ANALYSIS OF ALTERNATIVES

and

SOCIO-ECONOMIC ANALYSIS

Legal name of applicant(s):	Abbott Laboratories Limited
Submitted by:	Abbott Laboratories Limited
Substance:	4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated
Use title:	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.
Use number:	1

CONTENT

TABLES

FIGURES

LIST OF ABBREVIATIONS

4-tert-OPnEO	4-(1,1,3,3- tetramethylbutyl) phenol, ethoxylated
CLL	Chronic Lymphocytic Leukemia
CoRAP	Community rolling action plan
ED	Endocrine Disruptor
DNA	Deoxyribonucleic acid
FISH	Fluorescence In Situ Hybridisation
HLB	Hydrophile Lipophile Balance
IHC	Immunohistochemistry
ISH	In Situ Hybridisation
IVD	*In-Vitro* Diagnostic Device
IVDD	*In-Vitro* Diagnostic Device Directive
IVDR	*In-Vitro* Diagnostic Device Regulation
LDT	Laboratory Developed Test
NCCN	The National Comprehensive Cancer Network
NGS	Next Generation Sequencing
OP	Octyl phenol
PCR	Polymerase Chain Reaction
PMA	Pre-Market Approval
PRIO	Swedish Chemicals Agency database
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
ROW	Rest of World
RUO	Research Use Only
SIN	Substitute it Now
SOA	State of the Art
SSC	Citrate Buffered Sodium Chloride
SVHC	Substance of Very High Concern
UVCB	Unknown or Variable composition, Complex reaction products or Biological materials
ALK	Anaplastic lymphoma kinase
ALL	Acute Lymphocytic Leukaemia
ASCO	American Society of Clinical Oncology
CDx	Companion Diagnostics
CLL	Chronic Lymphocytic Leukaemia
CLP	Classification, Labelling and Packaging
CML	Chronic Myeloid Leukaemia
CMO	Contract Manufacturing Organisation
CRO	Contract Research Organisation
DAPI	4’,6-diamidino-2-phenylindole
FDA	Food and Drug Administration
HER2	Human Epidermal Growth Factor Receptor 2
IDH2	Isocitrate Dehydrogenase (NADP(+)) 2, Mitochondrial)
IHC	Immunohistochemistry
ISH	In Situ Hybridisation
MDS	Myelodysplastic syndrome
MLL	Mixed-Lineage Leukaemia

NGS	Next Generation Sequencing
NUS	Non-Use Scenario
PNEC	Predicted No Effect Concentration
SD	Sunset Date
STP	Sewage Treatment Plant

DECLARATION

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

Also, we request that the information blanked out in the "public version" of the Analysis of Alternatives and Socio-economic Analysis 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

1. SUMMARY

4-Tert-OPnEO is used in 1-10 b general reagents used to support FISH testing in 10-100 b FISH probe kits manufactured in the US by Abbott Molecular Division, covering approximately 400 assays, of which more than 100 b are classified as IVDs. FISH kits are used for diagnosing cancer, determining the type of cancer in a patient, the risk of recurrence, and for prescribing Companion Diagnostics (CDx) therapies. The products enter the EU market through a distribution centre in Wiesbaden and 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. The use is carried out by the Applicant's customers.

The 4-tert-OPnEO serves solely a detergent function (within the wash buffer), principally to wash unbound DNA and other unbound biological components originating from the specimen (including proteins). Removal of unbound components is required to eliminate critical non-specific signal to ensure the precision, accuracy and specificity of the test. 4-tert-OPnEO provides a very effective washing detergent function for use in FISH assays, due to a number of key properties.

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, Abbott Gmbh. A positive opinion was adopted on the application on 19 May 2020, and the European Commission made a positive decision on 16 November 2021, with a review period of 4 January 2028. Special transitional provisions (Article 127GA 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 application for 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.

As part of the process of applying for authorisation under EU REACH, the EU Distributor identified a potential alternative to 4-tert-OPnEO for use in its FISH tests, and proposed to adopt this alternative if its technical feasibility could be demonstrated. Since EU authorisation was granted under EU REACH, the EU Distributor has successfully completed the technical feasibility phase of its Substitution Plan. However, delays have been encountered as a result of the COVID pandemic. The remaining parts of the substitution plan include application for regulatory approval, an implementation phase – including scale-up to full manufacture of the new product and change control procedures mandated by regulations (e.g. amendment of documentation for all assays, including package inserts, kit labels and a large number of internal quality documents) – and a customer conversion phase to enable all of its f EU customers to make necessary requalification to approve the new products.

c

c [redacted]

If authorisation is granted, the EU Distributor will continue with its substitution plan, and the Applicant will introduce 4-tert-OPnEO-free versions of its FISH tests as soon as they become available. In the meantime, the Applicant will continue to supply 10,000-100,000 b [redacted] FISH tests to its 10-100 f [redacted] GB customers annually, with a value of £0.2-10 million b [redacted] Assuming customers discharge all waste 4-tert-OPnEO to the sewer (as was assumed as a worst-case scenario in the EU REACH authorisation application) would imply releases of 1.64 kg of OPnEO per year in GB, which translates into 0.54 kg of releases of OP per year. However, as part of the conditions for the EU REACH authorisation, EU customers are now being advised that they should not dispose of waste 4-tert-OPnEO to the sewer, but should use a method (e.g. incineration) which minimises releases. It is likely the Applicant will extend this condition to the supply of its FISH kits to GB customers also. This will effectively cut releases to zero.

If authorisation was not granted, the Applicant would stop supplying its FISH tests to GB customers as soon as the Secretary of States direction was received – assumed to be by the beginning of 2024. The EU Distributor would continue with its substitution plan, and the Applicant would propose to introduce 4-tert-OPnEO-free versions of its FISH tests to GB customers as soon as they become available. It is assumed that the Applicant's customers would on average lose one year's worth of testing, which would result in a loss of profits to the Applicant of around £0.05-2.7 million b [redacted], with additional profit losses to the Applicant's supply chain (the EU Distributor and the US parent company). Downstream users are assumed to switch to comparable tests supplied by competitors where available. However, there would still be impacts on patients due to the assumed absence of one year's worth of tests, as well as a potential loss of performance. The implied cost-effectiveness ratios (assuming releases of 1.64 kg) are 800-0.45m b [redacted] (OPnEO) and 2,778-1.5m b [redacted] (OP). If releases were reduced to zero through incineration (or similar) of waste 4-tert-OPnEO, these cost-effectiveness ratios would be effectively infinite.

The results of this analysis suggest that authorisation for continued use of 4-tert-OPnEO in the Applicant's FISH tests in GB is justified. This conclusion is robust to reasonable sensitivity analysis.

The review of progress on substitution demonstrates that the Applicant's parent company has successfully tested the technical feasibility of the identified alternative to 4-tert-OPnEO. However, delays have been encountered due to the COVID pandemic, c [redacted] The Applicant must also compile two sets of technical documentation to secure compliance with the UKCA product certification system. It is possible that the remaining stages of the substitution process can be completed within the existing review period granted to the EU authorisation. c [redacted] It would not be a valuable use of the Applicant's funds or the UK CA's resources to have to process a review report for this

application if it turned out the Applicant did, in fact, need an extra two years to complete its substitution. As a result, it makes sense to incorporate this (potential) additional time requirement into the current review period. As a result, the Applicant requests authorisation with a review period until January 2030.

2. AIMS AND SCOPE

2.1. Regulatory background for 4-tert-OPnEO

4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated, covering well-defined substances and UVCB substances, polymers and homologues (henceforth "4-tert-OPnEO" or "the substance") was included in Annex XIV of EU Regulation 1907/2006 (EU REACH), because it was identified as meeting the criteria of Article 57(f) of EU REACH, through its degradation to 4-(1,1,3,3-tetramethylbutyl)phenol, which is known to be an endocrine disruptor for the environment. 4-tert-OPnEO thus has probable serious effects on the environment, which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of article 57 of EU REACH.

4-tert-OPnEO was included in the 5th ECHA recommendation of substances for inclusion in the EU REACH Authorisation List, on 6 February 2014. The substance was included in that Authorisation List on 4 July 2017. The Latest Application Date (LAD) for 4-tert-OPnEO was 4 July 2019, 24 months after inclusion in the Authorisation List. The Sunset Date (SD), beyond which no use in the EU without an Authorisation is allowed, was on 4 January 2021, 18 months after the LAD.

Table 1 shows the EU REACH Annex XIV entry for the substance.

Table 1 EU REACH Annex XIV substance details

Entry No	Substance	Intrinsic properties	Latest Application Date	Sunset Date
42	4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated (covering well-defined substances and UVCB substances, polymers and homologues	Endocrine disrupting properties (Article 57(f) - environment)	4 July 2019	4 January 2021
Source: Annex XIV, Official Journal of the EU				

The existing EU Authorisation List has been retained under UK REACH, and the same LAD and SD apply for 4-tert-OPnEO, meaning that an authorisation is now required for the use of the substance in Great Britain (GB).

4-Tert-OPnEO is used by Abbott Molecular Division in 1-10 b general reagents used to support FISH testing in 10-100 b FISH probe kits, covering approximately 400 assays, of which more than 100 b are classified as IVDs. FISH kits are used for diagnosing cancer, determining the type of cancer in a patient, assessing the risk of recurrence, and for prescribing Companion Diagnostics (CDx) therapies. The products enter the EU market through the distribution centre in Wiesbaden and from there are imported into GB by 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. The use is carried out by the Applicant's customers.

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 made a positive decision on 16 November 2021, with a review period of 4 January 2028. Special transitional provisions (Article 127GA 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 application for authorisation for continued use under UK REACH within 18 months of the end of transition period, i.e. by 1 July 2022.

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, the Applicant, owned by subsidiaries of Abbott Laboratories Inc, and a UK legal entity.

2.2. The applicant and downstream users

Abbott is a US headquartered, global healthcare company that produces and supplies diagnostics products, medical devices, nutritionals and branded generic pharmaceuticals to over 150 countries. It employs approximately 103,000 employees, and in 2018 had a combined sales value of $30.6 billion.

Abbott Molecular Division is the division of Abbott that manufactures and sells FISH assay kits. It is a market leader in Europe for these products. Its manufacturing site is based in Des Plaines, Illinois in the US and the FISH products are sold around the world through the commercial hub in Wiesbaden, Germany.

Abbott Diagnostics GmbH is the main distribution centre for customers located within and outside the EU and provides products to distribution centres located outside the EU, for downstream use of 4-tert-OPnEO in FISH kits used by professionals. The Distributor produces and sells approximately 400 FISH assays in Europe, carried out using 10-100 ([redacted]) different FISH probe kits. The products are distributed to hundreds of customers in Europe (and dozens in GB) by the Distributor for its European customer base, professional end users of FISH assay test kits.

The applicant is Abbott Laboratories Limited, based in Maidenhead, England, which is the distributor for Abbott's FISH products in the UK.

The Applicant serves a number [redacted] of customers in GB. Sites of use for Abbott's FISH products include the following:

- Reference diagnostic laboratories that perform FISH testing when requested by medical professionals (hospital-associated and/or private physicians),
- Private diagnostic laboratories that perform FISH testing when requested by in-house and/or unaffiliated medical professionals (hospital-associated and/or private physicians),
- Hospital-based and academic institution-based diagnostic laboratories that perform FISH testing when requested by medical professionals (hospital-associated and/or unaffiliated private physicians); usually these laboratories are associated with medium to large hospitals.

2.3. Temporal and geographical boundaries

Use of the substance, and hence of Abbott's assays, by GB downstream users is currently authorised under the EU distributor's EU REACH authorisation application. Although this application has been granted by the EU Commission, this occurred after the UK had left the European Union, and hence the authorisation itself does not have legal standing in the UK. Under the transitional provisions of UK REACH, the LAD for the substance is extended until 1 July 2022. Assuming it takes 18 months to come to a decision, any use covered by an authorisation application made before this date will be permitted until around 1 January

2024 (after which it is assumed use will be able to continue if the authorisation is granted, or it must stop). If this authorisation application is submitted in June 2022, it is assumed that, if it were to be rejected, this rejection would be made around December 2023.

The SEA will examine the impacts of the decision on authorisation from now until the end of 2029, during which UK REACH transitional provisions will have ended and it is planned that 4-tert-OPnEO will be substituted in all FISH products.

Abbott has identified an alternative for 4-tert-OPnEO in the use within all the FISH assay kits, and a Substitution Plan for its implementation was included with the EU REACH authorisation application. Due to factors such as the COVID pandemic, some delays have been experienced in implementing that plan. The Substitution Plan included with the current application envisages complete substitution by the end of 2029. [redacted]

Abbott's FISH assay kits are produced outside Europe and are distributed to GB customers through the GB distributor (the Applicant), which in turn receives kits from the EU distribution centre in Wiesbaden, Germany. Only the GB use of the kits is of relevance to this AfA, but the decision will also affect non-GB stakeholders. The manufacturer of the kits, Abbott Molecular Division, is based in the US and all production of FISH kits takes place there. Any disruption in the GB supply of products caused by a refused authorisation will impact the US manufacturing plant(s).

In case of a refused authorisation, there will also be impacts to supply within GB, particularly to GB customers, i.e. medical laboratories, hospitals, academic centres and cancer care facilities, and to patients. Without the access to Abbott's kits, these health care facilities will be unable to test patient samples for certain cancer types. Patients within GB will be affected, as FISH testing is considered an essential tool by doctors in the correct diagnosing of cancer and particularly as Companion Diagnostics for personalised medicine. Finally, EU and GB employees of the Distributor, mostly in commercial and marketing operations will also be negatively affected if sales of FISH marketing in GB stop.

3. ANALYSIS OF ALTERNATIVES

3.1. SVHC use applied for

3.1.1. Description of the function of the Annex XIV substance and performance requirements of associated products

3.1.1.1. Fluorescent In Situ Hybridisation (FISH)

FISH stands for fluorescent in situ hybridisation. It is a test that looks for changes in genes in cells. Genes are made of DNA and control the behaviour of the cell, including when it grows and reproduces. Changes in genes can make a cancer cell produce particular proteins, stop making a particular protein, or make more of a particular protein than normal. This can make a cancer cell grow or reproduce more than normal. Some cancer treatments target specific proteins. FISH tests look for specific genes or parts of genes, and can help to identify whether a cancer has a particular change in its genetic make-up, which in term can help to inform whether a particular treatment is likely to work on that cancer.

FISH is a well-established technique developed in the 1980s and used to detect and locate the presence or absence of specific DNA sequences or chromosomes. It can be used to discover deletions or duplications that cannot be seen under a standard microscope and detect the amounts of a certain type of chromosome that may be present. FISH is used to look at one specific part of the chromosome – the fluorescent probe used only binds to the specific part of the chromosome that has a high degree of sequence similarity. Fluorescence microscopy is used to detect where the fluorescent probe has bound to the chromosome. When DNA is heated the two strands break away from one another and the probes hybridise to the complementary sequence in the DNA. If a deletion is present in the region which is complementary to the probe, the probe will not hybridise; if there is a deletion complementary to the probe, more of the probe will hybridise. The fluorescent probe must be constructed so that it is long enough to hybridise specifically to the target and not so long that general hybridisation will take place. The probe must be tagged with fluorophores with targets containing antibodies.

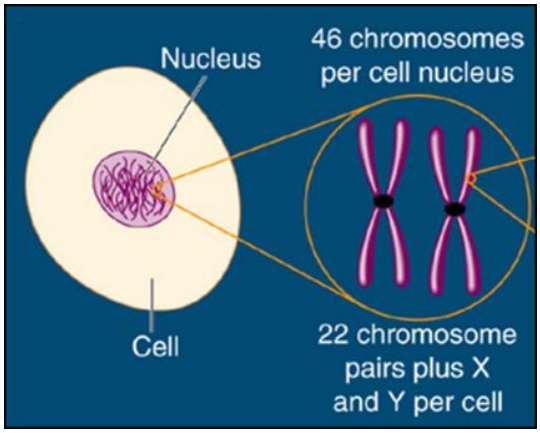


Figure 1: Normal Diploid Cell

FISH detection using fluorescence microscopy is fundamentally qualitative (i.e. results are yes or no i.e. is a signal present, or not); the observed fluorescent signal intensity is not evaluated quantitatively against a standard curve. Instead, results are reported as the

number of FISH signals of each colour present in each cell; 1-5 fluorophores, each with different specific colour, may be included in the probe formulation. However, quantitative aspects do apply in FISH signal enumeration, e.g., copy number assessment where the disease state is related to chromosomal copy deletion (resulting in nullisomy or monosomy) or amplification (e.g., trisomy) versus the normal diploid state (2 copies per cell). A normal diploid cell is given in Figure 1.

Abbott's FISH assay kits are used to diagnose and monitor genetic conditions (e.g. in oncology) related to gene or chromosome copy number variations, rearrangements or translocations. FISH results may be used to support treatment decisions, such as patient stratification into presumed responder/non-responder groups to determine suitability for prescription of approved therapies; or for enrolment in clinical trials, where no approved therapy currently exists.

3.1.1.2. Function of 4-tert-OPnEO in FISH assays

Abbott's FISH assays use 4-tert-OPnEO surfactant exclusively for the ancillary wash buffers consisting of citrate buffered sodium chloride (SSC) at a range of concentrations (0.4X to 2X SSC), with 0.1% or 0.3% 4-tert-OPnEO. Wash buffers are either supplied as pre-formulated kit components or as filled (neat liquid) and 20 x SSC (powder) components that are formulated into aqueous wash buffers by the clinical laboratory customer at point of use. Surfactants have a long history of use in molecular biology to improve the signal to noise ratio of in situ hybridisation (ISH) assays, such as the classical bacteriophage plaque nitrocellulose replica filter assay using P32-labelled DNA probes [9]. Post-hybridisation wash buffers containing 4-tert-OPnEO have been used in FISH technology since at least 1986 to support the target-specific detection of FISH probes hybridised to human chromosomes.[1] Analytical specificity is recognized as a key performance parameter for FISH assays used in clinical diagnostics.[2]

The 4-tert-OPnEO serves solely a detergent function (within the wash buffer), principally to wash unbound DNA and other unbound biological components originating from the specimen (including proteins). Removal of unbound components is required to eliminate critical non-specific signal to ensure the precision, accuracy and specificity of the test. 4-tert-OPnEO provides a very effective washing detergent function for use in FISH assays, due to a number of key properties:

- Surfactant type: The existing wash buffer contains 4-tert-OPnEO which is a non-ionic surfactant. This means there are no charged groups in the buffer which might generate undesirable interactions.
- Solubility: 4-tert-OPnEO is soluble in water. Water solubility is an important property of the surfactant. It aids the removal of unbound probe in the solution.
- Surface tension: Surfactants influence the interaction of diagnostic reagents with surfaces. A surface tension of 28-38 mN/m will prevent protein binding on slides

[1] Pinkel,D., Straume,T. and Gray,J.W. (1986) Cytogenetic analysis using quantitative, high-sensitivity fluorescence hybridization. *Proc. Natl Acad. Sci. USA* 832934-2938. Available online at: http://www.pnas.org/content/83/9/2934.short

[2] Wiktor A, Stupca P, Van Dyke DL, Dewald G (2006) Preclinical validation of fluorescence in situ hybridization assays for clinical practice. Genetics in Medicine 8(1):16-23

and coverslip surfaces, it will also aid the removal of the coverslip post hybridisation.

- Hydrophilic Lipophilic Balance: HLB is a numerical system used to describe the relationship between the water soluble and oil soluble parts of the hydrophilic and hydrophobic moieties of a surfactant molecule. If a surfactant has an HLB of 1 it is very oil soluble, whereas an HLB value of 15 is very water soluble. It is also a measure of the level of ethoxylation of the molecule. The 4-tert-OPnEO HLB value is >13.5 that produces clear emulsions making it ideal for detergency.

These properties enable 4-tert-OPnEO to perform five parallel functions in post hybridisation washing that are the same in all of Abbott's approximately 400 FISH assays. These functions and their relation to the substance properties are summarised in Table 2.

Table 2 Relationship between key substance properties, function and performance

Technical function	**Substance properties**	**Impact on product performance**	**Performance criteria**
Reduces nonspecific interactions between probe and specimen matrix	HLB Surface tension	Reduces non-specific signal generation from probe	Accuracy Precision Specificity
Reduces nonspecific interactions between probe and off-target DNA sequences	HLB Surface tension	Reduces non-specific signal generation from probe	Accuracy Precision Specificity
Prevents binding leading to aggregation on slide target or coverslip surfaces	HLB Surface tension	Eases coverslip removal, thereby reducing damage to the target specimen	Accuracy
Prevents self-aggregation and/or co-aggregation of probe with proteins background and/or specificity issues	HLB	Reduces generation of non-specific signal, thus minimising fluorescence	Precision Specificity
Promotes solubility	Surfactant type HLB	Increases removal of unbound material during washes, improving detection of specific hybridisation	Specificity

A further relevant property relates to the stability of wash buffer, as measured by the cloud point. The cloud point of a non-ionic surfactant is the temperature at which the mixture starts to phase separate with the surfactant forming its own structural phase. This behaviour is characteristic of non-ionic surfactants containing polyoxyethylene chains.

Table 3 Physical properties of 4-tert-OPnEO impacting assay performance

Property	**Value, description**
Surfactant Type	Non-ionic surfactant
Solubility in water	Soluble
Surface Tension	33 (dynes/cm at 1%)
HLB value	13.45(10-15 good wetting)
Pour point	1°C
Cloud Point	66 °C, 1 wt.% actives aqueous solution
Source: Supplier Safety Data Sheet, available at: www.sigmaaldrich.com/	

These properties and associated parameter values which explain the strong performance of 4-tert-OPnEO in FISH wash buffer are summarised in Table 3.

Any alternative(s) to the use of 4-tert-OPnEO in FISH wash buffer would be required to provide an equivalent set of functions to ensure the optimal wash effectiveness of the specimens and to ensure accurate interpretation of results of patient specimens across its approximately 400 molecular diagnostic products.

3.1.1.3. Description of the use of the wash buffer in the FISH assay

At a typical customer site, the end user, a professional laboratory technician, uses 4-tert-OPnEO as a component of post-hybridisation wash buffers, as part of the FISH diagnostic technique. A typical FISH assay kit is shown in Figure 2. The following gives a brief overview of how a typical FISH assay is performed by customers.

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% NP-40)

d. 1 x 66g SCC salt for wash buffer

Figure 2: Vysis CLL FISH probe kit

1. Sample Preparation

The specimen is loaded onto a microscope slide and the probe is applied. A cover slip is added to preserve the sample (Figure 3 and Figure 4)

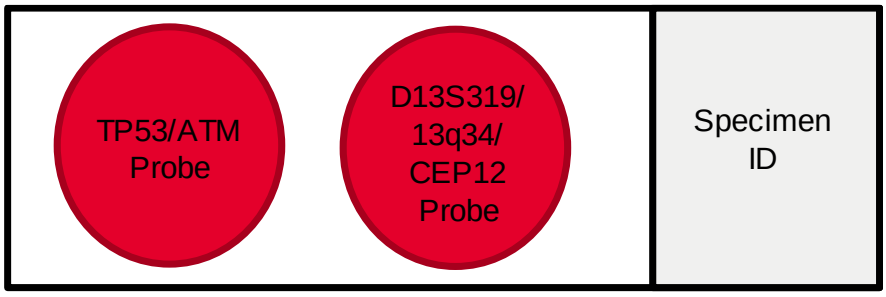


Figure 3 Fish assay slide preparation

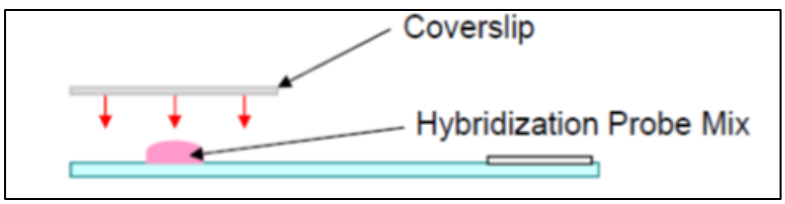


Figure 4 Probe application

2. Denaturation

The specimen and FISH probe DNA are then denatured at high temperatures in order to separate the two sets of complementary DNA strands.

3. 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. This is called hybridisation. The specimen is covered using a glass cover slip to seal the target to avoid evaporation of the probe solution and to protect the physical integrity of the sample (ensuring chromosomes and associated cell matrix structures remain intact and immobilised on the slide surface) (see Figure 5).

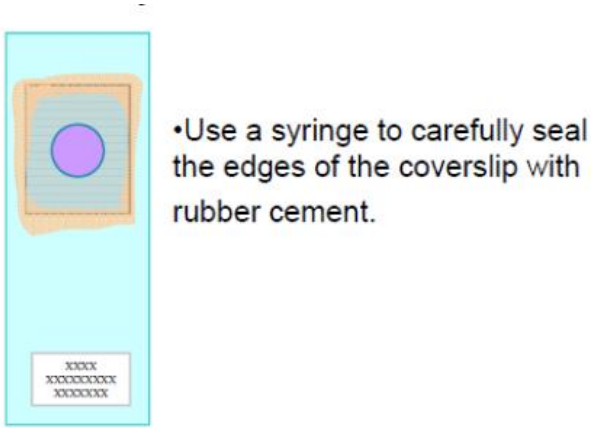


Figure 5: Seal hybridisation

4. Post-hybridisation wash

Following hybridisation, unbound probe is removed from the slide by a series of washes, using a surfactant containing 4-tert-OPnEO at different concentrations.

The two wash concentrations recommended are 0.3% and 0.1% 4-tert-OPnEO. 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 first wash in the next step. This reduces the overall amount of surfactant used.

The end user of the FISH assay kit has the option to use either the neat 4-tert-OPnEO supplied by the Distributor to make up wash buffer at point of use or to use the pre-formulated buffer (0.1% and 0.3% 4-tert-OPnEO) also supplied by the Distributor. The wash buffers are used in manual assays and/or with automation, as per Abbott's approved IVD/RUO labelling or the laboratory's own validated LDT procedures; as applicable.

5. Counter stain

4,6'-diamino-2-phenylindole DAPI, a DNA-specific die that fluoresces blue, is added to the nuclei to provide a clear backdrop ("counterstain") against which the orange and green DNA probes can be identified.

6. Visualisation and interpretation

Hybridisation of the probe with the cellular DNA target site(s) is visualised by direct detection using fluorescence microscopy (Figure 6). The adequacy of the final slide is first evaluated by the technologist, using the following criteria:

- *Specificity* The probe signal should only illuminate the target chromosome and regions thereof and not hybridise to other chromosomes or the nuclear and cellular membrane residue or otherwise interfere with accurate interpretation of the signal.
- *Background* Ideally, the background is essentially black and uniform across the entire target. The background level should not interfere with target interpretation. No or minimal visible fluorescent particles or haziness should be present on the target.
- *Intensity* Bright, distinct (well resolved) signals of the probe-specific colour should be seen at the intended chromosomal target region(s) so that the user can easily evaluate the interphase cells within the target.
- *Cross-hybridisation* Ideally, the probe hybridises only to the targeted region(s) of the specific chromosome(s). On the metaphase chromosome spreads present within the target, there must not be a level of cross-hybridisation visible to the user that would interfere with target interpretation.

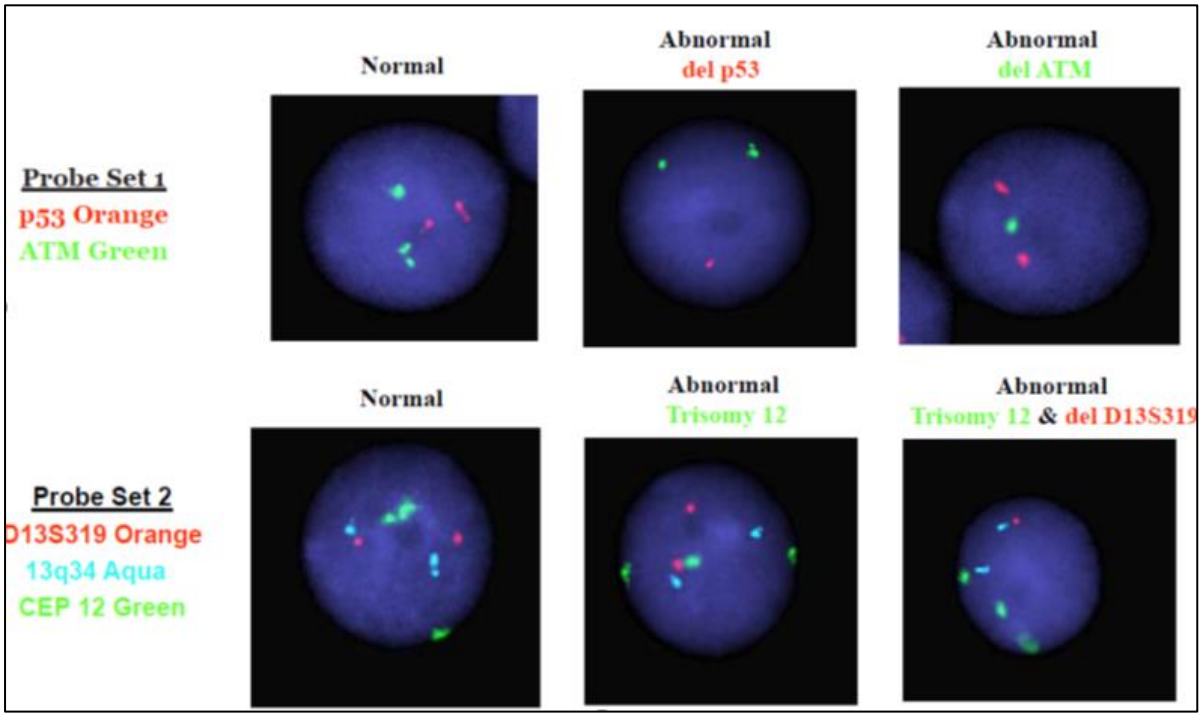


Figure 6: FISH probe hybridisation to patient samples under fluorescent microscope

Figure 6 presents the optimal appearance of cells under a fluorescent microscope, following hybridisation and post hybridisation washing using 4-tert-OPnEO. The figure compares a normal chromosome to those showing abnormalities i.e. the absence of a signal corresponding to a specific deletion.

3.1.2. Market analysis of products manufactured with 4-tert-OPnEO

3.1.2.1. FISH kits

The Applicant is supplying GB customers with FISH assay kits, which contain all the necessary components to carry out the required analyses. These kits are modular products, which contain several separate components. Some of these components are specific to a particular assay, while others can be used in more than one.

The main components of a FISH assay are:

- A set of fluorescent DNA probes, which bind on the desired section of the chromosome and can be detected by microscope. The number and position of the probes in the treated specimen can indicate if abnormalities are present in the patient's chromosomes and whether this is an indication of cancer or not.
- Wash buffer contains 4-tert-OPnEO. The solution is used to repeatedly wash the slides with the hybridised DNA specimen to remove the unbound probe before examination.
- 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 buffer, which is used for washing the unbound DNA and other unbound biological components that originated from the specimen (including proteins). In this way, non-specific signals are removed from the specimen and the test's precision, accuracy and specificity are increased.

The wash buffer performs the same post-hybridisation functions in all of the Applicant's FISH assays. Without the buffer, the visual inspection of the hybridised specimen with fluorescence microscopy would have a high amount of noise and would not produce reliable results.

As an example, Abbott's Vysis Chronic Lymphocytic Leukaemia (CLL) FISH Probe Kit is a test to detect the most common form of adult leukaemia in the developed world. The National Comprehensive Cancer Network (NCCN) Practice Guidelines™ for Non-Hodgkin's Lymphoma, which are the consensus recommendations of leading US oncology experts, states that FISH (including abnormalities tested for with this kit) is informative for both prognosis and therapy determination.[3] The guidelines recommend use of FISH at the time of diagnosis as well as re-evaluation by FISH at the time of relapse to direct treatment options (including abnormalities tested for by this kit). Furthermore, the leading European professional organisation for medical oncology, European Society for Medical Oncology (ESMO), indicates that genomic aberrations such as del(11q), del(17p) and mutations of TP53 help in defining clinical subgroups and are sufficiently predictive to be used for treatment decisions.[4]

Abbott's Vysis CLL FISH Probe Kit is a test to detect deletion of the LSI TP53 probe target (17p-) via FISH in peripheral blood specimens from patients with chronic lymphocytic leukaemia (CLL). The test is indicated for detecting deletion of the LSI TP53 probe target (17p-) as an aid in identifying those patients with CLL for whom treatment with VENCLEXTA® is indicated. Vysis CLL FISH Probe Kit received FDA 510(k) clearance in 2011 for prognosis of potential CLL patients at diagnosis.[5] Pre-Market Approval (PMA) for use of

[3] NCCN Guidelines (2016) Non-Hodgin's Lymphoma's. National Comprehensive Cancer network. Available online at: http://www.cancervisit.com/wp-content/uploads/2016/04/nhl.pdf

[4] Ghielmini M, et al (2013) ESMO Guidelines consensus conference on malignant lymphoma 2011 part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). Department of Medical Oncology, Oncology Institute of Southern Switzerland, Ospedale San Giovanni, Bellinzona, Switzerland. *PubMed*. Available online at: https://www.ncbi.nlm.nih.gov/pubmed/23175624

[5] FDA (2016) Vysis CLL FISH Probe Kit . Ref: 04N02-021. 30-608712/R1 Available online at: https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150041c.pdf

the Vysis CLL FISH Kit as a companion diagnostic for AbbVie's drug VENCLEXTA® was received April 11, 2016.[6]

3.1.2.2. FISH instruments

FISH assays tend to involve a lot of manual handling of the slides with the samples. Abbott provides instruments that automate and standardise this process, reducing manual intervention and potential for errors. These instruments may be supplied to customers using FISH 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.

Figure 7: VP 2000 processor system

Final filled (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 Vysis Thermobrite, the VP 2000® Processor and/or analyser (e.g., BioView® image analysis suite) in clinical laboratory settings.

Figure 8: VP 2000 basins for post hybridisation washing

The Thermobrite system is an open system that automates the denaturation and hybridisation steps in slide-based FISH procedures. This is a desk-mounted instrument

[6] Abbvie(2018) Venclexta (US), Venclyxto (EU), (ABT-199) is a selective small molecule Bcl-2 inhibitor being investigated for the treatment of multiple cancers. AbbVie Inc.North Chicago, Illinois, U.S.A. Available online: https://www.abbvie.com/our-science/pipeline/venclexta.html

that manually processes the slides to achieve the denaturation and hybridisation technique. Wash buffer is not used with this instrument.

The VP2000 Processor, in conjunction with the Vysis Thermobrite system for denaturation or hybridisation provides a modular systems approach to automated FISH testing. It is an integrated instrument that allows the user to perform specimen pre-treatment, staining, slide washing in a single instrument that is validated for use with the Applicant's Vysis FISH protocols. Figure 7 and Figure 8 provide an overview of the instrument system.

The VP2000 Processor easily processes slides using pre-programmed protocols for 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 (Figure 8). 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.

3.1.2.3. Applications of FISH assays

FISH is used for several diagnostic applications, and it is also widely used in research. Abbott offers FISH products for two main types of analysis, genetics and oncology. In genetics, FISH assays are used to pinpoint the location of specific DNA sequences in a chromosome. FISH was among the techniques used in the mapping of the human genome and it is still used in mapping genomes of other organisms.

The main use of FISH in recent years has been in clinical diagnosis, particularly for cancer. If there are mutations on the target chromosome, the probe will detect them. If a deletion has occurred, the chromosome will not hybridise, while if there has been an amplification, it will hybridise in more than one location. FISH assays for oncology can detect DNA abnormalities in the patient's specimen in locations that have been associated with a specific type of cancer.

The scope of FISH applications has broadened in recent years, with the discovery of numerous disease-related genes, such as HER2 amplification for breast cancer, ALK rearrangement for non-small cell lung cancer and BCR/ABL1 translocation for myeloid leukaemia. FISH is now considered an important component in diagnosis of genetic diseases, haematological malignancies and solid tumours, and in personalised medicine.

Results from FISH testing can be used to support treatment decisions on patients with different types of cancer, during every step of diagnosis:

- Identifying predisposition and risk of developing cancer, e.g. due to genetics or family history;
- Diagnosing the type and subtype of cancer of the patient;
- Describing severity of the cancer and predicting the chances of survival;
- Choosing the appropriate therapy or treatment for the patient, based on the type and subtype of cancer;
- Monitoring the patient after therapy for the possibility of recurrence of cancer;

- Re-evaluating the type and subtype of cancer in case of reappearance.

Correct and timely identification of cancer and of the responder/non-responder groups can be crucial for determining suitability for prescription of approved therapies, or for enrolment in clinical trials (where no approved therapy exists). This can increase the patient's chances of therapy, successful treatment and survival.

FISH detection with fluorescence microscopy is a qualitative technique, giving only a yes/no result. It is used to detect the presence or absence of a signal, but not, for example, the intensity of the observed signal against a standard curve. The researcher counts the number of FISH signals of each colour present in each cell.

FISH can be used for the diagnosis of different types of cancer. The products offered by the Distributor fall under the following categories, all of which use 4-tert-OPnEO:

- Haematological cancer: leukaemia (Acute Lymphocytic Leukaemia - ALL, Mixed-Lineage Leukaemia - MLL, Chronic Myeloid Leukaemia - CML, Chronic Lymphocytic Leukaemia - CLL), multiple myeloma, myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma and sex-mismatched bone marrow transplantation.
- Solid tumour: bladder cancer, breast cancer, gliomas, lung cancer, melanoma, prostate cancer, sarcomas, etc.

Abbott also offers FISH kits and probes for identification and characterisation of chromosome anomalies in pre-implantation, prenatal and postnatal genetics testing and research. The kits and probes can be used to detect such abnormalities that can cause genetic diseases and advise medical professionals on clinical decisions, thus improving the quality of life of the patient.

Some of the FISH assays offered by Abbott are unique in their function and utility and are the only available such test to assist the doctors in diagnosing some types of cancer. Abbott is offering the only FDA approved urine-based molecular test for bladder cancer. This test is intended for use in conjunction with current standard diagnostic procedures to aid in the diagnosis of bladder carcinoma in patients with haematuria and subsequent monitoring for tumour recurrence in patients previously diagnosed with bladder cancer. The test has been CE marked and is available in the EU to aid in the diagnosis of bladder cancer and subsequent monitoring of tumour recurrence. The test is also available in the UK.

The FDA approved FISH in 2001 for use in conjunction with cystoscopy to monitor for recurrence among those with previously diagnosed bladder cancer. Since then, several studies have confirmed the usefulness of including FISH analysis when monitoring for recurrence. Low-grade bladder cancers rarely demonstrate changes that can be detected by FISH analysis. Thus, a clinical benefit of FISH is its ability to identify the more aggressive bladder cancers earlier. Identifying the tumour type that may eventually become life-limiting allows the patient and health care providers to initiate a treatment plan that includes scheduled surveillance and pro-active or appropriate treatment.

3.1.2.4. FISH and companion diagnostics for personalised medicine

Cancer patients do not necessarily respond in the same way to any specific treatment. Their individual responses can often depend on various characteristics in each patient's genes. So identification of an appropriate treatment for each patient is a delicate exercise.

As cancer is generally caused by changes (mutations) in a person's genes (oncogenes), there is an increasing need for testing gene variants by doctors examining patients for cancer. For example, in certain types of cancer, e.g. breast cancer, there are certain types of drugs that are known to work only for women with particular genetic variations.

Personalised medicine is a relatively novel approach to healthcare, moving away from the traditional 'one size fits all' approach. It has the potential to tailor therapy with the best response and highest safety margin to ensure better patient care. Medical decisions are tailored to the individual patient, based on their susceptibility to disease or response to a particular treatment. A key component of personalised medicine includes testing of a patient's genetic information to help identify targeted treatment options.

CDx are laboratory tests, developed in parallel with particular drugs to help doctors decide which treatments to offer to patients. The companion diagnostic is thus essential to the safe and effective use of the drug. The tests are used to select which patients should be treated with that particular drug. Without the CDx, it is not possible to prescribe the particular drug. All of the Distributor's CDx assays have results linking to drug outcome.

Multiple companion diagnostic tests can be developed for a drug, but there are still some drugs for which only a single CDx exists. In Europe, the use of CDx to measure predictive biomarkers is recognised as a well-established method to select the right treatment for patients.

As of November 2021, the FDA in the US had approved 50 CDx tests by several companies, including five produced by Abbott. Three of the tests shown in Table 4 are (self-declared) CE marked and are marketed in the EU and UK. (Approval for the use of Tibsovo and Idhifa has not be sought in the EU and UK.)

Table 4 FDA-approved companion diagnostics marketed by the distributor

Test	**Treatment**	**Target disease**	**Comments**
Abbott RealTi*m*e IDH1	Tibsovo (ivosidenib)	Acute myeloid leukaemia	Not FISH – No CDx from a different company available
Abbott RealTi*m*e IDH2	Idhifa (enasidenib)	Acute myeloid leukaemia	
Vysis CLL FISH Probe kit	Venclexta (venetoclax)	B-cell chronic lymphocytic leukaemia	No CDx from a different company available
PathVysion HER-2 DNA Probe kit	Herceptin (trastuzumab)	Breast cancer	Several CDx available
Vysis ALK Break Apart FISH Probe kit	Xalkori (crizotinib)	Non-small cell lung cancer	The only approved FISH method – Several other CDx available
Source: FDA (2021), List of cleared or approved Companion Diagnostic Devices (*In Vitro* and Image tools). Available online (accessed on 14 March 2022) at: https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm,			

As can be seen in Table 4, for B-cell chronic lymphocytic leukaemia, Abbott is the only manufacturer offering CDx for predictive markers. For non-small cell lung cancer, Abbott is the only manufacturer offering FISH CDx.

3.1.2.5. FISH testing and companion diagnostics in the NHS

A brief literature search was undertaken to establish the status of FISH testing and companion diagnostics in the NHS in relation specifically to the different types of cancer targeted by products supplied by Abbott, specifically:

- Haematological cancer: leukaemia (Acute Lymphocytic Leukaemia - ALL, Mixed-Lineage Leukaemia - MLL, Chronic Myeloid Leukaemia - CML, Chronic Lymphocytic Leukaemia - CLL), multiple myeloma, non-Hodgkin's lymphoma.
- Solid tumour: bladder cancer, breast cancer, lung cancer, melanoma, prostate cancer.

The primary source of information was the website of the National Institute for Health and Care Excellence (www.nice.org), the organisation which approves and issues guidance on the treatment of disease and other conditions within the NHS. Other websites were consulted where relevant and useful.

Bladder cancer

NICE Guideline NG2 covers the assessment and management of bladder cancer.[7] The guidance makes reference to the cost-effectiveness of FISH testing in a number of areas. For instance, when considering the most effective endoscopic techniques for diagnosing bladder cancer, the evidence review found that a strategy which included FISH in both initial diagnosis and follow-up was most cost-effective compared with other strategies. In respect of follow-up strategies for high risk patients, the most cost-effective strategies included FISH or cytology to ensure patient health status could be monitored effectively over time.

The guideline makes its second key priority for implementation the testing of people with suspected bladder cancer via the transurethral resection of the tumour coupled with one of four diagnostic tests – Abbott's Urovysion FISH test for bladder cancer is mentioned specifically in this recommendation.

Breast cancer

FISH testing is done on breast cancer tissue removed during biopsy to see if the cells have extra copies of the HER2 gene. Cancers with more copies of the HER2 gene tend to grow and spread faster than other breast cancers but are much more likely to respond to treatment with drugs that target the HER2 protein.

According to breastcancer.org, the FISH test is not as widely available as another method of HER2 testing, immunohistochemistry (IHC), but is considered more accurate.[8]

The drug trastuzumab is recommended by NICE as an option for some people with tumours expressing HER2.[9] As noted in Table 4, Abbott's PathVysion HER-2 DNA probe kit is one of the CDx kits available for the diagnosis of HER2.

[7] NG2 Bladder cancer: diagnosis and management - full guideline (nice.org.uk)

[8] FISH (Fluorescence In Situ Hybridization) Test (breastcancer.org)

[9] 1 Guidance | Guidance on the use of trastuzumab for the treatment of advanced breast cancer | Guidance | NICE

Haematological cancer (leukaemia)

The addendum to the updated NICE guidance on the diagnosis and treatment of haematological cancer states:

"Whereas in the past the diagnosis of these conditions relied almost completely on microscopic morphological appearance, the developments in molecular medicine have led to a revolution in diagnostic techniques, many of which are now regarded as essential to the correct categorisation of the condition. These necessary techniques include:

- conventional histopathology and cytopathology
- flow cytometry and immunohistochemistry
- cytogenetics and Fluorescent In Situ Hybridisation (FISH)
- molecular genetics."[10]

Thus, the NICE guidance refers explicitly to the importance of the use of FISH testing in diagnosis, but no specific reference to Abbott's products was found in this review.

Lung cancer

NICE Guidance NG122 recommends treatments which are associated with FISH CDx provided by the applicant.[11] First-line systemic treatment for stage IIIB and IV non-squamous non-small cell lung cancer in people with the anaplastic lymphoma kinase (ALK)-positive gene rearrangement is governed by NICE technology appraisal guidance 406, on crizotinib, ceritinib and alectinib.[12] As per Table 4, Abbott's Vysis ALK Break Apart FISH probe kit is the only FISH test associated with crizotinib, although other companion diagnostics are available. The Evidence Review Group's report on crizotinib for untreated ALK-positive non-small cell lung cancer, produced by the University of York for technology appraisal guidance 406, states that the Vysis ALK Break-Apart FISH probe kit is "considered the gold standard for identifying the ALK fusion gene".[13]

Myeloma

The diagnosis and management of myeloma is governed by NICE guidance 35.[14] This does recommend the use of FISH testing, but not specifically Abbott's tests. For instance, it recommends FISH testing "to identify those adverse risk abnormalities which had been shown in multivariate analyses to be independent prognostic marker of high-risk disease." FISH testing is described as having been "validated in a large number of clinical trials and scientific studies as being the most practical and broadly applicable technique to identify acquired genetic abnormalities in myeloma."

[10] https://www.nice.org.uk/guidance/ng47/evidence/

[11] Lung cancer: diagnosis and management (nice.org.uk)

[12] https://www.nice.org.uk/guidance/ta406

[13] committee-papers-2 (nice.org.uk)

[14] NICE Guideline Template

Prostate cancer

No mention of FISH testing could be found in the various NICE guidance documents on the diagnosis and management of prostate cancer, although Cancer Research UK does refer to prostate cancer as one of the cancers which FISH testing is used to diagnose.[15]

Melanoma

NICE Guideline NG14 covers the assessment and management of melanoma.[16] Although NICE acknowledged that there was evidence that FISH testing could be useful in the diagnosis of atypical spitzoid lesions, it concluded that the evidence was not strong enough to justify a recommendation for its use. Instead, the guidance made a research recommendation on the topic. However, Cancer Research UK does mention melanoma as a target of testing via FISH (footnote 16).

Non-Hodgkin's lymphoma

NICE Guideline NG52 covers the assessment and management of non-Hodgkin's lymphoma.[17] It states that, based on their clinical experience, the Guidance Committee recommended that FISH should be "an integral part of patient information". The Committee considered that:

"Advanced molecular diagnostics will have a major impact on the diagnosis and stratification of all patients with lymphoma. Although the technologies are the same across lymphoma subtypes the data supporting its routine clinical application is greatest in high grade B-cell lymphomas."

The Committee considered that FISH testing should take the place of (some) IHC tests, and hence its recommendation would be largely cost-neutral.

Summary

This brief review confirms the important role of FISH testing and CDx in diagnosing and treating the cancers identified previously as targets for the Applicant's products, and demonstrates their use in the NHS, sometimes with recommendations of specific products. This is further reinforced by the description of the Applicant's customer base presented in the following sections.

3.1.2.6. Downstream users and customers in healthcare

The Applicant's customers are healthcare professionals that need to carry out patient specimen analyses daily. The customers all belong to the mainstream healthcare industry, which care for and treat cancer patients. In general, they fall into the following groups:

- Laboratory personnel (medical technologists) and laboratory management who will perform the FISH testing requested by medical professionals;

[15] https://www.cancerresearchuk.org/about-cancer/cancer-in-general/tests/fish

[16] NG14 Melanoma: assessment and management - full guideline (nice.org.uk)

[17] NG52 Full guideline (nice.org.uk)

- Qualified healthcare professionals (including pathologists, such as when it is required to pre-certify that a specimen is cancerous tissue prior to FISH testing) who will undertake the interpretation of FISH results for each specimen;
- Clinicians (physicians, medical doctors) who will request the tests from their local or regional laboratory, provide the required specimens to the laboratory, and use the results of the FISH tests performed (in conjunction with other available clinical information) to make patient management and associated therapy decisions for their patients, in consultation with the qualified healthcare professionals providing the interpreted FISH results.

The clinical interpretation of any FISH test results should be evaluated within the context of the patient's medical history and other diagnostic laboratory test results. FISH CE-marked IVD assays sold by the EU distributor are intended to be used in combination with additional biomarkers, patient demographics, history, tissue morphology, and other clinical information. Non-IVD FISH assays (and/or components thereof) sold by the Applicant must be validated for their intended use by the end user laboratory prior to use of the Lab Developed test (LDT) assay in clinical diagnostic testing for the purpose of making patient management and associated therapy decisions.

The Applicant serves dozens of customers in GB. Sites where the Applicant's FISH products are used include the following:

- Reference diagnostic laboratories that perform FISH testing when requested by medical professionals (hospital-associated and/or private doctors),
- Private diagnostic laboratories that perform FISH testing when requested by in-house and/or unaffiliated medical professionals (hospital-associated and/or private doctors),
- Hospital-based and academic institution-based diagnostic laboratories that perform FISH testing when requested by medical professionals (hospital-associated and/or unaffiliated private doctors); usually these laboratories are associated with medium to large hospitals.

Table 5 lists the Applicant's GB customers in 2021, by type of customer and the number of kits supplied. (Names withheld for confidentiality reasons.) As can be seen, some customers took only a small number of kits last year, and might not use kits every year, which explains why the list of customers can vary year-by-year. It also shows the large number of NHS hospitals which use a significant number of the Applicant's kits, although that list is dominated by four hospitals (and one in particular, which is well known for its diagnostic capabilities). There is a small list of commercial laboratories, but this too is dominated by two customers, the biggest of which describes itself as the largest independent provider of highly specialised pathology/clinical laboratory services in the UK.

The total number of kits sold to GB customers in 2021 was 1-10,000 (b). Each of the kits can be used for several tests, although some patients might be tested more than once. Nevertheless, the figures suggest that the Applicant's kits are used in the treatment of many thousands (b) of patients each year.

Table 5 Customers by Type and Kit Quantity, 2021

Customer	Customer Type	Kits
f		b
f		b
f		b
	b	b

3.1.2.7. Patients

Genetics FISH assays are used in identifying and monitoring genetic abnormalities in embryos and new-borns, helping to identify genetic diseases, thus allowing for timely and appropriate treatment to improve quality of life.

The Applicant's oncology FISH assays are not used independently, but rather they are requested to support data from other tests and examinations on a patient. Doctors normally ask for additional FISH information before they make any treatment decision on

a patient as the FISH assays can help identify the type and sub-type of cancer the patient has. This is particularly the case for Companion Diagnostics which are associated exclusively with a specific drug. This type of information plays a significant role in the selection of personalised medicine that increases the chances of successful therapy or effective treatment. FISH assays are also used for monitoring the progress of tumours of patients and can also be used to identify possible reappearance of cancer that was cured in the past. Abbott's FISH assays can be used for different types of cancer, both haematology malignancies (leukaemia, etc.) and solid tumours (e.g. lung, breast).

Table 6 shows information on cancer patients in the UK for selected types of cancer, which are targeted by the Applicant's FISH assays.

Incidence refers to the number of new cases (per 100,000) of a type of cancer arising in a year. Mortality is the number of deaths (per 100,000) from that type of cancer in the same year. Finally, five-year survival is the proportion of people diagnosed with a particular cancer who are still alive five years later. Higher percentages indicate cancers which people survive for longer, lower numbers indicate cancers which people survive for relatively short periods after diagnosis.

Table 6 Incidence, mortality and 5-year prevalence of cancer in the UK in 2018

Cancer type	Incidence		Mortality		5-year survival	
	Men	Women	Men	Women	Men	Women
Bladder (C67)	22.93	8.43	11.34	5.26	56	44
Breast (C50)	1.15	166.01	0.29	34.41		87
Leukaemia (C91-C95)	18.22	11.87	8.43	5.82	53	53
Lung (C33-C34)	77.60	69.53	57.04	47.85	8	12
Melanoma Skin Cancer (C43)	25.73	24.99	4.33	2.82	88	92
Myeloma (C90)	10.58	7.49	5.25	4.11	53	52
Non-Hodgkin Lymphoma (C82-C86)	24.08	18.92	8.60	6.51	68	69
Prostate (C61)	160.38	0.00	36.49		85	-
All Cancers Combined	678	550	321	225	49	59
Source: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type						

New cancer cases (incidence) can be diagnosed more accurately and faster with the help of the Applicant's FISH assays. In the UK, new cases of cancer in 2020 were estimated to number 0.46 million. FISH products are also important for all those living with cancer, both during therapy and once they are in remission, to monitor their progress. The population of those living with cancer five years after the first diagnosis in the UK was estimated at approximately 1.5 million in 2020.

More than 90% of women diagnosed with breast cancer at the earliest stage survive their disease for at least five years compared to around 15% for women diagnosed with the most advanced stage of disease.[18] More than 80% of lung cancer patients will survive for at least a year if diagnosed at the earliest stage compared to around 15% for people diagnosed with the most advanced stage of disease. Missed or delayed diagnoses often

[18] Cancer Research UK (2019) Why is early diagnosis important? Available online at: https://www.cancerresearchuk.org/about-cancer/cancer-symptoms/why-is-early-diagnosis-important

result in higher downstream costs for treating a disease that has advanced more progressively.

The financial implications of misdiagnosis can be substantial. Treatment options have become more effective, especially with the emergence of personalised medicine. The cost for treatment increases as diseases progress to more advanced stages. For example, treatment of stage-4 colon cancer can cost three times more than the cost to treat stage 1 of the disease. Therefore, missed or delayed diagnosis can result in significant financial costs.[19]

The World Health Organization (WHO) recently prioritised patient safety areas in primary care and included diagnostic errors as a high-priority problem. Cancer was among the conditions that were most commonly misdiagnosed in the US, as reported by a number of unconnected studies.[20] WHO's report recommended improved access to diagnostic tests as a potential intervention to reduce diagnostic errors, especially for cancer patients.

In 2021, the Applicant supplied oncology FISH assay kits in GB that could be used in 10,000-100,000 ([redacted]) tests. These were used to test patients in all stages of cancer, i.e. for diagnosis, during therapy and during monitoring.

3.1.2.8. Applicant's sales value and market share for FISH assays

In 2017, Abbott's global sales value for oncology FISH products was approximately £10-100 million [redacted]. Approximately 0-25% [redacted], i.e. £1-20 million [redacted] of those sales are in the EU region. FISH genetics products sales are approximately 50% of the oncology sales, so the estimated EU sales in 2017 were £1-20 million [redacted]. In 2021, FISH sales in GB were equal to £0.2-10 million [redacted]. Table 7 shows the global and EU sales for Abbott's genetics and oncology product lines in 2017, and GB sales in 2021.

Table 7 Sales of Abbott's FISH products in 2017 and 2021 (£m)

Product family	Global sales, 2017	EU Sales, 2017	GB Sales, 2021
Genetics	10-100 [redacted]	1-20 [redacted]	0.1-5 [redacted]
Oncology	10-100 [redacted]	1-20 [redacted]	0.1-5 [redacted]
Total	20-200 [redacted]	2-40 [redacted]	0.2-10 [redacted]

The EU REACH authorisation application presented evidence on the EU Distributor's market position and sales forecasts. It reported the findings of a market research study carried out in 2015, according to which the global market for In Situ Hybridisation (including FISH and other techniques) was estimated at $554.4 million in 2014. These figures included all hybridisation techniques, both DNA or RNA and fluorescent (FISH) or chromogenic (CISH). DNA FISH accounted for the largest segment of the market, approximately 55.7% in 2014. The global market value of FISH was thus calculated at approximately $309 million in 2014. The total ISH market was expected to reach $681 million in 2019; the European

[19] Khullar, D., Jha, A. K., & Jena, A. B. (2015). Reducing Diagnostic Errors--Why Now? *The New England Journal of Medicine*, 373(26), 2491-3. Available online at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4943217/

[20] Singh H, Schiff GD, Graber ML, et al The global burden of diagnostic errors in primary care. *BMJ Qual Saf* 2017;26:484-494. Available online at: https://qualitysafety.bmj.com/content/26/6/484

market was expected to grow by 4.3% during that period. Unfortunately, updates on these figures are not available.

Abbott's own assessment was that demand for its oncology products might increase by 1-10% b by 2019. This was expected to be driven in part by the increase in demand for testing associated with personalised medicine in oncology (Companion Diagnostics). However, as a more conservative approach, and considering the irregularity in the trend of the EU Distributor's sales in years prior to the application (as evidenced by the consumed quantities of 4-tert-OPnEO shown in Table 8), it was decided in the EU REACH SEA to assume that sales revenue in future would be stable at the 2017 value. The EU REACH CSR assumed that use of 4-tert-OPnEO would be equal to the (higher) average of use over the 2016-2018 period.

The EU REACH application noted that the market report named Abbott among the five major companies operating in the global FISH market at the time, along with F. Hoffmann-La Roche Ltd (Switzerland), Affymetrix, Inc. (U.S.), ThermoFisher Scientific, Inc. (U.S.), and Agilent Technologies, Inc. (U.S.). According to internal validations, the EU Distributor was the largest supplier of FISH assay kits in Europe, holding a market share of approximately 20-50% b of the oncology market in the EU. The market share of individual products ranged from 5% to 50%, depending on the product. Individual country market shares ranged from 25-50%.

Unfortunately, no further update of this information is available for the current application, although the Applicant and the EU Distributor do not believe there has been any significant shift in the market for FISH products in the EU (and GB) since the EU REACH application was compiled. c [redacted] As with the EU REACH application, it will be assumed that sales and profits going forward will remain constant, but at 2021 levels. This assumption has also been made in the CSR. Any deviations from this assumption do not change the overall conclusions of the benefit-risks comparison, since benefits and risks are both linearly related to sales values and volumes.

Table 8 Annual use of 4-tert-OPnEO in fish assay kits in the EU and GB

Year	EU use (kgs)	GB use (kgs)
2015	a	a
2016	a	a
2017	a	a
2018	a	a
2019	a	a
2020	a	a
2021		a
2022 -2030 per year		a

3.1.3. Annual volume of the SVHC used

The EU Distributor imports finished FISH assay kits from the US and distributes them from its distribution warehouse in Wiesbaden, Germany. Typical FISH assay kits contain the wash buffer in 2000ul vials within the kit assembly. The annual volume of 4-tert-OPnEO contained in the wash buffer exported for EU and GB customer use is given in Table 8.

This places annual use in the tonnage band 0-0.1 tonnes per year (0-100kg/yr).

3.2. Efforts made to identify alternatives

As described in Section 2.1, 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. That application described the efforts the EU Distributor's parent company had undertaken to find and implement an alternative for the use of 4-tert-OPnEO in the general reagents supporting FISH testing in its FISH probe kits, covering approximately 400 assays. It reported that an alternative had been identified, and a period of time was required to test this alternative fully and, if its performance was successful, to implement it (following regulatory approval) in its FISH test products. A positive opinion was adopted on the application on 19 May 2020, and a positive decision was made by the European Commission on 16 November 2021 to authorise continued use until 4 January 2028.

The special transitional provisions specified in Article 127GA of UK REACH) specify that an application for authorisation of continued use under UK REACH must be submitted within 18 months of the end of transition period, i.e. by 1 July 2022. This authorisation application seeks time for the use of Abbott's FISH test products containing 4-tert-OPnEO to continue in GB while the substitution described in the original authorisation application is completed. As a result, this application will repeat without alteration the information provided in the original EU application relating to the identification of the preferred alternative. It will then provide an update on Abbott's substitution activities, including an updated timetable for substitution.

3.2.1. Research and development

R&D activity has been based on a combination of laboratory studies and literature review and focused on the options that were considered to be the most promising for successful substitution. Abbott consulted R&D teams within its own organisation as the company has been progressing substitution of 4-tert-OPnEO from a range of IVD products. The primary source of information on potential alternatives was from the supplier of 4-tert-OPnEO surfactants. The supplier published a concise guide on alternatives to 4-tert-OPnEO that provided specific advice on potential alternatives in different use applications. Three approaches to substitution were considered – reduction/elimination of 4-tert-OPnEO, alternative techniques to FISH, and alternative surfactants to 4-tert-OPnEO. The activities and their activities under each heading are summarised below.

3.2.1.1. 4-tert-OPnEO reduction and elimination

Abbott completed a series of laboratory tests on representative FISH assays to assess the impacts of the eliminating and reducing the concentrations of 4-tert-OPnEO in the wash buffer on the assay performance. These tests are described fully in the EU application for

authorisation. The conclusion was that elimination and reduction of the surfactant in the wash buffer was not feasible given the difficulties encountered with coverslip removal. Ease of coverslip removal ensures the target's unique cellular morphology remains unperturbed – if the specimen is compromised, the results cannot be accepted. This option was therefore not considered further in the previous application.

3.2.1.2. Alternatives to the FISH technique

There are alternatives to the FISH technique in molecular diagnostics. Abbott does not supply alternatives to FISH to the market but did complete a comparative performance assessment of some techniques under the 'State of the Art' review required under the IVD regulatory framework. This review is required to demonstrate that the intended benefits of the IVD and safety are achieved. Abbott completed a thorough literature review of public and academic information to assess the continuing relevance of FISH in molecular diagnostics. The review focused on the comparative results obtained using FISH and the other commercially available IHC, NGS methods for a specific gene detection. These demonstrated that some alternative techniques have the capacity to act as a technically feasible alternative for at least some tests covered by the company's FISH assays, but that no single technique could act as a 'like for like' replacement.

Abbott concluded that, according to the best current judgement, FISH remains an essential technique within the medical diagnostic repertoire. Moreover, informed medical decision making often relies on results obtained from orthogonal techniques to identify and confirm the best approach to patient treatments, and therefore that all methods are considered relevant and complementary. The development of alternative techniques to FISH was therefore not pursued in this research and the EU application for authorisation.

3.2.1.3. Alternative surfactants

Abbott carried out a literature review and consulted supplier information on potential alternative surfactants to octyl phenol ethoxylates. An internal consultation was also conducted to seek information on the experience of the use of different surfactant types within the wider organisation. Experience within the organisation, gained from the use of other surfactant types, made a significant contribution to the identification process. The research focused on commercially available surfactants in order to maximise success of substitution.

This process resulted in a list of potential alternative surfactants which was used as a starting point for the identification of a potential alternative for use in FISH assay wash buffer. The subsequent screening and technical feasibility assessment focused on the capacity of the alternatives to reduce the risk to the environment, and their physicochemical properties compared with those of 4-tert-OPnEO presented in Table 3.

Screening 20 potential alternative substances using substance physicochemical properties and Abbott's previous experience resulted in the identification of three surfactants which could potentially act as an alternative for 4-tert-OPnEO in FISH post hybridisation wash buffer. These three surfactant types were not considered to have hazardous properties that would impact their selection for future use in line with Abbott's policies on substitution. Since the basic principles for all the company's FISH assays are similar, and the functions performed by the post-hybridisation wash buffers containing 4-tert OPnEO are also substantially the same, across all FISH kits (IVD, RUO and their LDT equivalents), it was

concluded that one surfactant should act as an alternative in all Abbott's approximately 400 FISH assays. As such, initial feasibility testing was carried out comparing the three-short listed potential substitutes across seven representative assay types. This design was chosen to maximise the efficiency and increase the probability of finding an alternative in product by product testing.

Initial feasibility assessments of potential alternatives were completed using seven FISH assays selected to represent the most common post-hybridisation conditions that use 4-tert-OPnEO in the wash buffer. For each assay, control slides were prepared at standard conditions in parallel, using the three short listed surfactants, and a comparative assessment of the hybridised slides was conducted in accordance with the evaluation criteria for each product-specific quality procedure.

The evaluation procedure comprised individual ratings of assay performance by two reviewers. Since FISH assay interpretation is not quantitative, the performance criteria used in the study focused on the quality of the signal generated compared with that of the 4-tert-OPnEO against the following criteria:

- Specificity
- Background
- Intensity
- Cross-hybridisation

The results of the initial feasibility studies demonstrated that all three alternative surfactants provide acceptable results as a substitute for 4-tert-OPnEO in FISH wash buffer. As a result, Abbott chose the alternative with the best hazard profile (Polysorbate 20) for the remaining verification studies. Polysorbate 20 was also used in assessment studies for the universal pre-treatment buffer in-development, and therefore it was considered prudent and advantageous to maintain consistency across the assay components. In what follows, Polysorbate 20 is the alternative which is described in more detail and which is the subject of the substitution activities described in the original EU REACH authorisation application (updated here).

3.2.2. Consultations with customers and suppliers of alternatives

Subject matter experts in various departments of the company were consulted during the data mining phase of the alternative selection process. The 4-tert-OPnEO supplier was also consulted about alternative techniques and surfactants.

3.2.3. Data searches

For the EU REACH authorisation application, Abbott carried out data searches using online resources and internal consultations for the alternative selection and screening process under the following headings:

Identification of alternative surfactants

For the identification of possible alternatives, Abbott performed an online search for "Octylphenol ethoxylates-alternatives" and used the information available from a major producer of 4-tert-OPnEO. The producer offers specific guidance on the alternatives to the octylphenol ethoxylates across a large number of different applications. The guidance document Alternatives to Alkyl Phenol Ethoxylate (APE, APEO) Surfactants, available from

http://msdssearch.dow.com/, was used as a starting point for the generation of the initial list of potential alternatives to 4-tert-OPnEO.

Assessment of alternative techniques

Abbott completed a literature review of public databases and research papers for the purpose of a 'State of the Art' (SOA) review, intended to assess 'a current' SOA status for FISH CE marked products, literature searches were carried out to identify published research papers referencing FISH or variants thereof. Searches were performed using key phrases:

- Clinical utility of FISH
- FISH comparison studies
- FISH diagnosis

Screening of alternative surfactants

For the specific information on screening alternatives using physical-chemical properties Abbott used the following resources:

- Suppliers' product information (e.g. technical datasheets)
- Suppliers' SDS library: www.sigmaaldrich.com/
- ECHA dissemination pages www.echa.europa.eu/information-on-chemicals

Hazard assessment of alternatives

For the screening and hazard assessment of the shortlisted alternatives, the following resources were consulted:

- ECHA website www.echa.europa.eu
- SIN list by ChemSec https://chemsec.org/sin-list/
- Swedish Chemicals Agency PRIO database https://www.kemi.se/en/prio-start/search-in-the-database
- Swedish Chemicals Agency Restricted Substances Database https://webapps.kemi.se/begransningsdatabasen/Sok.aspx
- Yordas Hive https://www.yordasgroup.com/hive/

3.2.4. Identification of alternatives

As described in Section 3.2.1, the EU Distributor's parent company considered substance reduction/elimination, alternative techniques and alternative substances for the use applied for in its EU REACH authorisation application. Only alternative substances were considered feasible substitutes in the context of this use and the company's business.

3.2.5. Shortlist of alternatives

As described in Section 3.2.1.3, the results of the initial feasibility studies demonstrated that three alternative surfactants provide acceptable results as a substitute for 4-tert-OPnEO in FISH wash buffer. As a result, Abbott chose the alternative with the best hazard profile (Polysorbate 20) for the remaining verification studies. Polysorbate 20 was also used in assessment studies for the universal pre-treatment buffer in-development, and therefore it was considered prudent and advantageous to maintain consistency across the assay components. Polysorbate 20 is the alternative which is the subject of the substitution activities described in the original EU REACH authorisation application (updated here). This alternative is described in more detail in the next section.

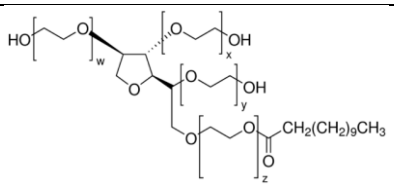
3.3. Assessment of shortlisted alternatives

3.3.1. Alternative 1: Polysorbate 20

3.3.1.1. General description of Alternative 1

Alternative No. 1 is a polysorbate surfactant with a fatty acid ester moiety and a long polyoxyethylene chain. Such surfactants are generally considered gentle as they do not affect protein activity and are effective in solubilisation. Polysorbate 20 is routinely used as an emulsifier in wash-off products, and in washing agents in immunoblotting and ELISA in order to minimise nonspecific binding of antibodies and to remove unbound moieties. Table 9 provides the identification and properties of Alternative No. 1.

Table 9: Substance identification

Substance Name(s)	**IUPAC Name**	**CAS Number**
Polysorbate 20 PEG (20) sorbitan monolaurate	polyoxyethylene sorbitan monolaurate	9005-64-5
Molecular Formula	**Structural Formula**	**Surfactant type**
$C_{58}H_{114}O_{26}$		Non-ionic
Classification and Labelling	Not classiified	
Source(s) Supplier SDS[13],ECHA[16]		

3.3.1.2. Availability of Alternative 1

Alternative No. 1 is a commercially available, general-purpose surfactant. It is already in use by Abbott in a number of other products and applications. Future use of the alternative is not likely to be subject to any licensing or access rights based on commercial availability.

The substance has been registered in the EU at 100-1000 tonnes/yr and the EU Distributor has completed a member registration for the importation of this substance within the EU. Additional volumes resulting from the replacement of 4-tert-OPnEO are not expected to move the volume to a higher tonnage band.

However, since any change to the FISH wash buffer requires approval from IVD regulatory bodies within the EU and globally, Alternative No.1 could not be considered available for substitution at the time of the preparation of the original EU REACH authorisation application. Authorisation was requested and granted for a period up to January 2028, which was the date by which it was expected that all of the Distributor's EU customers would have fully transitioned to products based on the alternative surfactant. This has not changed, and hence the alternative will not be available by the time the Secretary of State would be expected to have made a final decision on this application (approximately 36 months after the end of the transition period, i.e. by 1 January 2024). Alternative No. 1 cannot therefore be considered available for substitution by the end of the current transitionary arrangements.

3.3.1.3. Safety considerations related to using Alternative 1

The key advantages of moving to Alternative No. 1 with respect to reduction in risk are described in this section and summarised briefly below. Alternative No.1 is:

- Not an SVHC according to REACH regulation (Regulation (EC) No 1907/2006) or UK Statutory Instrument 2020 No. 1577
- Not listed in the ECHA CoRAP list of substances
- Not listed in the ECHA PACT list
- Not listed in the Chemsec SIN list
- Not listed in the Swedish Chemicals Agency PRIO database
- Not listed in the Swedish Chemicals Agency Restricted Substances Database

The conclusion is that Alternative No. 1 does not meet the criteria for being identified as a SVHC under REACH, and moving from 4-tert-OPnEO to Alternative No. 1 would result in a reduction in risk to human health and the environment.

Classification according to Regulation (EC) No. 1272/2008

There is no harmonised classification for polysorbate 20 in Annex VI to CLP. According to the ECHA data dissemination database a registration dossier for CAS Number 9005-64-5 has been submitted in the 100-1000 tonnes per year tonnage band. The lead and member registrants have registered the substance as not classified.

Table 10: Comparison of hazard classification Of 4-tert-OPnEO, OP and Polysorbate 20

	4-tert-OPnEO	**octyl phenol (OP)**	**Polysorbate 20**
EC No / CAS No	9036-19-5/9002-93-1	205-426-2/140-66-9	500-018-3/9005-64-5
Endocrine disruption	ED compound for environment By degradation to OP*	ED compound for environment	Not classified
Physicochemical	None	None	Not classified
Human health	Skin Irritant 2 (H315) Eye Damage 1 (H318) Acute oral toxicity 4 (H302)	Skin Irritant 2 (H315) Eye Damage 1 (H318)	Not classified
Environmental	Aquatic acute 1 (H400) Aquatic chronic 1 (H410) M factor = 10	Aquatic acute 1 (H400) Aquatic chronic 1 (H410) M factor = 10	Not classified
Source(s)	Supplier's SDS[13]	Harmonised classification (Index No: 604-075-00-6) [15]	ECHA Dissemination database[16]
Notes: *Classification of 4-tert-OPnEO is based on the classification of the degradation product, namely 4-tert- octylphenol (OP).			

Toxicology

4-tert-OPnEO is listed on Annex XIV because of its degradation to octyl phenol (OP), which has endocrine disrupting properties for the environment. Table 10 compares the hazard classifications of 4-tert-OPnEO, its degradation product OP and Alternative No. 1.

While 4-tert-OPnEO is not listed on Annex XIV for human health properties, human health hazards are relevant for the use of the substance by the Applicant's customers. Comparison of all hazards is discussed in addition to the environmental classification to show that the proposed alternative does not pose any additional risk. OP is only relevant

for its environmental hazards, as it is expected to be present only in the environment during the waste phase.

Endocrine disruption 4-tert-OPnEO was added to the Authorisation list because it degrades to OP, which has been shown to be an endocrine disruptor for environmental species. Polysorbate 20 is not an endocrine disruptor and its degradation product substances do not have endocrine disrupting properties.

Physicochemical hazards Polysorbate 20 is not classified for physicochemical hazards. The same applies to 4-tert-OPnEO and OP.

Human health hazards The registration dossier indicates the substance is not classified for human health hazards

Environmental fate-degradability The registration dossier indicates the substance is readily biodegradable.

Bioaccumulation The registration dossier indicates the substance is not bioaccumulative.

Environmental hazards The registration dossier indicates the substance is not classified for environmental hazards.

Conclusions on reduction of overall risk due to transition to the alternative

Owing to the lack of classification for the substance, all hazards, human health, environmental and physicochemical are lower than that of 4-tert-OPnEO.

As a conclusion, after comparing the hazard profiles of 4-tert-OPnEO and OP with that of Alternative No. 1, the overall reduction in risk to human health and the environment after transition to the alternative will be conclusive, with the risk from endocrine disruption being eliminated completely.

3.3.1.4. Technical feasibility of Alternative 1

Preliminary technical feasibility studies concluded that the FISH wash buffer containing Polysorbate 20 performs comparably with the current wash buffer in the post hybridisation washing procedure in seven representative assays (see Section 3.2.1.3). In accordance with the regulatory requirements and the EU Distributor's substitution and phase-out plan set out in the existing EU Reach authorisation application, real-time stability studies on seven worst case assays commenced in June 2016 and continued through to the end of 2020. The results of these tests have confirmed that the alternative is technically feasible and can successfully substitute for 4-tert-OPnEO in the company's FISH assays.

Technical feasibility can only be finally confirmed once kits using the alternative have received regulatory approval, and customers have successfully completed any revalidation they might need of their own processes with the new products. As a result, it is not possible to declare Alternative No. 1 fully technically feasible at this point.

3.3.1.5. Economic feasibility of Alternative 1

In evaluating the economic feasibility of moving from the existing substance to the most likely alternative, the following cost categories were evaluated by the EU Distributor for the original EU REACH authorisation application:

- R&D costs: Costs to identify, verify and implement the alternative;
- Regulatory costs: Costs to prepare the necessary documentation to receive marketing authorisation for the products containing the alternative;
- Raw material costs: Cost of the new alternative and of any other additional raw materials that may be required after reformulation of the reagents.

The R&D costs included identifying the alternative, pursuing small scale technical feasibility trials, design verification activities and the implementation costs. As discussed previously, extensive testing has been required in order to verify the alternative meets the acceptability specifications for all approximately 400 assays. Feasibility studies have indicated that a like-for-like replacement is suitable and hence the concentration of the alternative in the wash buffer will not increase or decrease.

The cost of completing this R&D activity was estimated in the EU REACH application at €100,000-€200,000 e [redacted] over the course of the requested seven-year review period. Regarding the cost of regulatory submissions, a review of the regulatory requirements for each country in which the EU Distributor places the products on the market was completed. A fee is required for each country in which a submission would be required. Implementation costs are associated with the development of revised protocols, labels and inserts for newly formulation product. Together regulatory and implementation were estimated at being €0.5million-€1.5million e [redacted]. Headcount costs for R&D activities, regulatory submissions and implementation activities were estimated at €0.5-€1.5million e [redacted].

The raw material cost of Alternative No. 1 is higher than that of 4-tert-OPnEO, at €173 per litre compared with €86 per litre. However, the volumes used are so small that this will have a minimal impact on the cost of the FISH products to customers.

Overall investments and resources needed to develop and implement the substitution of 4-tert-OPnEO have been estimated at €1.1-3.2 million e [redacted]. A GB-specific share of this might be calculated as €0.05-0.6 million e [redacted], based on the GB share of kits sold in the EU b [redacted].

In conclusion, the substitution of 4-tert OPnEO with Alternative No. 1 is not economically feasible, as defined by the ECHA Committee for Socio-Economic Analysis.[21] However, the EU Distributor decided that the substitution was affordable over the course of the seven-year review period requested, and granted by the European Commission, and hence proposed to adopt the alternative.

3.3.1.6. Suitability of Alternative 1 for the applicant and in general

As part of the preparation of the original EU REACH authorisation application, the EU Distributor identified an alternative surfactant, Alternative No. 1 for the substitution of 4-tert-OPnEO from its approximately 400 FISH assays. The alternative was not suitable at the time, due to cost, the need for technical development, and because of the time taken for introduction (including regulatory approval). However, the EU Distributor proposed to adopt the alternative if technical performance could be demonstrated and time was allowed for implementation. (Cost was considered affordable.) Feasibility studies have continued, and have demonstrated that Alternative No. 1 provides comparable performance to 4-tert-OPnEO in the FISH post hybridisation wash buffer across Abbott's large range of molecular diagnostic assays. However, regulatory approvals are still required and the overall substitution process has been delayed due to the COVID pandemic c [redacted]. A further eight years is considered necessary before the alternative can be fully implemented. (See Section 4.1.3). This is after the date at which the Secretary of Stage might be expected to make a decision on an application for

[21]https://echa.europa.eu/documents/10162/17091/seac_authorisations_economic_feasibility_evaluation_en.pdf

authorisation submitted by 1 July 2022. Hence, the alternative currently remains unsuitable as defined under REACH.

4. SOCIO-ECONOMIC ANALYSIS

4.1. Continued use scenario

4.1.1. Summary of substitution activities

If authorisation is granted, the EU Distributor will continue to pursue substitution of 4-tert-OPnEO in its FISH assays with the alternative identified in Section 3. Since authorisation was granted under EU REACH, the EU Distributor has successfully completed the technical feasibility phase of its Substitution Plan. However, delays have been encountered as a result of the COVID pandemic. The remaining parts of the substitution plan include application for regulatory approval, an implementation phase – including scale-up to full manufacture of the new product and change control procedures mandated by regulations (e.g. amendment of documentation for all assays, including package inserts, kit labels and a large number of internal quality documents) – and a customer conversion phase to enable all of its f [redacted] EU customers to make necessary requalification to approve the new products. According to the substitution plan presented in the EU REACH authorisation application, substitution is expected to be fully competed by the end of 2027. (Authorisation has been granted until January 2028.) c [redacted] Once regulatory approvals have been obtained, the EU Distributor and Applicant will undertake an implementation programme, including updates to documentation relating to production, quality control and customer information. Finally, customers must be assisted with their conversion processes, which might require revalidation within their own quality procedures. Further to this validation, a 21-month shelf-life expiration timeframe allows for all existing FISH buffer containing 4-tert-OPnEO to be phased out by its customers.

4.1.2. Conclusion on suitability of available alternatives in general

The EU Distributor is a major supplier of FISH test products in the EU and GB, but there are other suppliers of similar products, as well as other techniques available than FISH. These products and techniques are generally proprietary, and it is not generally known whether they use 4-tert-OPnEO or not. Therefore, it is not possible to say whether there are suitable alternatives generally available. However, the significance of this is moot, because Abbott has already committed to substituting its use of 4-tert-OPnEO with Alternative No. 1 as soon as possible. Accordingly, the next section provides an update on the substitution plan provided in the original EU REACH application.

4.1.3. Substitution plan

This section presents an update of the substitution plan submitted by the EU Distributor with the original EU REACH authorisation application.

4.1.3.1. Factors affecting substitution

Technical feasibility

Abbott is at an advanced stage in its R&D programme to substitute 4-tert-OPnEO from the FISH assay wash buffer with the chosen alternative surfactant. The process of establishing technical feasibility for any given product involves a complex multi-step IVD manufacturing process and the introduction of any change in the formulation of wash buffers requires a series of verification testing and revalidation activities. The verification of the technical feasibility of Alternative No. 1 has involved the completion of real-time stability studies on seven representative assays. These studies were due to be completed in 2019, but factors including the COVID pandemic meant that this work was subject to delays. However, the tests were completed in June 2020 and have demonstrated that the chosen alternative is technically feasible and can successfully substitute for 4-tert-OPnEO in Abbott's FISH test products.

Regulatory approvals

To implement Alternative No. 1 in its FISH test products, Abbott must compile and submit extensive documentation on each product to multiple regulatory agencies across the world. It is the EU Distributor's experience that a single bundled submission to one regulatory authority is more time efficient than multiple submissions. However, a bundled submission for 400 products is not feasible. As such, the company plans to complete the required document updates and all associated regulatory approval submissions in successive stages; each stage will cover a small group of products that are manageable within the organisation. Some regulatory authorities require significant time to review and approve product changes. In the company's experience, some countries require 24 months to return approvals.

In addition, the EU Distributor has developed a regulatory approval strategy to minimise delays from the requirement to develop duplicate approval submissions for the new IVDR and existing IVD systems. As the regulatory approval phase will be driven by the longest approval times, the EU Distributor will start with the highest volume products sold to customers and work through the product portfolio. Once products receive marketing approvals, the change implementation process can be executed so products will complete substitution in a staged manner rather than at once.

The UKCA (UK Conformity Assessed) marking is a UK product marking used for certain goods, including medical devices, being placed on the Great Britain market (England, Wales and Scotland). Manufacturers of medical devices can use either the UKCA marking or the CE marking on devices they place on the GB market until 30 June 2023. From 1 July 2023, a UKCA marking will be required in order to place a device on the Great Britain market. The applicant's FISH tests are classed as Class IIa general medical devices. As such, no UK approved body involvement is required in the conformity assessment process. However, the Applicant must prepare extensive technical documentation and ensure that the manufacturing process follows the principles of quality assurance. Because the 4-tert-OPnEO-free tests will not be available by 1 July 2023, it will be necessary for the Applicant to prepare and file this technical documentation twice, once for the existing products and again for the 4-tert-OPnEO-free products. Although a lot of the information will be the same for the two versions, it will be necessary to allow time for this compilation to be completed.

Customer conversion

Once the re-formulated FISH wash buffer is launched commercially, time must be given to allow customers to implement required changes to adopt the new formulation. They may need to re-validate the substituted buffer within their own quality procedures for each assay they use. Further to this re-validation, a maximum of 21-month shelf-life expiration is required to allow for all existing stock of FISH buffer containing 4-tert-OPnEO to be phased out. The Applicant and the EU Distributor will communicate with their customers as products are substituted alerting them to the changes and the need for conversion.

c

c

EU REACH authorisation

The EU distributor has been authorised to continue marketing its FISH test products using 4-tert-OPnEO until January 2028. Delays have been encountered in the substitution process associated with the COVID pandemic, c

4.1.3.2. List of actions and timetable with milestones

Identification of potential alternatives phase – Complete

Technical feasibility phase – Complete

c

The technical feasibility phase has demonstrated that the alternative meets the product performance requirements. In the original EU REACH authorisation application, the next stage was to commence the acquisition of regulatory approvals for the products with the new surfactant. c

Regulatory approval phase – Scheduled start mid-2022 through end 2025

The change to the new surfactant will require regulatory approval before products can be marketed. Such approval from regulatory bodies is necessary to ensure the conformity of the product with the relevant quality, safety and efficacy regulations in each of the countries where the product is marketed. The EU Distributor markets FISH products in 64 different countries. c

c [redacted] Current expectation is that applications will be determined by the UK MHRA after approximately one year.[22]

Implementation phase – Scheduled start 2025 through mid 2028

Change implementation is also a significant activity in the substitution and phase out plan for 4-tert-OPnEO in FISH wash buffer. The implementation phase includes the scale up to full manufacture and involves significant activity related to change control procedures mandated by regulations. For this single change the EU Distributor must amend documentation for all of its assays including package inserts, kit labels and a large number of internal quality documents. This effort is significant and requires input across many functional areas. In addition, documentation for all manufacturing, quality control testing marketing and medical writing must be updated prior to marketing the substitution FISH wash buffer. In total, up to 400 different documents will require amendment or creation.

Table 11: Substitution plan action list summary

Ref.	Milestone	Actions	Status	Timescale
1	Identification of Potential Alternatives	– Literature search – Consultation with suppliers – Screening based on physicochemical properties	Complete	Completed - 2014 to end 2015
2a	Preliminary feasibility	– Identification of model assays – Manufacture of FISH test lots – Completion of lab studies	Complete	Completed - 2016 to end 2017
2b	Design Verification	– Run Realtime stability studies – Assess product performance at time intervals	Complete	Completed - 2016 to end 2020
c [redacted]	c [redacted]	c [redacted]	c [redacted]	c [redacted]
3	Regulatory Approval	– Develop strategy for global submissions – Develop documentation – Complete submissions – Await approvals	Planned	12-30 months; 36-54 months with IVDR impact included but no additional impact to requested Authorisation period
4	Change Implementation completed	– Labelling – Package inserts – Safety data sheets – Operations manuals	Planned	End of Reg approval + 36 months
5	All Customer Conversions Completed	– Inform customer – Expire stock – Validate procedures – Risk assess the new formulation containing 4-tert-OPnEO	Planned	Longest expiration 21 months

Customer conversion phase – Scheduled 2027 through end 2029

The EU Distributor applied for authorisation on behalf of its f [redacted] EU customers, f [redacted] GB customers, of its FISH assays. In order to ensure a seamless conversion for its customer base, the EU Distributor plans to communicate the substitution of 4-tert-OPnEO from its products in the form of customer letters. These communications will be sent on a rolling basis as each product set completes regulatory approval.

[22] https://www.gov.uk/government/statistics/medicines-licensing-time-based-performance-measures

Finally, when the product is launched commercially, time must be given to allow customers to implement required changes to adopt the substituted FISH wash buffer. Customers may be required to validate the substituted buffer within their own quality procedures. Further to this validation, a 21-month shelf-life expiration timeframe allows for all existing FISH buffer containing 4-tert-OPnEO to be phased out by its customers.

Table 11 provides a summary of the milestones and actions completed and outstanding in the substitution plan.

4.1.3.3. Monitoring of the implementation of the substitution plan

The EU Distributor previously established a programme level organisation dedicated to identifying and implementing alternatives for 4-tert-OPnEO in its approximately 400 FISH assays. Individual project managers have been put in place for each stage of the substitution plan with responsibility for tracking and reporting progress. A programme management office is in place to provide overall monitoring of the implementation of the substitution plan with periodic reporting to executive management. Table 12 summarises the project management plan, resources and the risks and mitigations identified and the monitoring arrangements in place.

Table 12 Monitoring of the remaining substitution plan

Phase	**Action**	**Ownership / Resources**	**Status / Timescale**	**Monitoring Progress**	**Identified risks**	**Mitigation / Escalation**
C [redacted]	C [redacted]	C [redacted]	C [redacted]	C [redacted]	C [redacted]	C [redacted]
Regulatory Submissions Approval (RSA)	Develop strategy for global submissions Develop documentation Bundle regulatory submissions Await approvals	Medical Writing (MW), Regulatory Affairs (RA)	6-24 months; Planned 36-54 months with IVDR impact included but no additional impact to requested Authorisation period	Quarterly review of progress versus timeline	MW / RA resources or design data needed to support RSA insufficient; RSA cycle times too long (can be 2-3 years) *	Management review (yearly) to assess need for strategy changes and/or increased resource allocation
Implementation (Create updated DMR documents)	Labelling Package inserts Safety data sheets Operations manuals ("DMR" documents)	Technical Product Support (TPS) / Manufacturing Operations (OPS)	36 months-Planned	Quarterly review of DMR update progress versus timeline	TPS / OPS resources insufficient to meet DMR / RS timeline	Management review (yearly) to assess need for strategy changes and/or increased resource allocation
Customer Conversion (CC)	Inform customer Expire stock Validate procedures Risk assess the new formulation containing 4-tert-OPnEO	The Distributor Downstream users	37 months-Planned	Quarterly review of customer inquiries, complaints	Customer acceptance and/or assay validation progress inconsistent with timeline	Consider additional customer communication and/or training activities

4.1.3.4. Conclusions

Abbott has demonstrated its commitment to substituting the use of 4-tert-OPnEO in its FISH test kits, by identifying and ascertaining the technical feasibility of an alternative, and proceeding with its adoption. C [redacted] the plan is to seek regulatory approval of all products using the alternative by the end of 2025, with full phase-out of 4-tert-OPnEO by the end of 2029.

4.2. Risks associated with continued use

4-tert-OPnEO is considered as being of an equivalent level of concern to an endocrine disruptor substance, according to Article 57(f) of the EU REACH Regulation, because it degrades to 4-tert-OP, which is a known environmental pollutant and an endocrine disruptor for the environment. The degradation product has a CLP classification of Aquatic acute 1 and Aquatic chronic 1 and can adsorb to sediment from where it may be slowly released to the aquatic environment.

4.2.1. Impacts on humans

Not relevant for this substance.

4.2.2. Impacts on environmental compartments

If an Authorisation is granted, 4-tert-OPnEO will continue to be used by the Applicant's GB customers and also by Abbott's non-GB plant during formulation of the solutions. The Applicant has 10-100 f [redacted] customers across GB, so use and releases of 4-tert-OPnEO are not concentrated in any particular location. Information collected for some of the highest volume customer sites showed that the widespread practice is discharging used solutions and instrument wastes directly to the sewer, where they would be treated at a municipal sewage treatment plant (STP). In some cases, the leftover, neat solution is disposed of by incineration, but this is expected to be a very small share of the total usage.

4.2.2.1. Description of releases

4-tert-OPnEO in the Applicant's FISH assay kits is used in GB by laboratory workers in medical labs of clinics and hospitals to test patient samples for cancer indicators. They formulate the wash buffer, if needed, and then carry out the washing of the slides, as described in Section 9.1.1 of the CSR. Work is being carried out both manually and automatically, the latter in case the customer is using one of the instruments supplied by the Applicant. In both cases it is assumed the used wash buffer is discharged to the sewer with no further treatment at the customer site.

All 4-tert-OPnEO that is imported into GB in the Applicant's FISH kits is used by their customers and it is assumed that they are discharged to the sewer as wastewater. Stable usage of 4-tert-OPnEO in GB is assumed throughout the whole review period, so the quantities of 4-tert-OPnEO emitted to wastewater will be 1.64kg per year, spread over all of the Applicant's customers in GB.

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 less than 20 tests to over 10,000 tests per year). Most of the customers use low or very low numbers of tests.

As a worst-case approach, it is assumed that all 4-tert-OPnEO will be released to wastewater and will reach the local STP. There, it will degrade to endocrine disrupting substances. The degradation products will be released to the environment either through the liquid outflow or sewage sludge. Eventually, it is assumed that everything will degrade to 4-tert-OP. Emissions to the environment will be proportional to the quantity of 4-tert-OPnEO used, but the environmental concentration will vary due to different dilution factors in and after the STP (e.g. due to different sizes of STPs and to different receiving bodies of water, both in type and volume).

Sewage sludge is often used as fertiliser in agricultural soil. 4-tert-OP may adsorb to the soil and be slowly released to the environment. 4-tert-OP does not have any systemic

hazards for human health and 4-tert-OPnEO has been included in the Authorisation List due to endocrine disruptive properties for the environment, so it is unlikely that there will be risks to humans via their diet.

4.2.2.2. Environmental concentrations

4-tert-OPnEO was included in the Authorisation List because it degrades to 4-tert-OP in the environment. As shown in the CSR, it was not possible to derive a Predicted No Effect Concentration (PNEC) for the substance. Therefore, the impact of releases from the use of the Applicant's IVD kits cannot be determined accurately and are only discussed qualitatively.

In the CSR, local and regional concentrations of 4-tert-OP have been calculated, based on the expected use of the FISH assays by GB customers, the efficiency of wastewater treatment plants and appropriate dilution factors in the receiving bodies of water.

The downstream use of the Applicant's products is considered to be widely dispersive in nature since it occurs at 10-100 (f) 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 (b) tests per day for the higher volume users.

Due to the wide-spread dispersive nature of the use, the Applicant assessed the exposure scenario of all downstream users in GB through the ECHA Guidance R16 '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' (ECHA, 2016).

Furthermore, to verify the validity of a wide-dispersive use assessment, specific use information was collected for three downstream user local areas representative groups to cross reference the wide dispersive use value.

The majority of the customers are located in large cities/towns due to downstream users located in laboratories / hospitals / blood banks, etc.). Customers were grouped according to their geographical location (where more than one customer was located in a city or town) as it was assumed that customers in a certain city/town may discharge to the sewage treatment plant (STP), and hence, the same body of water. Due to the number of customers, 27 locations (local areas) were identified separated into three representative emission types ('high', 'medium' and 'low' releases), based on their daily test usage. Calculated expected 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.

As described below in Table 13, the table summarises the findings of the exposure assessment. Note that the releases reported do not account for any removal in the modelled biological STP.

The calculated environmental concentrations are very low, with the highest freshwater local concentration being approximately 0.483 ng/L. This does not indicate absence of any adverse effect, as the substance is non-threshold. However, it shows that the environmental concentrations caused by the customer use of the Applicant's FISH kits are very low. Highest concentrations were observed in the highest usage sites.

Table 13 Environmental concentrations (calculated)

Assessment	WDU	1- t	2- t	3- t

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	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sediment (marine water) mg/kg/dw*	-	1.01E-6	-	n.a.	-	n.a.	-	n.a.
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

*Only freshwater representative sites (conservative approach).

Conservative assumptions were made when selecting the most appropriate STP for each site, as well as the flow rate of the receiving body of water. More specifically, a single STP was used for the high-volume sites, where it is more reasonable to assume that treatment of waste is spread over several ones. The single STP was selected as a worst-case assumption, which may contribute to higher environmental concentrations in these high-volume sites. This approach may result in higher calculated environmental concentrations than the actual ones.

4.2.2.3. Minimisation of releases

The quantities of 4-tert-OPnEO used in GB in the Applicant's FISH assays are very low and spread over numerous customers and locations. The Applicant cannot control or monitor directly how each customer uses their product or handles the 4-tert-OPnEO waste generated from using the wash buffer in the FISH assay. The Applicant includes directions for handling waste in the FISH kit package inserts and the VP2000 processor's instrument manual advice and local regulations also apply. Additionally, the products have hazard labels in accordance with CLP and GHS.

As a condition of granting the EU REACH authorisation, 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. 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.

The EU REACH authorisation application also proposed an additional measure to change customer behaviour by educating them on how to implement optimised FISH test batching to reduce liquid waste generation. This involves customers' grouping tests and running them in fewer sessions, to take advantage of using the same buffer solution for multiple tests before disposal. This is a readily available alternative RMM (minimising the generation of waste containing 4-tert-OPnEO) that does not incur increased disposal cost or require DU customers to revalidate their FISH assays. Each FISH laboratory has validated a maximum number of FISH slides (individual assays) that can be washed before each set of wash buffers must be discarded and replaced with a fresh set of wash buffers (e.g. 16 slides per set in the most restrictive case; see section 9.1 of the CSR for additional details). Thus 4-tert-OPnEO waste minimisation can be achieved by maximising utilisation of each set of aliquots of the wash buffers containing 4-tert-OPnEO on each day of FISH testing.

This measure has been adopted by the EU Distributor, and product documentation has been updated to include instruction as to how to minimise use of 4-tert-OPnEO through batch-testing. It is expected that this measure will also be incorporated into GB product guidance.

80-100% f [redacted] of the Applicant's DU customers are users of low quantities, running a small number of tests each day. It is believed that the practice of optimal FISH test batching represents the most effective additional RMM available until substitution can be implemented.

4.2.3. Compilation of human health and environmental impacts

Table 14 summarises the remaining releases of OPnEO to the GB environment from customers' uses of the Applicant's FISH assays in the applied-for use scenario.

Table 14: Summary of remaining releases to the environment

	Per year
Total releases/emissions (in kg per period)	1.64 kg

1.64 kg of OPnEO releases per year translates into 0.54 kg of releases of OP per year.

4.3. Non-use scenario

4.3.1. Summary of the consequences of non-use

The Applicant's current use of 4-tert-OPnEO is governed by EU REACH application for authorisation number 11-2120816695-47-0000, which permits use of the substance in GB until 1 July 2022. For the purposes of determining the impacts of non-use scenario, it is assumed that the Secretary of State would be expected to have made a final decision on this application approximately 18 months after submission (assumed before July 2022),

or around 36 months after the end of the transition period, i.e. by the beginning of 2024. Thus, under the non-use scenario, it is assumed that use of 4-tert-OPnEO in the Applicant's FISH kits would be effectively banned from the end of 2023.

Sales in GB are a relatively small proportion of Abbott's global b and EU b sales of FISH kits, so changes in GB sales are not expected to have significant impacts on global or EU operations. GB sales of FISH kits are also not a large proportion of the Applicant's total sales b. As a result, it is not expected that the non-use scenario would have significant impacts on the immediate supply chain for the Applicant's FISH products. The Applicant, the EU Distributor and the US parent company would, of course, lose profits on any lost sales.

There could be significant impacts on the Applicant's downstream users/customers and, ultimately, patients. Downstream users (hospital labs and ultimately doctors diagnosing patients) would be affected more or less depending on whether comparable alternative tests are available on the market (and currently in use).

If there are no comparable tests on the market, downstream users will most likely be obliged to continue without the Abbott tests, thereby losing their diagnostic capabilities, with consequent effects on patient care (and outcomes).

If there are comparable tests on the market, but these are not currently in use, the downstream user might elect to adopt them, and will probably need to incur approval and validation costs to do so. These tests might also be expected to be less effective and/or more costly than the Abbott versions (which would explain why the Abbott versions were preferred in the first place), implying further costs of adopting them. Any reductions in effectiveness might have implications for diagnostic performance and hence patient outcomes. Additional tests used by Abbott's downstream users would be expected to generate sales and profits for the suppliers of these alternative tests.

If there are comparable tests on the market, and they are already in use, the downstream user will simply lose the additional benefits from being able to use the Abbott tests alongside them. This also would be expected to affect diagnostic and patient outcomes.

Once Abbott's 4-tert-OPnEO-free FISH tests become available, one would expect them to be adopted by downstream users who used Abbott's previous tests and do not have comparable tests available. Where comparable tests are available, downstream users might decide to adopt the new Abbott tests, depending on the costs of so doing and their comparative performance advantage. Where the new tests are adopted, one would expect diagnostic and patient outcomes to improve and/or financial costs to fall (which would be the motivation for adopting them). Sales and profits would rise or fall for the Applicant and its competitors in line with changes in the volumes of each's tests used following introduction of Abbott's 4-tert-OPnEO-free FISH tests.

4.3.2. Identification of plausible non-use scenarios

For the purposes of determining the most plausible non-use scenario, it is assumed that the Secretary of State would be expected to have made a final decision on this application for authorisation approximately 18 months after submission (assumed before July 2022), or around 36 months after the end of the transition period, i.e. by the beginning of 2024.

c . Regulatory approval for the new products

will not have been obtained by this time. Therefore, the EU Distributor (and hence the Applicant) will not have any products available which do not use 4-tert-OPnEO, and hence these products would need to be removed from the market and could not be immediately replaced with non-4-tert-OPnEO alternatives.

There are essentially two alternatives. The first would be to remove all the products from the GB market permanently, and not replace them. The second would be to remove the products from the GB market, and replace them with non-4-tert-OPnEO-based alternatives once they have been approved and implemented.

The GB market is relatively small compared with the EU market b [redacted]. Authorisation has already been granted to the EU Distributor to continue marketing its 4-tert-OPnEO-based FISH products in the EU until 4 January 2028 (with the possibility of extension if necessary and justified). Therefore, a rejection of this current GB authorisation application, and a ban on the sale of the products in GB from (assumed) 2024, would not impact the EU Distributor's plans to substitute 4-tert-OPnEO and replace the existing products with alternatives in the EU by 2028.

Therefore, there will be 4-tert-OPnEO-free products to introduce into the GB market as a result of the EU-driven substitution, even if marketing of existing products in the GB was forced to cease in 2024. The question then is whether the returns to (re-)introducing its FISH products into GB would be sufficient to justify the costs. This depends on two principal factors: the costs of reintroduction (largely the costs of obtaining regulatory approval for marketing in GB) and the profits on sales of the product once reintroduced. In turn, sales of the reintroduced product will depend on the extent to which alternative tests are available and have been adopted instead (temporarily or permanently) by testing laboratories.

The costs to Abbott of introducing 4-tert-OPnEO-free products are largely the same in the applied-for and non-use scenarios, so it can be assumed that the only difference between the two scenarios will be driven by customers. In practice, customers' response to non-availability of the Applicant's FISH tests would be likely to be a mixture of two possibilities. Where comparable alternative tests are available, customers would be likely to incur conversion costs and switch to these alternative tests permanently. Where comparable alternatives are not readily available, customers would be likely to make do with the next best alternative – or simply not do the particular Abbott test – and then reintroduce the non-4-tert-OPnEO Abbott test when it becomes available.

4.3.3. Conclusion on the most likely non-use scenario

The preceding discussion suggests that, if it would be worth Abbott introducing 4-tert-OPnEO-free products into the EU and GB in the applied-for use scenario, it would also be worth doing in the non-use scenario also. The key difference between the two scenarios, therefore, would be determined by the behaviour of customers. It is argued that, where comparable alternative tests are available, customers would be likely to incur conversion costs and switch to these alternative tests permanently. Where comparable alternatives are not readily available, customers would be likely to make do with the next best alternative – or simply not do the particular Abbott test – and then reintroduce the non-4-tert-OPnEO Abbott test when it becomes available.

4.4. Societal costs associated with non-use

4.4.1. Approach to valuing the impacts of non-use

Following the discussion in the previous section, the most likely non-use scenario would be expected to comprise the following responses by different segments of the Applicant's customer base:

1. Customers who stop using Abbott's tests until 4-tert-OPnEO-free versions are available, and do not use comparable tests in the meantime;
2. Customers who stop using Abbott's tests until 4-tert-OPnEO-free versions are available, and continue to use comparable tests in the meantime;
3. Customers who stop using Abbott's tests until 4-tert-OPnEO-free versions are available, and introduce additional comparable tests in the meantime;
4. Customers who stop using Abbott's tests and introduce additional comparable tests, and do not adopt Abbott's 4-tert-OPnEO-free versions when they become available.

For segments 1 and 2, the principal impacts would be temporary reductions in sales and profits for the Applicant (and its upward supply chain), and reductions in diagnostic effectiveness and patient outcomes. Note that sales and profits from the supply of tests reflect the value of those tests to users. (Producer surplus is essentially a financial transfer from customers to producers.)

For segments 3 and 4, there would be reductions in sales and profits for the Applicant (and its upward supply chain), and some increases in sales and profits for the suppliers of comparable alternatives. There would also be expected to be additional costs associated with qualifying new tests, and the need to undertake this qualification might prevent the switch from Abbott tests to 'comparable' tests from being seamless. (It is assumed that the volumes of tests involved are small enough that they can be supplied within competitor companies' existing production capacities.) These changes might be temporary or permanent. There would also be expected to be reductions in diagnostic effectiveness and patient outcomes (because users would be unable to use their preferred tests).

It is not known what proportion of the Applicant's customers are in which segment 1-4 above. However, the segments do represent bounds on the size of the costs associated with the non-use scenario. For instance, comparing segment 1 and 2, customers in segment 1 lose the value of Abbott's tests for the entire duration of the gap in supply until 4-tert-OPnEO-free versions are available; customers in segment 2 also lose that value, but have comparable (complementary) tests already in use alongside – it might be considered that this means the loss would be more costly for segment 1 users than segment 2 users (since the additional benefit of the Abbott tests are perhaps smaller if one already uses comparable tests). Nevertheless, both sets of customer pay the same amount for their tests (generating the same producer surplus), so this difference in additional user value implies differences in consumer surplus. Overall, the loss in value is the reduction in producer surplus (the same for both segments) and the reduction in consumer surplus (assumed higher in segment 1 than segment 2).

With segment 4, customers are likely to spend some time without using tests, while new (comparable) tests are qualified. Consumer and producer surplus will be lost during any such period of interruption (although it is possible that some customers might qualify in

advance to ensure no interruption to test availability). Abbott's 4-tert-OPnEO-free tests would have needed (re-)qualification anyway, so the costs of qualifying new (comparable) tests could be said to be brought forward, rather than incurred additionally. Consumer surplus gained from the use of the 'new' tests would be expected to be lower than previously (as the 'new' comparable tests were not customers' preferred ones).

The objective of this AoA/SEA is to compare the benefits and risks of the continued use of 4-tert-OPnEO in the Applicant's FISH tests in GB. If benefits are greater than risks, authorisation is justified in principle. If it can be demonstrated that benefits are greater than risks under assumptions which do not exaggerate (and might underestimate) the benefits of continued use, this provides confidence that the result is robust to changes to the assumptions.

The substitution plan outlined in Section 4.1.3 anticipates customer conversion running for a period of 36 months until the end of 2029. This includes time for (re-)qualification, the exhaustion of inventories where they exist, and the full roll-out of the 4-tert-OPnEO-free tests to all customers. Some customers can be expected to have adopted the 4-tert-OPnEO-free tests relatively quickly after the start of customer conversion at the beginning of 2027. Assuming this timetable would be unchanged in the non-use scenario, this would mean an absence of users of Abbott FISH tests of between three (2024-2026 inclusive) and five years (2024-2028 inclusive).

For the purposes of this assessment, and to ensure estimates of the benefits of continued use are not exaggerated, it will be assumed customers will be without Abbott or 'comparable' tests for a maximum of one year. Due to a lack of data, consumer surplus losses will be considered only qualitatively. Customer conversion costs brought forward or duplicated will not be considered.

4.4.2. Economic impacts on the applicant

The Applicant's FISH kits are manufactured by its parent company in Des Plaines, Illinois, USA. The EU Distributor imports the kits into its distribution centre in Wiesbaden, Germany, and from there distributes them throughout Europe, including GB. The Applicant acts as the GB distributor All revenues and profits associated with the sale of the kits in GB are shared between the Applicant, the Distributor in the EU and its parent company in the USA.

As seen in Table 7, sales of the Applicant's FISH products in GB were £0.2-10 million b [redacted] in 2021. According to the company accounts for the year to 31 December 2020 (the latest available),[23] Abbott Laboratories Limited had total sales of £394.4 million in 2020, with gross profit of £105.6 million and operating profit of £14 million, giving profit rates of between 3.5% and 26.8%. Given the relatively small share of the Applicant's sales accounted for by FISH products, it is reasonable to assume that operating costs would be little affected by changes in the value of FISH sales, and hence the higher gross profit rate of 26.8% is a fair indicator of producer surplus earned by the Applicant. A loss of one year's worth of profit in the non-use scenario would be equal to £0.05-2.7 million b [redacted].

[23] https://find-and-update.company-information.service.gov.uk/company/00329102/filing-history

4.4.3. Economic impacts on the supply chain

A reduction in sales for the Applicant would also imply a reduction in sales, and hence profits, for the EU Distributor and the US parent company. Internal figures suggest this could add an additional 10-60% b in margin, or £0.005-1.62 million b. These are losses which would accrue outside of GB.

As discussed in Section 4.4.1, the Applicant's customers would be expected to incur costs through the need to requalify tests earlier and/or to requalify additional tests. They would also by implication suffer a reduction in consumer surplus through the inability to use their preferred tests. This latter impact would be expected to manifest itself in less favourable diagnostic performance of one year's worth of tests b. The information does not exist to put monetary values on these impacts.

4.4.4. Economic impacts on competitors

In Section 4.4.1 it was explained that, as a best-case scenario, it would be assumed that there would be a one-year interruption in the supply of FISH tests following the ban on the use of 4-tert-OPnEO in the Applicant's tests after January 2024. This assumption effectively implies that the Applicant would lose profits for the entire duration of the non-use scenario (2024-2029), competitor companies would gain profits from increasing supply of their tests for the non-use scenario less one year (2025-2029). The net change, therefore, is a loss of one year's worth of profits, as calculated in Section 4.4.2 (and 4.4.3), and impacts on competitors have already been accounted for implicitly.

4.4.5. Wider socio-economic impacts

4.4.5.1. Impacts on employment

In the original EU REACH authorisation application, it was assumed that supply of the distributor's FISH products to the EU would stop in the NUS for the duration of the review period analysed (2021-2027). It was estimated that this would cause a significant drop in sales volume and hence production output, which would have material implications for employment at the parent company's US production facility and at the distributor's EU offices.

The NUS in the current case envisages a cessation of supply of the distributor's FISH products in GB from approximately the beginning of 2024 until the supply of 4-tert-OPnEO-free products restarts around 2029. It is possible that no change in employment would occur over this period, and staff will simply be redeployed temporarily to cover the gap in supply. However, it is also possible that staff would be made redundant and then re-employed later as necessary. GB sales represent only a minor part b of EU sales. A *pro rata* reduction in employment in manufacturing and distribution would mean a loss of around 0-30 d jobs in total (based on an estimated total of 15-150 d in the EU REACH application). It is not expected that any of these losses would be in GB. As a result, the costs of any such (temporary) unemployment are not quantified for this application.

4.4.5.2. Impacts on patients

As described in Section 4.4.1, it has been assumed that the non-use scenario would be associated with a net reduction in one year's worth of tests b. This is comprised of a loss of six years' worth of tests supplied by the Applicant, and an increase

of five years' worth of tests by competitor manufacturers. In addition, it can be assumed that tests supplied by the Applicant provide a better combination of price and performance than those supplied by competitors (which is why the Applicant's customers prefer them). These price and performance advantages would be lost in the non-use scenario.

4.4.5.3. Distributional impacts

There is well-established evidence of socio-economic disparities in cancer outcomes – diagnosis, treatment and survival.[24] A recent study by Arik *et al*. (2021) looked at the impact of social deprivation (as measured by the Index of Multiple Deprivation) on cancer incidence and mortality rates in England in 2001 and 2016. [25] The authors found that the impact of deprivation had declined between 2001 and 2016 for some cancer outcomes (e.g. lung cancer mortality in men, although not statistically significantly in all areas), but had risen for others (e.g. lung cancer mortality in women, significantly in all areas). Moreover, statistically significantly higher incidence and mortality rates were found in higher deprivation groups in all areas in England in 2016, for both men and women for all cancers examined.

The non-use scenario would be expected to result in a reduction (even if temporary) in the number of diagnostic tests for cancer performed in GB. This in turn would be expected to be associated with delays in patients receiving (accurate) diagnoses and treatment, and hence potentially with negative cancer outcomes. Consistent with the evidence just discussed, these negative outcomes would be expected to be borne more heavily by patients from more deprived groups. As a result, the NUS would have negative distributional impacts.

4.4.6. Compilation of socio-economic impacts

Table 15 summarises the preceding sections and summarises the societal impacts of the non-use scenario in quantitative and qualitative terms.

Table 15 Societal costs associated with non-use

Description of major impacts	
1. Monetised impacts	**£ Over 6 years**
Producer surplus loss due to ceasing the use applied for	£0.05-2.7 million b
Relocation or closure costs	N/A
Loss of residual value of capital	Not quantified
Social cost of unemployment	Non-GB unemployment not quantified
Spill-over impact on surplus of alternative producers	Included in producer surplus loss figure
Please specify	N/A
Sum of monetised impacts	£0.05-2.7 million b

[24] https://www.cancerresearchuk.org/health-professional/cancer-statistics/survival/socio-economic-group

[25] https://doi.org/10.1371/journal.pone.0253854

2. Additional quantitatively assessed impacts	**Over 6 years**
Reduction in diagnostic tests	10,000-100,000 b
3. Additional qualitatively assessed impacts	
Loss of consumer surplus due to use of inferior comparable tests	
Reduced patient outcomes due to use of inferior comparable tests, leading to reduced diagnostic performance	

4.5. Combined impact assessment

Table 16 combines the estimates of the societal costs of the non-use scenario with estimated releases over the applied-for use scenario. The estimate of the societal costs of non-use is £0.05-2.7 million b . The estimate of OPnEO releases per year in Table 8 is 1.64 kg, or 9.84 over six years. This translates into OP releases of 3.24 kg over six years. This implies cost-effectiveness ratios of 800-0.45m b (OPnEO) and 2,778-1.5m b (OP).

Table 16 Costs of non-use per unit of release

	Over 6 years
Total costs (£)	£0.05-2.7 million b
Total releases OPnEO (kg)	6-60 b
Ratio (£/kg)	800-0.45m b
Total releases OP (kg)	1.8-18 b
Ratio (£/kg)	2,778-1.5m b

There are no benchmark figures against which to compare these cost-effectiveness ratios. Oosterhuis and Brewer (2015) did review some regulations of PBT substances,[26] and this study has been cited by ECHA's SEAC as a possible starting point for comparisons for OPnEO.[27] They concluded:

"The available evidence suggests that there is a wide 'grey zone' (orders of magnitude between EUR 1000 and EUR 50,000 per kg avoided PBT use/presence or emission) within which the cost of a measure can either be 'acceptable' or 'too high'."

b

4.6. Sensitivity analysis

[26] https://echa.europa.eu/documents/10162/13647/R15_11_pbt_benchmark_report_en.pdf

[27] https://echa.europa.eu/documents/10162/17229/seac_ed_approach_opneo_npneo_en.pdf

Two key assumptions have been made in this analysis. The first is that the entire volume of 4-tert-OPnEO used in the Applicant's FISH tests is discharged to wastewater and is free to enter the environment via STPs. However, as described in Section 4.2.2, a condition of the EU REACH authorisation is that users are instructed to ensure that all waste 4-tert-OPnEO is disposed of in such a way as to minimise releases (via, e.g., incineration). This instruction has been included in product guidance to EU customers and is likely to be included in guidance to customers in GB too. If this guidance is followed, as would be expected, releases will be cut effectively to zero. As a result, the estimates of the releases of 4-tert-OPnEO used in the analysis above, albeit still low, are drastically exaggerated. This would mean that the cost-effectiveness ratios estimated in Section 4.5 are significantly underestimated, and could even approach infinity.

The second key assumption is that the non-use scenario would result in only a single year's worth of impacts on the profits of the Applicant's supply chain and on its patients. This assumption was made for conservative reasons because of uncertainty regarding the market positioning of the Applicant's FISH products and the availability of comparable tests from alternative manufacturers. It was therefore designed to underestimate the true benefits of continued use. It happens to coincide with the lower bound proposed by SEAC for the application of its approach to estimating producer surplus losses.[28] For situations where there is a suitable alternative generally available, SEAC recommends two years' worth of profit losses as a rule of thumb; where no such alternative exists, profit losses over four years are proposed. As a result, it could be argued that profit losses could justifiably be multiplied a number of times, which would have concomitant impacts of estimated cost-effectiveness ratios. In addition, it has not been possible to quantify impacts on patients from not having preferred tests available.

C [redacted]

Accordingly, the results of this analysis are considered to be robust to reasonable variations in assumptions.

4.7. Information in support of the review period

As previously noted, an EU REACH authorisation application has already been granted for the continued use of 4-tert-OPnEO in the EU Distributor's FISH tests in the EU. This authorisation was granted until 4 January 2028 (a 'normal' review period of seven years). Use of 4-tert-OPnEO is currently authorised in the Applicant's FISH tests in GB under the original authorisation application, but this needs to be 'replaced' by a UK REACH authorisation. This is the objective of this current application.

[28] https://echa.europa.eu/documents/10162/0/afa_seac_surplus-loss_seac-52_en.pdf

The analysis presented here demonstrates that the original conditions of the EU REACH application are also met in the GB context. In fact, it is highly likely that emissions of OPnEO will be much lower in practice in the applied-for use scenario than assumed (and could approach zero). At the same time, the costs of non-use for patients and the Applicant's supply chain would be significant. Authorisation is clearly justified on this basis.

The review of progress on substitution, provided in Section 4.1.3, demonstrates that the Applicant's parent company has successfully tested the technical feasibility of the identified alternative to 4-tert-OPnEO. However, delays have been encountered due to the COVID pandemic, [redacted]
The Applicant must also compile two sets of technical documentation to secure compliance with the UKCA product certification system. It is possible that the remaining stages of the substitution process can be completed within the existing review period granted to the EU authorisation. [redacted] It would not be a valuable use of the Applicant's funds or the UK CA's resources to have to process a review report for this application if it turned out the Applicant did, in fact, need an extra two years to complete its substitution. As a result, it makes sense to incorporate this (potential) additional time requirement into the current review period. As a result, the Applicant requests authorisation with a review period until January 2030.

5. CONCLUSION

4-Tert-OPnEO is used by Abbott Molecular Division in 1-10 b general reagents used to support FISH testing in 10-100 b FISH probe kits, covering approximately 400 assays, of which more than 100 b are classified as IVDs. FISH kits are used for diagnosing cancer, determining the type of cancer of a patient, and for prescribing Companion Diagnostics (CDx) therapies. The products enter the EU market through the distribution centre in Wiesbaden and from there are imported into GB by 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. The use is carried out by the Applicant's customers.

The 4-tert-OPnEO serves solely a detergent function (within the wash buffer), principally to wash unbound DNA and other unbound biological components originating from the specimen (including proteins). Removal of unbound components is required to eliminate critical non-specific signal to ensure the precision, accuracy and specificity of the test. 4-tert-OPnEO provides a very effective washing detergent function for use in FISH assays, due to a number of key properties.

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 made a positive decision on 16 November 2021, with a review period of 4 January 2028. Special transitional provisions (Article 127GA 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 an 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.

As part of the process of applying for authorisation under EU REACH, the EU Distributor identified a potential alternative to 4-tert-OPnEO for use in its FISH tests, and proposed to adopt this alternative if its technical feasibility could be demonstrated. Since EU authorisation was granted under EU REACH, the EU Distributor has successfully completed the technical feasibility phase of its Substitution Plan. However, delays have been encountered as a result of the COVID pandemic. The remaining parts of the substitution plan include application for regulatory approval, an implementation phase – including scale-up to full manufacture of the new product and change control procedures mandated by regulations (e.g. amendment of documentation for all assays, including package inserts, kit labels and a large number of internal quality documents) – and a customer conversion phase to enable all of its f customers to make necessary requalification to approve the new products.

c

[redacted]

If authorisation is granted, the EU Distributor will continue with its substitution plan, and the Applicant will introduce 4-tert-OPnEO-free versions of its FISH tests as soon as they become available. In the meantime, the Applicant will continue to supply 10,000-100,000 [redacted] FISH tests to its 10-100 [redacted] customers annually, with a value of £0.2-10 million [redacted]. Assuming customers discharge all waste 4-tert-OPnEO to the sewer would imply releases of 1.64 kg of OPnEO per year, which translates into 0.54 kg of releases of OP per year. However, as part of the conditions for the EU REACH authorisation, EU customers are now being advised that they should not dispose of waste 4-tert-OPnEO to the sewer, but should use a method (e.g. incineration) which minimises releases. This would effectively cut releases to zero.

If authorisation was not granted, the Applicant would stop supplying its FISH tests to GB customers as soon as the Secretary of States direction was received – assumed to be by the beginning of 2024. The EU Distributor would continue with its substitution plan, and the Applicant would propose to introduce 4-tert-OPnEO-free versions of its FISH tests to GB customers as soon as they become available. It is assumed that the Applicant's customers would on average lose one year's worth of testing, which would result in a loss of profits to the Applicant of around £0.05-2.7 million [redacted], with additional profit losses to the Applicant's supply chain (the EU Distributor and the US parent company). Downstream users are assumed to switch to comparable tests supplied by competitors where available. However, there would still be impacts on patients due to the assumed absence of one year's worth of tests, as well as a potential loss of performance. The implied cost-effectiveness ratios are 800-0.45m [redacted] (OPnEO) and 2,778-1.5m [redacted] (OP). If releases were reduced to zero through incineration (or similar) of waste 4-tert-OPnEO, these cost-effectiveness ratios would be effectively infinite.

The results of this analysis suggest that authorisation for continued use of 4-tert-OPnEO in the Applicant's FISH tests in GB is justified. This conclusion is robust to reasonable sensitivity analysis.

The review of progress on substitution demonstrates that the Applicant's parent company has successfully tested the technical feasibility of the identified alternative to 4-tert-OPnEO. However, delays have been encountered due to the COVID pandemic, [redacted] The Applicant must also compile two sets of technical documentation to secure compliance with the UKCA product certification system. It is possible that the remaining stages of the substitution process can be completed within the existing review period granted to the EU authorisation. [redacted] It would not be a valuable use of the Applicant's funds or the UK CA's resources to have to process a review report for this application if it turned out the Applicant did, in fact, need an extra two years to complete its substitution. As a result, it makes sense to incorporate this (potential) additional time requirement into the current review period. As a result, the Applicant requests authorisation with a review period until January 2030.