figure 1: Probe Application

Step 2: Hybridisation - Following denaturation, the single-stranded fluorophore-labelled DNA probe is allowed to anneal to the complementary target sequence within a specimen attached to a microscope slide (hybridisation). The specimen is covered using a glass cover slip to seal the target.

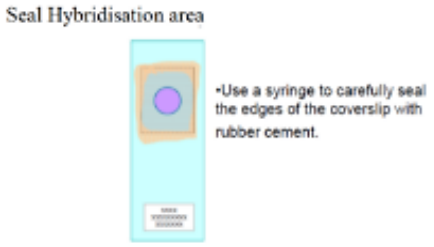

Figure 2: Seal Hybridisation

Step 3: Post hybridisation wash - Following hybridisation, unbound probe is removed from the slide by a series of washes, using a surfactant like 4-tert-OPnEO.

Step 4: Counterstain - The nuclei on the washed slide are counterstained with DAPI, a DNA-specific stain that fluoresces blue.

Step 5: Examination - Hybridisation of the probe with the cellular DNA target site(s) is visualized by direct detection using fluorescence microscopy.

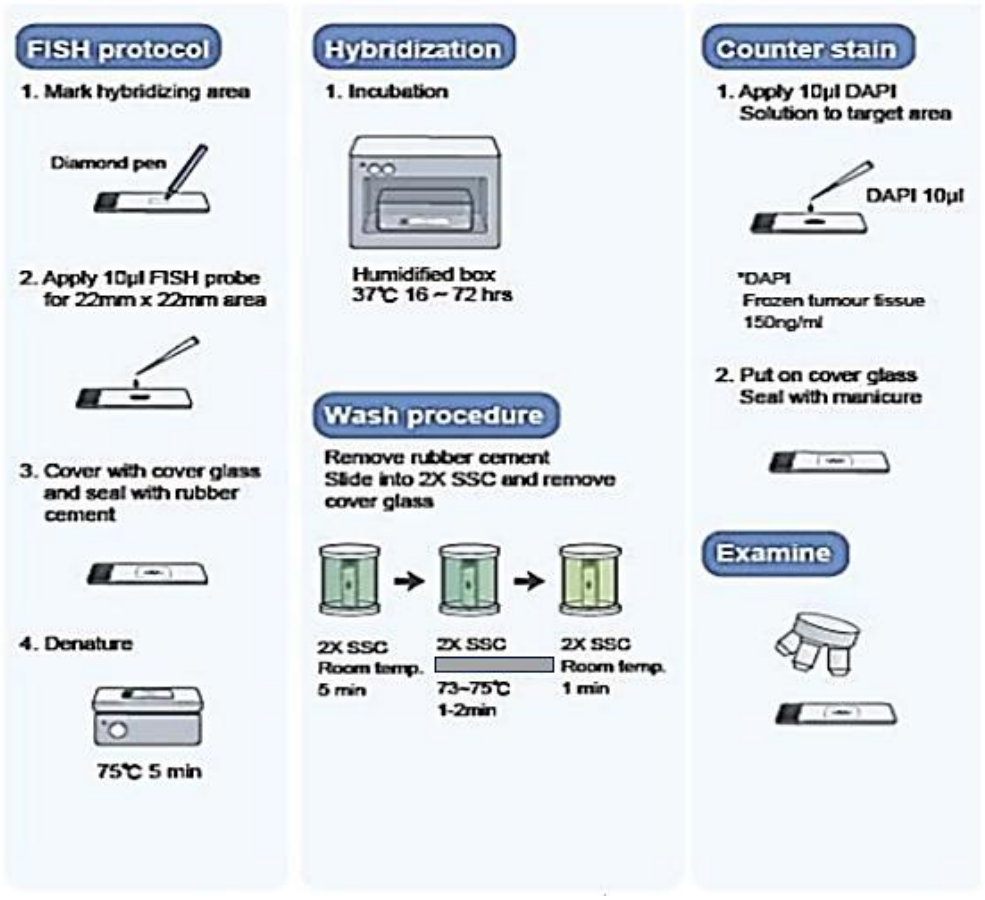

Figure 3: Overview of FISH process (BioGenex, 2016)

FISH diagnostic products may be used in manual assays, or in partially automated assays where some slide processing steps may be performed using dedicated equipment (e.g., pre-treatment to remove excess protein and/or other cellular debris; co-denaturation and hybridisation of sample and FISH probe; post-hybridisation

washes to remove unbound excess probe; raw microscopy data collection; and/or enumeration of final FISH imaged results).

For manual operations, the post hybridisation wash procedure (which contains the 4-tert-OPnEO) is likely to occur on the laboratory bench alongside a water bath. FISH assays tend to involve a lot of manual handling of the slides with the samples. The Applicant provides instruments that automate and standardise this process, reducing manual intervention and potential for errors. Such instruments are Thermobrite which allows for processing the slides with samples for hybridisation and VP2000, which allows for pre-treatment and staining of specimens.

This FISH assay process can also be carried out with the support of semi-automated instrumentation. The Applicant supplies the automated FISH testing platforms to make pre-hybridisation, hybridisation and post-hybridisation processing of slides more streamlined, and to substantially automate final FISH slide quality examination (rating, signal enumeration): as mentioned the FISH assays tend to involve a lot of manual handling of the slides with the samples. These instruments can work with predefined protocols for different staining and specimen pre-treatment procedures. They are designed to be used with all FISH assays supplied by the Applicant and are specific to the Applicant's products.

Automated FISH assays require instrument platforms developed and supplied by the Applicant (including VP2000® for pre-hybridisation and post-hybridisation processing, ThermoBrite® for hybridisation, BioView® for final slide quality examination), and/or those from other equipment suppliers. The VP2000 Processor, in conjunction with the Vysis Thermobrite System for denaturation / hybridisation provides a modular systems approach to automated FISH testing. The two systems can be used separately. These instruments may be supplied to customers using FISH Assay Kits as part of the contract or independently. They are not necessary for using the FISH assay kits, but they can increase the cadence of tests and reduce the possibility of errors by automating some of the steps in the FISH test process.

As per the manual process, 4-tert-OPnEO performs the same function in improving the ease of cover slip removal, through reduced surface tension and wetting action in the automated instrument platforms described above.

Figure 4: The Applicant's Automated Processors

The VP2000 Processor easily processes slides using pre-programmed protocols for fluorescence in situ hybridisation (FISH) for applications such as paraffin removal and the specimen pre-treatment protocols for various FISH Probe Kits. The reagents are stored within basins in the main processor. See figure 5 below.

Figure 5: Basins for storage of reagent within the processing unit

In the automated system, reagent basins are used for wash buffer solutions. The numbered reagent basins are each removed from the instrument, and then filled (or refilled) one at a time; to the appropriate depth needed for processing according to the processing map selected and the specific protocol provided in the reagent package insert. Each filled basin is returned to the appropriate numbered carrier position within the processor unit. Upon completion of all required processing protocol(s) at end of day, used reagents in the basins must be disposed of appropriately.

For both the manual and the automated systems described above, all hazardous materials, including wash buffer components containing 4-tert-OPnEO, should be disposed of according to the institution's guidelines for hazardous disposal in accordance with local, state and federal regulations. Empty Ambient Reagent Vessels may be cleaned with most detergent solutions, using a dampened cloth; then wiped a second time to rinse, using a cloth dampened with water. The Heated Reagent Basins are made of stainless steel and should be cleaned with detergent or organic solvents whenever they are emptied.

A typical FISH assay kit is presented below.

a. 2 x 200ul vials of fluorophore labelled DNA probes (orange and green)
b. 600ul vial DAPA II counterstain
c 2 x 2000ul vials containing 4-tert-OPnEO (100% OPnEO)
d. 1 x 66g SCC salt for wash buffer

Figure 6: Vysis CLL FISH Probe Kit

Tonnage information:

Annual tonnage band: 0 – 0.1 ([redacted]) tonnes/yr.

Tonnes/yr. directly imported: 0 – 0.1 ([redacted]) tonnes/yr.

Tonnage supplied per market sector:

In 2021, the Applicant's GB total tonnage was 0.5 - 5 L ([redacted] L or [redacted] kg) of 4-tert-OPnEO. The 2021 tonnage represents a conservative estimate of future use during the entire review period (until January 2030).

The following table provides the tonnage per use and the local tonnages used in the assessment for each environmental contributing activity.

The following table lists all the exposure scenarios (ES) assessed in this CSR.

Table 9.1 Overview of exposure scenarios and contributing scenarios

Identifiers*)	**Titles of exposure scenarios and the related contributing scenarios**	**Tonnage (2021)**
ES1-PW	Professional Use as a surfactant, in Wash Buffer components used in conjunction with an In Vitro Diagnostic Device (IVD), FISH test kits and/or their Laboratory Developed Test (LDT) equivalents, in clinical diagnostic use for medical analysis of human tissue and blood samples to identify characteristic genetic abnormalities related to specific disease conditions.	0 – 0.1 ([redacted])
*) Manufacture: M-#, Formulation: F-#, Industrial end use at site: IW-#, Professional end use: PW-#, Consumer end use: C-#, Service life (by workers in industrial site): SL-IW-#, Service life (by professional workers): SL-PW-#, Service life (by consumers): SL-C-#.)		

9.0.2 Introduction to the assessment

9.0.2.1 Environment

Scope and type of assessment:

The substance was added in Annex XIV of REACH (Authorisation list) because it breaks down to a substance (4-tert-Octylphenol) that is an endocrine disruptor for the environment. The approach taken in the CSR will be based on a non-threshold approach and will focus on the exposure estimates for the substance.

The exposure assessment presented in this CSR focuses on environmental exposure as the substance has been added to the Authorisation list based on its impact to the environment. This is because the concern for the substance is as an endocrine disruptor in environmental species and thus its effects and impacts on the environment are of relevance and are only considered relevant. The criterion for selection as an Article 57(f) substance is fulfilled by the substance's toxicity to aquatic organisms in particular Endocrine disruption. The exposure of workers is not assessed here since it is not considered relevant in the Annex XV report (BAuA, 2012).

The focus of the environmental exposure assessment is based upon the professional, widespread dispersive use of the Applicant's GB FISH Assay customers. The assessment also carried out representative customers' sites located in GB, for comparison purposes against the WDU approach taken by the Applicant. The scope of the exposure assessment and type of risk characterisation required for the environment are described in the following table.

Table 9.2 Type of risk characterisation required for the environment

Protection target	Type of risk characterisation	Hazard conclusion (see section 7)
Freshwater	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Sediment (freshwater)	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Marine water	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Sediment (marine water)	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Sewage treatment plant	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Air	Qualitative	PEC/Clocal value estimation Chesar 3.7.1
Agricultural soil	Qualitative	PEC/Clocal l value estimation Chesar 3.7.1
Predator	Not required	No hazard identified

Comments on assessment approach:

A worst-case mass balance approach is employed in the environmental exposure assessment assuming that all quantities of 4-tert-OPnEO released to the environment are ultimately converted to 4-tert-OP. In accordance with the Guidance on Risk-related considerations in applications for authorisation for endocrine disrupting substances for the environment, specifically OPnEO and NPnEO, which was agreed by the Risk Assessment Committee (RAC 43), Applicants may assume, "as a worst case scenario, that all 4-tert-OPnEO released to the environment will eventually be present as 4-tert-OP". Information provided by the supplier indicated that the concentration of ethoxylates is </= 100%. Therefore, for the purpose of this risk assessment it is assumed that all material supplied and used by the Applicant is 4-tert-OPnEO and subsequent releases to the environment after degradation are 4-tert-OP. Hence, the input parameters used in CHESAR to develop the predicted exposure concentrations and risk assessment are for 4-tert-OP.

The environmental concentrations arising from the uses of 4-tert-OPnEO will be calculated based on the releases and the appropriate environmental fate parameters of the substance. Distribution sites, including those owned and operated by the Applicant and/or third-party organizations that distribute the Applicant's products to end-user laboratories, will generate no 4-tert-OPnEO waste under normal operating conditions; barring the occasional accidental spill which only in rare cases may result in 4-tert-OPnEO environmental release. The vast majority of 4-tert-OPnEO impact to the environment results from professional use of the Applicant's FISH Assay products at end-user laboratory sites.

The following substance properties are used in the environmental fate estimation completed by EUSES. They correspond to the "value used for CSA" reported in sections 1 and 4 of the CSR. Please note that these values are for 4-tert-OP, on which the risk assessment is based. Values for 4-tert OP10EO are also listed for comparative purposes to illustrate differences in the modelled distributions in the biological sewage treatment plant.

Table 9.3 Key physicochemical property data used in the assessment for 4-tert-octylphenol

Substance property	4-tert-OP	4-tert-OP10EO
Molecular weight used for the assessment	206.3	646.9
Vapour pressure	0.001k Pa at 20 °C	$0.24 \cdot 10^{-11}$ Pa
Partition coefficient (Log Kow)	4.8 at 22 °C	2.39 at 20 °C
Water solubility	19 mg/L at 22 °C	189 mg/l at 20 °C (critical micellar concentration)
Melting point	84.5°C	Not available
Boiling point	289°C, at 101kPa	Not available
Biodegradation in water: screening tests	inherently biodegradable	inherently biodegradable
Bioaccumulation: BCF (aquatic species)	740 dimensionless	Not available
Adsorption/Desorption: Koc at 20 °C	10,000	18.20

Fate (release percentage) in the modelled biological sewage treatment plant (STP)

In a standard (modelled) biological STP, the emissions are distributed as shown in Table 9.4.

Table 9.4 Fate (release percentage) in the modelled biological sewage treatment plant

Substance property	4-tert-OP	4-tert-OP10EO
Release to water	43.0%	98.7%
Release to air	4.8%	1.1%
Release to sludge	52.2%	0.2%
Release degraded	0%	0%

The above fractions are calculated by the SimpleTreat model integrated in EUSES 2.1.2. They are calculated for 4-tert-OP, as it is assumed that, in a worst-case scenario, all 4-tert-OPnEO will be converted to 4-tert-OP in the STP and distribution to different compartments is based on 4-tert-OP's physicochemical properties. Information collected in the Annex XV dossier for 4-tert-OPnEO indicates that some removal of the substance takes place in the STP and that it degrades to 4-tert-OP at a later stage.

A conversion factor of 0.33 is applied to quantities of 4-tert-OPnEO released to the environment in order to express releases in terms of 4-tert-OP. The conversion factor was calculated based on the relative molecular weights of 4-tert-OP (MW = 206.3) and 4-tert-OPnEO with an average of 9.5 ethoxylate units (MW = 625). A single conversion factor of 0.33 based on 4-tert-OPnEO with an average of 9.5 ethoxylate units is applied, as a worst-case, to the total tonnage in order to have representative analysis.

For comparison, the SimpleTreat model was also run for 4-tert-OP10EO, which is closest to the average ethoxylation for the 4-tert-OPnEO used by the Applicant (average n = 9.5), with an assumption of no degradation in the STP. The model predicts that the substance would be present almost exclusively in the liquid outflow of the STP, mainly due to the very low adsorption / desorption coefficient (Koc). In practice, the situation is expected to be somewhere between these two extremes, with only partial degradation to 4-tert-OP occurring in the STP. The degradation products, according to information in the Annex XV dossier (BAuA 2012) would most likely be 4-tert-OP1/2EO, 4-tert-OPnEOs and 4-tert-OP. Their distribution to water, air and sludge varies. All these degradation products are expected to degrade to 4-tert-OP over time. The current approach in the CSR is carrying out the analysis using the values for 4-tert-OP. Therefore, it is likely that the share of 4-tert-OP released in water would be higher than what is assumed using the SimpleTreat model and the

share released to air and sludge would be expected to be lower. The current approach tends to overestimate releases of 4-tert-OPnEO to agricultural soil via application of sludge from STPs and underestimate the releases to surface water and sediment.

9.0.2.2 Man via environment

Scope and type of assessment:

The scope of this assessment focuses on the environmental exposure only. The type of risk characterisation required for man via the environment is not evaluated in this application as the substance has been onto UK REACH (The REACH etc. (Amendment etc.) (EU Exit) Regulations 2019; SI 2019 No. 758) based on its endocrine disrupting properties in environmental species and thus its effects and impacts on the environment are only of relevance. Thus, worker exposure is not relevant for this application as determined in the Annex XV dossier. In addition, according to ECHA's Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.16 on Environmental Exposure Assessment): "An assessment of indirect exposure of humans via the environment is generally only conducted if: a) the tonnage >1 000 t/y or b) the tonnage >100 t/y and the substance is classified b1) as STOT RE 1; or b2) as a carcinogen or mutagen (any category); or b3) as toxic to reproduction (categories 1A or 1B)." 4-tert-OPnEO is used below 1 tonne and is not classified with any of the mentioned hazard categories, as stated in Section 3 of this chemical safety assessment or as reported on ECHA's public Dissemination Portal, therefore the risk assessment for humans via the environment is not evaluated.

9.0.2.3 Workers

Scope and type of assessment:

The scope of this assessment focuses on the environmental exposure only. The type of risk characterisation required for workers is not evaluated in this application as the substance has been placed in Annex XIV of EU Regulation 1907/2006 (REACH), and transposed to REACH (The REACH etc. (Amendment etc.) (EU Exit) Regulations 2019; SI 2019 No. 758) based on its endocrine disrupting properties in environmental species and thus its effects and impacts on the environment are only of relevance. Worker exposure is not relevant for this application as determined in the Annex XV dossier (BAuA 2012).

Comments on assessment approach related to toxicological hazard:

Not relevant - worker toxicological hazards are not subject to assessment in this CSR

Comments on assessment approach related to physicochemical hazard:

Not relevant - physiochemical hazards are not subject to assessment in this CSR

General information on risk management related to toxicological hazard:

Not relevant- worker risk management measures (RMM) are not subject to assessment in this CSR. RMM related to the environment are detailed in Conclusion on risk characterisation (minimisation of emission/exposure).

9.0.2.4 Consumers

Scope and type of assessment:

Exposure assessment is not applicable as there are no consumer-related uses for the substance.

9.1 Exposure scenario 1 for Environment

Professional use as a surfactant, in Wash Buffer components used in conjunction with Fluorescence In Situ Hybridisation (FISH) test kits and/or their Laboratory Developed Test (LDT) equivalents, in clinical diagnostic use for medical analysis of human tissue and blood samples to identify characteristic genetic abnormalities related to specific disease conditions.

Environment contributing scenario(s): ERC 8a Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)

Products Category: PC21 Laboratory Chemicals

Sector of use: SU 20 Health Services

Worker/Consumer contributing scenario(s):*

PROC15 Use as laboratory reagent

Technical function of the substance: Surfactant

*Exposure Scenario 1: Involves the professional use of wash buffer containing 4-tert-OPnEO to be used with final test kits at laboratory scale with less than or equal to 1 litre or 1 kg present at workplace.

Description of the activities and technical processes covered in the exposure scenario:

Explanation on the approach taken for the ES:

Description of the activities and technical processes covered in the exposure scenario:

- At the customer site, the end user, a professional laboratory technician, uses the post-hybridisation wash buffers as part of the FISH diagnostic technique. The process involves putting the specimen loaded microscope slides through a series of washes in buffer to remove unbound probe DNA before examination. The Applicants FISH assays use 4-tert-OPnEO surfactant exclusively for these ancillary wash buffers, consisting of citrate buffered sodium chloride (SSC) at two concentrations: 0.1% or 0.3% of 4-tert-OPnEO. Wash buffers are either supplied as pre-formulated kit components or as filled (neat liquid) and 20X SSC (powder) components that are final formulated into aqueous Wash Buffers by the clinical laboratory customer at point of use.

- Two wash concentrations of 4-tert-OPnEO are recommended: 0.3% and 0.1%. The higher concentration (0.3%) is deemed necessary for active removal of unbound / non-specifically bound fluorescent probe; the lower concentration (0.1%) is sufficient to wash off any remaining liquid from the 1st wash in the next step; thus, reducing the overall amount of surfactant used based on the lower concentration.

- Wash buffers are either supplied as pre-formulated kit components or as filled 4-tert-OPnEO (neat liquid) and 20X SSC (powder) components that are formulated into aqueous wash buffers by the clinical laboratory customer at point of use. End users can manually make up the wash buffers on site at the recommended concentrations or use preformulated wash buffer kit components.

- Where the Applicant has supplied 4-tert-OPnEO (100%) in vials, professional laboratory technicians will prepare the post-hybridisation wash buffers at the point of use. Whether the Applicant supplies vials or pre-formulated wash buffer components containing of 4-tert-OPnEO, the end user laboratories

will either follow the Applicant-supplied instructions (IVD / RUO kits only) or their own validated LDT procedures; as appropriate.

- Typical steps in the production of a FISH IVD/ RUO/ GPR wash buffer reagent kit performed in the end user laboratory by professional clinical laboratory technicians include the following steps:

1. 20X SSC Reagent Concentrate Preparation:
    a. Dissolve 20X SSC salt raw material in aqueous solution, measure the pH, adjust if necessary and then add purified water to required batch size. Then perform terminal filtration. There is no 4-tert OPnEO added at this step.
2. FISH Wash Buffer Preparation: Wash buffers are typically made up for use in 500 mL or 1 Litre quantities.

    a. Mix the 20X SSC Reagent Concentrate made in step 1, with 4-tert-OPnEO and purified water. Mix thoroughly; measure pH, adjust pH to target if necessary; and then add purified water to necessary batch size to achieve the desired ingredient concentrations. Perform terminal filtration.

    b. Manual filling and labelling of bulk reagents prepared in end user laboratory may be performed at customer's discretion.

    c. Wash Solutions used in the assay must be discarded at the end of each day. Unused Wash Solutions must be discarded after 6 months, or sooner if solution appears cloudy or contaminated.

In manual operations

1. FISH laboratory technicians will use the wash buffers according to the Applicant's protocols. The slides are washed in 60 to 120 mL basins (Coplin jars) containing 50 to 100 mL wash buffer.

2. First post hybridisation wash is completed in 0.3% 4-tert-OPnEO wash buffer, slides are immersed in the basin at 73 ± 1 degrees C for 2 minutes ± 30 seconds.

3. The second post hybridisation wash is completed in 0.1% 4-tert-OPnEO wash buffer for 5 seconds to 1 minute at room temperature.

4. After each test is completed, the contents of the Coplin jars can be reused or are disposed of. At the end of the day, the Coplin jars are cleaned by the end user by rinsing several times with water. It is assumed, as a worst-case, that downstream user releases the waste (the used wash buffer solutions and associated jar rinse water aliquots) to wastewater which is connected to the facility wastewater stream.

Use in Automated Systems

Use of wash buffers in automated systems is substantially the same as that in manual assays. Buffers can be manually made as described above or come as pre-formulated. Final filled 4-tert-OPnEO (neat liquid) vials, bottled wash buffer components containing 4-tert-OPnEO and/or kits containing said components, are sold to customers to be run on specific automated slide processing instruments such as the Applicant's VP 2000® Processor and/or analyser (e.g., BioView® image analysis suite) in clinical laboratory settings.

Explanation on the approach taken for the ES:

Water

The downstream use of the Applicant's products is considered to be widely dispersive in nature since it occurs at over 10-100 (d) customer locations across GB. These sites are spread all over GB and vary in the level of IVD kit usage from a small number of tests per day to greater than 10-100 (c) tests per day for the higher volume users.

Risk management measures

Possible future conditions (safe disposal)

As a condition of granting the EU REACH authorisation (ECHA reference number 11-2120816695-47-0000), the European Commission has required the EU Distributor to inform users of its FISH tests that they must collect all liquid and solid waste for adequate treatment, which minimises releases to environmental compartments as far as technically and practically possible, specifying that release to the municipal STP does not constitute adequate treatment. The EU Distributor includes directions for handling waste according to instrument handling manuals and local regulations in the product instructions, and these directions have been updated to include this new requirement. It is expected that these new conditions will be incorporated into GB guidance also. However, for the purposes of the assessment, it is assumed that all quantities of 4-tert-OPnEO used in the FISH kits are released to waste water, and from there to the STP and the environment. This means that the results of the assessment are worst case, as it assumes that all guidance provided by customers will be disregarded to wastewater. Given that all users of the Applicant's tests are laboratory technicians (and similar), trained in applying controlled conditions, this assumption is clearly likely to overestimate emissions. If similar conditions are proposed by the UK authorities, emissions will be effectively zero.

For reference, the Applicant has instructed their EU downstream users that are covered under their EU AfA (ECHA reference number 11-2120816695-47-0000) that they shall collect all their liquid and solid waste for adequate treatment (e.g. incineration) and that release into the public sewer system is not considered to be adequate treatment. These instructions would be also communicated to GB customers if instructed as a condition of the Applicant's UK AfA application.

'Optimal batching' customer communication

Further to the instructions above, the Applicant has implemented another risk management measure within their EU AfA (ECHA reference number 11-2120816695-47-0000), to reduce the amount of use of 4-tert-OPnEO generated by their EU customers. The instructions provided to customers are described as 'optimal batching'. It encourages the maximising of utilisation of each FISH set of wash buffer aliquots to 'each day of FISH testing' (as the buffer can be used multiple times for samples for 24 hours). By batching a large number of assays once or twice a week, would result up to a five-fold reduction in the amount of 4-tert-OPnEO used. This will lead to a reduction in waste generated of 4-tert-OPnEO. This has been communicated to EU customers as having a 'corresponding environmental health benefit'. This batching communication has not been taken into account for the exposure scenario (which represents a worst case) where downstream users do not condense samples per wash buffer. However, this 'optimal batching instructions' will also communicated to the Applicant's GB customers.

Assessment for the Chemical Safety Report

As described above, a condition of granting the EU REACH authorisation (ECHA reference number 11-2120816695-47-0000), the European Commission has required the EU Distributor to inform users of its FISH tests that they must collect all liquid and solid waste for adequate treatment, which minimises releases to environmental compartments as far as technically and practically possible, specifying that release to the municipal STP does not constitute adequate treatment. As a result, the Applicant has instructed their EU downstream users that are covered under their EU AfA that they shall collect all their liquid and solid waste for adequate treatment (e.g. incineration) and that release into the public sewer system is not considered to be adequate treatment. However, although this application has been granted by the EU Commission, this occurred after the UK had left the European Union, and hence the EU authorisation itself does not have legal standing in the UK. The instructions for disposal of waste to the Applicant's EU customers would be also communicated to GB customers if instructed as a condition of the Applicant's UK AfA application.

For the exposure assessment, it is assumed that no conditions are applied and represent a worst-case scenario of a 10-100 ([redacted])% release rate of waste to municipal STPs. If the Applicant instructs their GB downstream users to collect all their liquid and solid waste for adequate treatment (e.g. incineration) and that release into the public sewer system is not considered to be adequate treatment, the release rate will be 0%.

Assessment assuming release of waste

4-tert-OPnEO in the Applicant's FISH assay kits is used in GB by laboratory workers in medical labs of clinics and hospitals. The use is carried out by trained professionals at multiple sites in GB. The customer sites vary in size and in the number of tests they run from < 20 tests to over 1,000 - 100,000 ([redacted]) tests per year. Most of the customers use low or very low numbers of tests. Due to the wide-dispersive nature of the use across GB, the Applicant conducted a wide-dispersive use assessment on releases. To demonstrate that this value is approximately conducive of the Applicant's use, three areas of customers' use in categories of High, Medium and Low were assessed to cross reference the wide dispersive approach.

The daily wide-dispersive use was estimated from the total annual usage for all downstream users in GB following ECHA Guidance R16 (ECHA, 2016). The total annual tonnage usage for all downstream users (laboratories / hospitals / blood banks, etc.) in GB was calculated as described above in section 9.1.1.

To verify the validity of a wide-dispersive use assessment, specific use information was collected for three downstream user local areas. Based on ECHA's R16 guidance, (section R16.1.4.1) the customer releases were grouped together (as applicable), based on their city/town location and proximity to each other resulting in an aggregate value for each location. Grouping of the customers is based on the expectation that all customer releases will enter the same sewage treatment plants, and hence, the same water bodies if they are in the same city/town (herein referred to as local areas), thus contributing to the overall environmental concentration.

The three local areas cover a range of release rates, categorized as high, medium, and low. Calculated environmental concentrations were based on test usage, amount of 4-tert-OPnEO per test, STP capacity and receiving body of water flow rate and other relevant available information. Measurement for 4-tert-OPnEO or relevant degradation products for downstream users was not considered suitable based on the complexities around measuring releases at DU customers as it may not be realistic in the terms of solely examining the Applicants releases. Measurements at a local STP level would take into consideration other emission not relevant to the Applicant. Based on the current use pattern and understanding of the Applicant's DU customers profile, it was concluded that a mass balance approach should be used as it provides more specific information on the use. Based on VP2000 usage per the ALK package insert, the wworst-case manual assay usage exceeds that calculated for worst-case automated usage (with VP2000) of 1.4 mL 4-tert-OPnEO /day (x 260 = 364 mL/year). But as it is not possible to determine the amount of overall annual tests that are conducted using the automated system versus the manual assay, the release rates are based on the worst-case manual calculations. Hence any potential differences between releases from manual and automatic procedures were not considered as it is not possible to capture accurately the DU user assay procedures at the individual use level.

The daily wide-dispersive use calculation as described by R.16 guidance (ECHA, 2016).

According to R.16.2.2.1.2. Estimation of tonnage for widespread uses, a default daily amount used in a standard town is estimated starting from the tonnage for the use, and taking into account:

• the fraction of the "tonnage for the use" used in the region (regional tonnage),
• the fraction of the regional tonnage used in the standard town (proportional to the ratio of number of inhabitants in a standard town (10 000) compared to the number of inhabitants in a region (20 000 000)),
• the number of days in a year: 365
• The resulting tonnage is multiplied by an assessment factor of 4 to take into account geographical or temporal peaks in the use of a substance.

The daily wide-dispersive use calculation for this assessment

For the purpose of this assessment, the daily wide-dispersive use was estimated per site starting from the total annual usage for GB and dividing it by:

• 10: Fraction of the total tonnage at EEA level used in the region,

• 2’000: Fraction of the regional tonnage used in the standard town of 10’000 inhabitants (versus 20’000’000 inhabitants in the region),
• 260 (day/a): number of operating days (see detailed explanation above on number of operational days) in a year defined per product group and multiplying it by a safety factor of 4 to take into account geographical or temporal variations in the use and releases as recommended in Guidance R16 (ECHA, 2016).

Daily amount (kg/day) = total annual usage for all GB customers × 4 / (10×2000×260 days) as determined in the Guidance R16 (ECHA, 2016). This release rate was subsequently used to determine wide dispersive daily release rate of 4.17 × 10-7 OP kg/day in CHESAR.

Therefore, all liquid waste containing the substance is assumed to be released to the wastewater and treated in a municipal Sewage Treatment Plant (STP) along with liquid waste from other users before it is discharged to the environment. As a result, measuring the local concentration of 4-tert-OPnEO or 4-tert-OP at the point of emission to the environment (i.e. after the STP) would not provide representative data or further refinement of the Applicants individual DU site emissions.

A mass balance approach has been taken by the Applicant based on their annual sales of FISH Assay kits and use patterns of the customers (number of kits per annum) to determine a worst-case number of tests run per day and subsequent DU customer emissions. The Applicant has utilized 2021 sales data to determine specific use information (number of possible tests run per day and kit sets per day) and daily release rates. This information provides meaningful information on the downstream users’ profile and has been used in the analysis and calculations of emissions of 4-tert-OPnEO at user sites in Chesar 3.7.1. As a result of this analysis a selection of customer locations have been further analysed to determine a possible range of emissions for the Applicant’s DU use.

The Applicant’s purchasing and distribution records show that Fish Assays are carried out by trained professionals in multiple towns and cities in GB. The professional use occurs at dozens of hospitals, clinical laboratories and laboratory sites distributed across GB. These sites are spread all over GB and vary in size and the level of IVD kit usage ranges from to 1-10 (c) kits per year to 100-1500 (c) kits per year. The average tests per day run by GB customer sites was calculated from the total number of kits sold to each customer site in the EU in 2021. The kits usage is dependent on the number of samples run, and it assumed that there are 20 tests per kit (the smallest, most common kit size); therefore, the ranges of tests run by customers is between 1-100 (c) and ≥ 5 (≥1 test) per work day, based on an assumption of 260 possible days of testing per year (52 weeks/year x 5 work days/week).

Table 9.5 Average tests per day run by professional personnel in GB customer sites

User group	No. kits per year	No of tests per year	No of local areas	% of local areas per user group
High	>500	>10,000 <25,000	1	(d) 1-10
Medium	>200 <500	>2,000 <10,000	2	(d) 1-10
Low	<200	<2,000	24	(d) 80-100
Total		< 20,000 to <200	10-100 (d)	

The majority of local areas contain low volume users 80-100 (d) %.

A worst-case 4-tert-OPnEO waste generation scenario for the Applicant’s FISH products distributed in GB was developed based on the assumption of all manual FISH Assays run at the largest GB user, a site that purchases approximately 1000 FISH Assay kits per year. The analysis indicated that for this worst-case waste scenario, Wash Buffer (WB I, II) waste containing 2.0 mL 4-tert-OPnEO per day (520 mL 4-tert-OPnEO per year) would be produced. The assumption behind this analysis is that there are 20 tests per kit which would result in 20,000 tests per year at this individual site. As these are used by professionals in clinics and hospital settings, it is

assumed that the tests can be run 260 days in the year which would result in approximately 80 tests per day. It was also assumed that the maximum number of FISH assays that can be run before the wash buffers are discarded and replaced with fresh wash buffer, is a set of 16 tests. The average size of the largest Coplin jar used to process the tests (slides) take 100 mL; therefore 100 mL of wash buffer I (0.3% 4-tert-OPnEO) and 100 mL of wash buffer II (0.1% 4-tert-OPnEO) per set are assumed; which would give a total amount 4-tert-OPnEO per 1 set of 16 tests of 0.4 mL (0.42g) 4-tert-OPnEO. This is a conservative estimate, as the package insert requirement states to discarded wash buffer at end of day. Based on the package insert requirement there would be approximately 5 sets of 16 tests that could be potentially completed per day before wash buffer is replaced.

9.1.1 Environmental contributing scenario 1

9.1.1.1 Conditions of use

Product (article) characteristics
▪ Physical form of substance: Liquid ▪ Substance in preparation: No
Amount used, frequency and duration of use (or from service life)
▪ Daily local widespread use amount: a tonnes/day OPnEO *Based on a wide dispersive use approach, assuming an estimation of tonnage applied to a default daily amount in a standard town (Standard wide dispersive use, R16 guidance, R16.2.2.1.2).*
▪ Annual usage: Widespread dispersive use a kg/year OPnEO Based on 2021 annual tonnage data (representative year until the end of the requested review period).
▪ Frequency and duration of use: Number of emission days per year: 260
Technical and organisational conditions and measures
▪ N/A
Conditions and measures related to sewage treatment plant
▪ Biological STP: Standard [Effectiveness Water: 56.99%] *Calculated by the SimpleTreat model embedded in Chesar* ▪ Discharge rate of STP: 2,000 m^3 /day (standard wide dispersive values, ECHA R.16 guidance) ▪ Application of the STP sludge on agricultural soil: Yes (standard wide dispersive values, R.16 guidance)
Conditions and measures related to treatment of waste (including article waste)
▪ Particular considerations on the waste treatment operations: No
Other conditions affecting environmental exposure
▪ Receiving surface water flow rate: 18,000 m^3 /day (standard wide dispersive values, R.16 guidance)
Additional good practice advice. Obligations according to Article 37(4) of REACH do not apply
▪ Outlined under conclusions and risk management measures

9.1.1.2 Releases

Table 9.6 Local releases to the environment

Release	Release factor estimation method	Explanation / Justification
Water	Measured Release Rates	**Initial release factor:** 10-100 ([b]) % **Final release factor:** 10-100 ([b])% **Local release rate:** Wide dispersive use 1.26 x 10^{-6} OPnEO kg/day (4.17 x 10^{-7} OP kg/day) Releases per the three example local areas are presented in Appendix II. **Explanation / Justification:** It is assumed, as a worst case, that all quantities of used kits solutions containing 4-tert-OPnEO are released directly to the facility's wastewater system and from there to the local sewage system. It is assumed that there are no environmental release conditions applied. The release rate has been determined based on the number of tests (1 to 16 tests per set) of which there could be 1 to 6 sets per day. Based on information collected from the Applicant's sales database, 1 to 16 tests per set can be run at any given time on any given day. Based on the worst-case amount of 4-tert-OPnEO (0.42)g used per 1 set, daily emission of approximately 0.42 g 4-tert-OPnEO per day was calculated for low-volume end users that run only 1 set/day. A daily amount for each of the customers was determined based on the number of sets per day that would need to be run (1 to 5) to accommodate their average daily number of tests run per day; this value was in turn multiplied by the average amount of 4-tert-OPnEO per 1 set (0.42) g. The 4-tert-OPnEO quantities are converted to 4-tert-OP quantities, as they are the ones for which the risk assessment is carried out. It is assumed that 4-tert-OPnEO will be completely degraded to 4-tert-OP in the STP. We used a conversion factor of 0.33 kg of 4-tert-OP per kg of 4-tert-OPnEO, based on an average degree of ethoxylation of 9.5.
Air	ERC Release Rates	**Initial release factor:** 0% **Final release factor:** 0 % **Local release rate:** 0 kg/day **Explanation / Justification:** The substance is not volatile and all operations take place at low temperatures. The reagent carrousel is cooled at a temperature of 7°C, with a range of 2-12°C. The vapour pressure of 4-tert-OPnEO at 20°C is practically zero ($0.24 \cdot 10^{-11}$ Pa). Furthermore, all operations involving 4-tert-OPnEO are in an enclosed instrument, so, until release to the drain, the substance is never in the open. Therefore, no releases to air are assumed.
Soil	ERC Release Rates	**Final release factor:** 0% **Explanation / Justification:** No direct releases to soil take place during use of IVD kits.

Releases to waste:

Water:

As a conservative approach, it is assumed that all quantities of 4-tert-OPnEO are discharged to the local municipal sewage systems. The release factor to the environment wastewater is assumed to be 10-100 ([redacted])% (it is assumed that there are no environmental release conditions applied), assuming that all quantities of FISH Assay wash buffer solutions distributed within GB are released to the environment either through the discarding of residual quantities and other unused material or through the instrument liquid waste stream which are discarded to waste water.

Wash solutions used in the manual or automated FISH assay must be discarded at the end of each day. Unused pre-formulated wash solutions supplied to customers by the Applicant must be discarded after 1 year, or sooner if solution appears cloudy or contaminated. If, however, the unused wash solutions were prepared on-site by the customer, then the unused amount must be discarded after 6 months, or sooner if solution appears cloudy or contaminated. As per the manual process the buffers used in the automated system are the same final concentrations of 0.3% and 0.1%.

All hazardous materials, including wash buffer components containing 4-tert-OPnEO, should be disposed of according to the institution's guidelines for hazardous disposal in accordance with local, state and federal regulations. The Coplin jars used to perform post-hybridisation wash incubations of the slide are cleaned after use by rinsing several times with water.

Potential releases from the use of the wash buffers from the IVD kits by professionals could result from:

1.spillage during installation and removal of system solution bottles from the instrument and/or from Coplin jars used in manual assays

2.discarding of residual quantities from spent containers and from expired and otherwise unused material

3.the instrument and/or manual assay liquid waste stream,

The Applicant does not consider spillage to be a significant source of emission. Operations manuals provide recommendations for waste handling, stating that each facility is responsible for labelling all waste containers and characterizing its waste stream to ensure waste is disposed of in accordance with the appropriate local, state, and national regulations. Products have hazard labels in accordance with CLP and GHS.

Release factor to waste from the process: 10-100 **([redacted])**%

Release factor to waste from onsite treatment: Not relevant

Table 9.7 Summary of 4-tert-OPnEO used and released expressed as 4-tert-OP (based on 2021 quantities)

Use 1	Total 4-tert-OPnEO kg/year used	Total 4-tert-OPnEO kg/year released	Total 4-tert-OP kg/year released
Total	1-5 ([redacted])	1.64	0.54

9.1.1.3 Exposure and risks for the environment and man via the environment

The following table provides Local (Clocal) emissions and Predicted Environmental Concentrations (PEC) for the three-example downstream user site locations and, for comparison, the wide dispersive use as evaluated for ES 1. Detailed information on the emissions for each individual location are reported in Appendix II.

Table 9.8 Clocal and PEC emission concentrations for emission categories within local areas & the wide dispersive use assessment

Assessment	WDU		1- [redacted]		2- [redacted]		3- [redacted]	
Emission category	NA		High		Med		Low	
Exposure assessment	Clocal	PEC	Clocal	PEC	Clocal	PEC	Clocal	PEC
Freshwater mg/L	8.83E-9	1.01E-8	4.81E-7	4.83E-7	7.86E-8	8.13E-8	7.1E-8	7.36E-8
Sediment (freshwater) mg/kg dw*	-	1.02E-5	-	4.84E-4	-	8.16E-5	-	7.39E-5
Marine water mg/L	8.83E-10	1.01E-9	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Sediment (marine water) mg/kg/dw*	-	1.01E-6	-	Not applicable	-	Not applicable	-	Not applicable
Sewage treatment plant mg/L	-	8.96E-8	-	5.01E-7	-	1.04E-6	-	1.38E-6
Air mg/m^3	5.56E-12	7.63E-11	7.91E-9	7.96E-9	1.67E-7	1.67E-7	6.41E-9	6.48E-9
Agricultural soil mg/kg dw	2.8E-6	2.8E-6	1.57E-5	1.57E-5	3.42E-5	3.42E-5	4.32E-5	4.32E-5

*For sediments, EUSES does not return a local concentration (without regional contribution) and thus no estimate is available (Guidance Chesar 2, Section 25.3)

Remarks on measured exposure:

As this is considered a widespread use, the Applicant examined a wide dispersive use calculation and verified this value against three example exposure scenarios. The widespread use assessment was carried out, as described in 9.1, and resulted in a predicted environmental concentration (PEC) of 1.01 E-8 OP mg/L for the freshwater compartment, and 1.01 E-9 OP mg/L for the marine compartment.

The Applicant then evaluated a subset of their downstream user profiles (high, medium, low emissions), receiving STPs with conservative environmental compartments. The range of these three examples resulted in a lowest predicted concentration of 7.36 E-8 OP mg/L for freshwater (local area #3), and a highest predicted concentration of 4.83 E-7 OP mg/L for freshwater (local area #1).

As can be observed by the information in Table 9.8, the resulting local concentrations and PECs for fresh and marine water are very low, in the range of ng/l or below.

The three local areas assessed illustrate examples of release rates expected for the downstream use where; resulting Clocal and PEC across GB would be expected to vary depending on the particulars of individual STPs and receiving bodies of water. The highest concentrations of emissions can be expected to be seen in areas that have higher usage such as large capital cities, with receiving water bodies that may not have high dilution rates (moderately flowing rivers). Higher concentrations could also be expected in medium usage local areas where waste is received by a small STP that discharges to rivers with low flow rates (low dilution). It should be noted that, in selecting these local areas and the parameters to use for the calculation of the environmental concentrations, conservative approaches were used. More specifically, a single STP was used for the very high-volume local area (local area #1), where it is more reasonable to assume that treatment of waste is spread over

several STPs. A single STP was selected as a worst-case assumption, as it leads to lower dilution and higher environmental concentrations.

Through both methods of calculations, local areas with specific individual data, or through wide dispersive use calculation, the predicated environmental concentrations for freshwater were in the magnitude of E-8 mg/L, with the high emission category (Local area #1), being one magnitude higher with E-7 mg/L. Local area #1 is considered a very-worst case, due to the conservative approach (a single STP selected, worst case assumption in terms of use). Furthermore, it represents a small percentage of customers 1-10% (d %). Most of the Applicant's downstream users will 80-100% (d %), are represented by the local area #3 assessment, measuring E-8 OP mg/L).

As a result of this assessment, it can be assumed that the predicated environmental concentrations reflected through the wide dispersive use assessment exposure scenario for ES1 are representative (conservative) of the Applicant's downstream user profiles and therefore was considered to be an appropriate value for use in the overall exposure assessment.

Conclusion on risk characterisation:

Overall, the hierarchy of control principles have been followed with the Applicant's implementation of their ongoing substitution plan as detailed in the Analysis of Alternatives and as noted in the conclusions on risk management. The hierarchy of control is a widely accepted system where control methods at the top of hierarchy are potentially more effective and protective than those at the bottom. Following this hierarchy normally leads to the implementation of inherently safer systems, where the risk of illness or injury or release has been substantially reduced. The Chemical Agents Directive (EU), Article 6, defines occupational 'hierarchy of control' in order of priority from the top down as: 1). Elimination or substitution, 2). Engineering controls, 3) Collective protection measures and 4). Individual protection measures. Though generally considered a safety and health concept, the same principles can be considered for environmental control strategies with substitution at the top of a hierarchy for environmental risk management (EU Commission, Directive 98/24/EC).

Substitution plan

Currently the most effective long term risk management measure is substitution, as this will completely eliminate 4-tert-OPnEO from the Applicant's FISH products and will eventually cover all DU customers distributed in GB. Due to the large number of affected FISH products and complexity of the regulatory applications required to enable commercial release of the corresponding updated OPnEO-free products, achieving substitution will require a multi-year new product rollout cycle once all stability verification studies are complete. As a result, it was not possible to substitute 4-tert-OPnEO in the Applicant's FISH Assays by the Sunset Date (EU/GB).

A detailed description for this process can be found in the Applicant's combined AoA/SEA. The review of progress on substitution, provided in Section 4.1.3 of the AoA/SEA, demonstrates that the Applicant's parent company has successfully tested the technical feasibility of the identified alternative to 4-tert-OPnEO.

Risk characterisation of releases

As described above, a condition of granting the EU REACH authorisation (ECHA reference number 11-2120816695-47-0000), the European Commission has required the EU Distributor to inform users of its FISH tests that they must collect all liquid and solid waste for adequate treatment. This minimises releases to environmental compartments as far as technically and practically possible, specifying that release to the municipal STP does not constitute adequate treatment. As a result, the Applicant has instructed their EU downstream users that are covered under their EU AfA ,that they shall collect all their liquid and solid waste for adequate treatment (e.g. incineration) and that release into the public sewer system is not considered to be adequate treatment. However, although this application has been granted by the EU Commission (EU AfA number: REACH/21/10/0), this occurred after the UK had left the European Union, and hence the EU authorisation itself does not have legal standing in the UK. The instructions for disposal of waste by FISH UK customers would be also communicated to GB customers if instructed as a condition of the Applicant's UK AfA application.

For the exposure assessment, it is assumed that no conditions are applied and represent a worst-case scenario of a 10-100 ([redacted])% release rate of waste to municipal STPs. If the Applicant instructs their GB downstream users to also collect all their liquid and solid waste for adequate treatment (e.g. incineration) and that release into the public sewer system is not considered to be adequate treatment, the release rate will be 0%, not 10-100 ([redacted])% as described in this Chemical Safety Report.

10. RISK CHARACTERISATION RELATED TO COMBINED EXPOSURE

10.1. Human health (related to combined, shift-long exposure)

10.1.1. Workers

No risk characterisation has been conducted for workers as it is not considered relevant for this substance.

10.1.2. Consumers

No risk characterisation has been conducted for consumers as it is not considered relevant for this substance as there is no consumer use.

10.2. Environment (combined for all emission sources)

10.2.1. All uses (regional scale)

10.2.1.1 Total releases

The total releases to the environment from the exposure scenario covered are presented in the table below. All quantities of 4-tert-OPnEO used in the GB by the Applicant's customers are discharged to liquid waste. From there, they are treated in municipal STPs and released to the relevant environmental compartments. Table 10.1 shows the quantities of 4-tert-OPnEO used by downstream users in GB, along with the releases from this use.

Table 10.1 Total releases to the environment per year from all life cycle stages.

Release route	Total releases per year of 4-tert-OPnEO
Water	1.64 kg/year
Air	0 kg/year
Soil	0 kg/year

Remarks:

Releases to wastewater were assumed to be directed to an STP in their entirety. It is assumed that 4-tert-OPnEO degrades to 4-tert-OP completely and it is discharged to the environmental compartments as predicted by the Chesar for the STP (see Table 9.4). Conversion of quantities of 4-tert-OPnEO to 4-tert-OP used a factor of 0.33, which applies for a 4-tert-OPnEO with an average of 9.5 ethoxylation units.

10.2.1.1. Regional exposure

No measured regional concentrations are available, as the Applicant is not the only user of 4-tert-OPnEO in GB. So, any measurements would not provide meaningful information for the contribution of the uses applied for to the total concentrations. The regional quantities were derived from a starting point of 10% of total emissions of 4-tert-OPnEO in 2021. So, the total regional releases of 4-tert-OPnEO are approximately 0.164 kg/year and the total regional emissions of 4-tert-OP were approximately 0.054 kg/year. Of these, 10% (0.0054 kg/year) are released directly to water, as a conservative assumption regarding STP coverage of the Applicant's customers. The remaining 0.0486 kg 4-tert-OP/year are treated in a STP and are released to water (43%), air (4.8%) and sludge (52.2%).

Environment

Table 10.2 Predicted regional exposure concentrations of 4-tert-OP (Regional PEC) and risks for the environment based on a wide dispersive use

Protection target	Regional PEC
Fresh water	**Regional PEC:** 1.94E-10 mg/L
Sediment (freshwater)	**Regional PEC:** 3.64E-7 mg/kg dw
Marine water	**Regional PEC:** 2.53E-11 mg/L
Sediment (marine water)	**Regional PEC:** 4.28E-8 mg/kg dw
Air	**Regional PEC:** 3.71E-11 mg/m³
Agricultural soil	**Regional PEC:** 1.95E-9 mg/kg dw

Remarks on measured regional concentrations:

The regional concentrations of 4-tert-OP were calculated using the Chesar 3.7.1 tool. The default physicochemical and environmental fate properties of the model were used. The only custom input were the releases of the substance to the different environmental compartments, assuming the default 10% regional use share. This factor is based on the widespread use of the IVD kits by the Applicant's customers in GB. A typical region for the purposes of regional risk assessment is assumed to have 20 million inhabitants and an area of 40,000 km2 , i.e. a 200 km square. The Applicant's customer site locations are scattered across GB and it is not likely that there will be such a region containing 10% of the total usage based on current use data. Nevertheless, the 10% regional tonnage share will be used as a conservative approach.

Remarks on risk characterisation for regional concentrations:

The regional environmental concentrations in water in Table 10.2 present a rather conservative regional concentration, as it largely assumes that 4-tert-OPnEO is being treated in standard STPs, which achieve a lower dilution than what has been observed during the environmental exposure assessment for individual example local areas in ES1. Furthermore, the Applicant's customers are hospitals, medical laboratories and clinics or research centres, therefore it was assumed that, as a conservative approach, 90% of the emitted quantities of 4-tert-OPnEO will be treated in an STP. From there, they will be distributed to the environmental compartments according to the release percentages for the model STP, which were calculated by the modelling tool, Chesar. The 10% that will not be treated by an STP is assumed that will be released directly to water.

Human via environment

The scope of this assessment focuses on the environmental exposure only and type of risk characterisation required for human health exposures is not evaluated in this application as the substance has been placed in Annex XIV to EU Regulation 1907/2006 (REACH), and transposed into REACH (The REACH etc. (Amendment etc.) (EU Exit) Regulations 2019; SI 2019 No. 758) on the basis of its endocrine disrupting properties in environmental species and thus its effects and impacts on the environment are only of relevance.

11. REFERENCES

1. BAuA, Federal Institute for Occupational Safety and Health (2011) Annex XV Report: Proposal for identification of a substance as a CMR Cat 1A or 1B, PBT, vPvB or a substance of an equivalent level of concern. Substance Name: 4-(1,1,3,3-tetramethylbutyl)phenol, 4-tert-Octylphenol Available online : https://www.echa.europa.eu/documents/10162/c26cbb7e-91f9-4454-a054-c2a731029219
2. BAuA, Federal Institute for Occupational Safety and Health (2012) Annex XV Report: Proposal for identification of a substance as a CMR Cat 1A or 1B, PBT, vPvB or a substance of an equivalent level of concern. Substance Name: 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated – covering well-defined substances and UVCB substances, polymers and homologues. Available online: Available online at: https://echa.europa.eu/documents/10162/397abe32-ecb8-451c-87d2-33af413687dd
3. BioGenex (2016) Fluorescent in-situ Hybridisation (FISH) Available online at: https://www.slideshare.net/vbiogenex/fluorescent-insitu-hybridization-fish
4. COMMISSION REGULATION (EC) No 790/2009 of 10 August 2009 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures. Official Journal of the European Union. 9002.9.5. L 235/1 Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:235:0001:0439:en:PDF
5. Directive 2000/60/EC establishing a framework for Community action in the field of water policy; supplemented by the Groundwater Directive (2006/118/EC) and the Environmental Quality Standards Directive (2008/105/EC). Implementing regulation available online at: https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF
6. Directive 98/24/EC - risks related to chemical agents at work of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC. Available online at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31998L0024
7. ECHA (2011) SVHC Support Document 4-(1,1,3,3-tetramethylbutyl)phenol, 4-tert-octylphenol. Available online at: https://echa.europa.eu/documents/10162/4c6cccfd-d366-4a00-87e5-65aa77181fb6
8. ECHA (2016) Guidance on information requirements and Chemical Safety Requirements. Chapter R.16 Environmental Exposure Assessment. Version 3.0. Available online at: https://echa.europa.eu/documents/10162/13632/information_requirements_r16_en.pdf
9. EPISUITE: Estimation software (version 4.1) (2011) Developed by Syracuse Research Corporation for the United States Environmental Protection Agency, Washington, DC
10. Eurostat website, 2019, Waste generation and management, incineration. Available online at: https://ec.europa.eu/eurostat/web/waste/waste-generation-and-management/management/incineration, Accessed January 2019
11. 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 (Text with EEA relevance). Available online at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1907-20180301
12. REACH (The REACH etc. (Amendment etc.) (EU Exit) Regulations 2019; SI 2019 No. 758)
13. Swedish Meteorological and Hydrological Institute (2019) Current river flow conditions with forecast based on open data. EU River Flows. Switch on. Available online at: http://riverinfo.eu/

14. Urban Waste Water Treatment Map, available online at: https://www.eea.europa.eu/themes/water/water-pollution/uwwtd/interactive-maps/urban-waste-water-treatment-maps; Accessed on 21 August 2018.

APPENDICES

Appendix I: Uncertainty Analysis

There are some uncertainties in the aforementioned analysis that may contribute to overestimation of the CLocal and PEC values; while every effort has been taken to avoid underestimation of the CLocal and PEC values, whenever possible. The above approach has taken a more worst-case overestimate approach based on some of the following points.

Appendix Table. 1 Uncertainty Analysis

Uncertainty	Impact for the CSR	Assumption
The exposure assessment is based off of a worst-case scenario, where in which there is a 10-100 (b)% release rate of waste to wastewater from the Applicant's downstream users. The Applicant has instructed its EU customers under the corresponding EU authorisation to collect their waste for adequate treatment. If instructed under this authorisation, the release rate would become 0%.	Predicted Environmental Concentrations and releases may not be accurate in this exposure assessment. It would present a significant overestimation of releases and Predicted Environmental Concentrations values.	It is assumed that 10-100 (b)% of releases at customer sites are discharged into wastewater. This could overestimate overall emissions and environmental concentrations.
There is uncertainty on the treated volume of wastewater and the flow rates in the rivers at some of the sites examined in the exposure assessment of the downstream user use.	Predicted Environmental Concentrations may not be accurate, which could lead to over- or underestimations.	The methodology for selection of the Sewage Treatment Plants and the values used in the analysis is described in under approach taken. Conservative assumptions were made where possible. The approach described in the R.16 Guidance document was followed when determining river flow rates. It is thus expected that uncertainty has been minimised.
Fate of 4-tert-OPnEO in the Sewage Treatment Plants. The substance is treated in a multitude of STPs across GB, with different characteristics and processing methods.	Releases of 4-tert-OPnEO and 4-tert-OP from the STPs would differ from location to location and it is not possible to determine a representative distribution of the substance to the environment. Environmental	A conservative approach was followed, by assuming that all 4-tert-OPnEO will degrade to 4-tert-OP in the STPs, without any removal.

	concentrations could vary. In addition, some Sewage Treatments Plants have sludge incineration in place, however this is dependent on the STPs. It is also possible that in some STPs, degradation of 4-tert-OP would also be observed, reducing the total emissions.	Distribution to the different compartments of outflow of the STP (water, air, sludge) was determined on the basis of the 4-tert-OP properties, instead of 4-tert-OPnEO. This approach has a greater share of the substance in sludge and agricultural soil. It was used as it was assumed that all emissions would be in 4-tert-OP.
There is uncertainty in how the FISH assay kits and solutions are used by DU customers, such as the number of slides they run within each batch of FISH assays; possible reuse of the solution for more than 16 tests per set; or when customers buy standalone wash buffer to make up their own solutions separate to the kit.	This could potentially lead to an overestimation in the Predicted Environmental Concentrations. But the impact is not considered to be significant as a worst-case use calculation where used that should address this.	A conservative assumption has been used to determine use per day and corresponding worst case emissions. The usage of 4-tert-OPnEO for the calculations has been made based on assumptions for the maximum number of FISH assays that can be run before the customer's wash buffers must be discarded and replaced with fresh wash buffers;.
There is uncertainty in whether low volume DU use batching in their scheduling of FISH assays and if so how if so to what extent batching is used.	This could potentially lead to an overestimation in the Predicted Environmental Concentrations.	The assessment of the data for low volume DU customers has taken a worst-case analysis approach to reflect the uncertainty due to this variability.

Appendix II Individual Local Areas and WDU Assessment Data

Appendix Table.2 Local areas assessment data

Local Area No.	P.eq □	STP discharge (m^3/day) *	Receiving water body	Receiving water body flow (m^3/day) †	Total Daily Sum		Total Annual Sum	
					OPnEO (kg/day)	OP (kg/day)	OPnEO (kg/yr.)	OP (kg/yr.)
High release local areas◊								
1 (d[redacted])	3,578,977	715,795	River	5,640,624	2.53E-03	8.34E-04	0.6568	0.2168
2 (d[redacted])	574,000	114,800	River	5,241,600	8.42105E-04	2.77895E-04	0.218947368	0.072252632
3 (d[redacted])	216,00	43,200	River	2,476,800	4.2105E-04	1.38947E-04	1.095E-01	3.612632E-02
Wide Dispersive Use	Default	Default	Default	Default	-	4.2 × 10-7	-	0.5419

◊ Daily Emission ranges. High emission range 0.0008 to 0.0003, Medium 0.0003 to 0.0001, Low emission range <0.0001 OPnEO kg per day.

□ Data was obtained from the EU Urban Waste Water Treatment Map reported by Member States in 2015 (containing data from GB treatment plants). Available online at: https://www.eea.europa.eu/themes/water/european-waters/water-use-and-environmental-pressures/uwwtd/interactive-maps/urban-waste-water-treatment-maps.

*Data was obtained from the EU Urban Waste Water Treatment Map reported by Member States in 2015 (containing data from GB treatment plants). Available online at: https://www.eea.europa.eu/themes/water/european-waters/water-use-and-environmental-pressures/uwwtd/interactive-maps/urban-waste-water-treatment-maps If information was not available, R16 guidance (Section A.16-3.3, Equation R.16-18) was applied.

† River flow figures were taken from the Swedish Meteorological and Hydrological Institute website (2019) for river flows. Available online at: http://riverinfo.eu/. R16 Guidance for average river flow calculations: A.16-3.3.3 Calculation of PEClocal for the aquatic compartment (freshwater). Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r16_en.pdf. All volumes are in m^3/day values are averages & divided by 3 as per r16 guidanc