Marine Management Organisation

Development of criteria to define exempt tracers

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Development of Criteria to Define Exempt Tracers

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Glossary

Bioaccumulation - The accumulation of substances in the fatty tissue of living organisms, including humans, which can lead to adverse effects.

Bioconcentration Factor - The ratio of the average concentration of test chemical accumulated in the tissue of the test organism (under steady state conditions) to the average measured concentration in the water

Carcinogenicity - The ability or tendency for a chemical substance or a mixture of chemical substances to induce cancer or increase its incidence.

Ecotoxicity - The degree to which a substance can have damaging effects on a living organism, typically measured through endpoints such as growth, reproduction and survival. In terms of microbes, this primarily considers the ability to exert pathogenicity in humans and/or wildlife through disease.

EC_{50} (Effect Concentration) - The concentration of test chemical which will affect 50% of the sample size/population.

LC_{50} (Lethal Concentration) - The concentration of test chemical which will be lethal to 50% of the sample size/population.

Lowest Observed Effect Concentration (LOEC) - The lowest concentration of a toxicant to which an organism is exposed that causes observable adverse effects on the test organism (e.g. the lowest concentration of a toxicant for which the values for the observed responses are statistically significantly different from the controls).

Mutagenicity - The ability or tendency of an agent, such as a chemical, ultraviolet light, or a radioactive element, to induce or increase the frequency of mutation in an organism.

No Observed Effect Concentration (NOEC) - The highest concentration of a toxicant to which an organism is exposed that causes no observable adverse effects on the test organism (e.g. the highest concentration of a toxicant for which the values for the observed responses are not statistically significantly different from the controls).

Octanol-water partition coefficient (log K_{ow}) - The ratio of the compound's concentration in a known volume of n-octanol to its concentration in a known volume of water after the octanol and water have reached equilibrium.

Persistence - The property of a substance or organism to remain intact or viable for exceptionally long periods of time.

Reproductive toxicity (Reprotoxicity) - The ability or tendency for a chemical substance to exert a toxic effect on the process of reproduction.

Executive Summary

The Marine Management Organisation (MMO) is responsible for licensing the use of marine tracers released directly to the marine environment by vehicle, vessel, aircraft or marine structure under the Marine and Coastal Access Act 2009. Article 17 of The Marine Licensing (Exempted Activities) Order 2011 (as amended) provides for the MMO as the English marine licensing authority to define exemptions for the use of tracers in the marine area.

Materials and substances can be released into the marine environment to allow scientists and engineers to directly track the movement of water and water-transported media. Collectively, these materials are known as tracers and can vary from inert particles and soluble fluorescent dyes to radioactive/biocidal substances and bacterial/microbial cells. Their deployment allows for the investigation of water and sediment movement and transport including issues such as dispersion, dilution, stratification and mixing. Tracers are used in a wide range of marine based projects including construction/engineering, dredging, flood/coastal defence, leak detection and water quality studies.

The purpose of this study has been to develop a process for identifying marine tracers that can be included on an approved tracer list for exemption and a process for determining exemptions for potential new tracers in the future. Development of an appropriate exemptions list and process will contribute to sustainable development in the marine environment by ensuring that regulatory decisions are proportionate and adequately protect existing environmental, social (including human health) and economic interests in line with the principles of better regulation.

This process for defining exemptions for existing tracers has taken account of the full range of potential risks posed by the different types of tracer including risks to the natural environment and to social (including human health) and economic interests. For those tracers which are considered to be potentially suitable for exemption, a series of additional criteria have been identified to control the amount of tracer used, its discharge rate and the manner in which it is released and monitored to ensure that the marine environment is adequately protected.

An initial list of 70 tracers, including dyes, microbiological tracers, particle tracers, radiological tracers and other well documented tracer types was identified based on a review of existing licences for use of tracers, previous reports and wider literature searches. This list was prioritised for the purposes of this project based on a review of tracers licensed over the last few years on the MMO public register and expert judgement. A final list of 24 tracers was identified for review. Based on a detailed risk assessment, 15 of these tracers were identified as being potentially suitable for exemption, subject to tracer specific controls on the amount of tracer to be released and the discharge rate. These substances have been included in the first list of tracers that are considered suitable for exemption (the ‘approved tracers list’).

The study has also developed a methodology for adding new tracers to the approved tracers list based on the methods used to develop the first list.

1. Introduction

The Marine Management Organisation (MMO) is responsible for licensing the use of marine tracers released directly to the marine environment by vehicle, vessel, aircraft or marine structure under the Marine and Coastal Access Act 2009. Article 17 of The Marine Licensing (Exempted Activities) Order 2011 (as amended) provides for the MMO as the English marine licensing authority to define exemptions for the use of tracers in the marine area.

The purpose of this study has been to develop a process for identifying marine tracers (including chemical dyes, particle tracers and microbial tracers) that can be included on an approved tracer list for exemption under The Marine Licensing (Exempted Activities) Order 2011 (as amended). Development of an appropriate exemptions list and process will contribute to sustainable development in the marine environment by ensuring that regulatory decisions are proportionate and adequately protect existing environmental, social (including human health) and economic interests in line with the principles of better regulation. The objectives of the study are:

1. To produce a table of approved tracers and associated conditions for exemption from marine licensing:

    - Establish appropriate criteria for the inclusion of marine tracers on an approved list for exemption. This may include, but is not limited to, consideration of potential impacts (e.g. persistence, bioaccumulation, toxicity, smothering and pathogenicity) on the marine environment, human health and commercial interests and establishing appropriate thresholds for tracer characteristics.
    - Develop standard conditions for use of the approved tracers. This may include, but is not limited to, restrictions on quantity, concentration, frequency of use, location of use and mandatory notification of appropriate authorities. A mechanism to facilitate prevention of potential cumulative/in-combination effects of tracers must be considered within the conditions.
    - Criteria and conditions must be based on best-available evidence and must provide appropriate protection to the environment (in particular Marine Protected Areas) and commercial interests (e.g. shellfisheries). Criteria and conditions must encompass all aspects of tracer use (e.g. access to the deposit site, method of deposit, tracer sampling subsequent to deposit).

2. To establish a process/guidelines for the addition of new tracers and associated conditions to the approved tracers table:

    - The process should be based on the criteria and conditions developed under Objective 1. The project should look to ensure these are applicable to the potential inclusion of novel tracers going forwards.
    - Internal and external guidance should be produced describing the process for tracer exemption. Internal guidance will be in the form of desk notes for marine case officers. External guidance should be summarised in the form of website text suitable for publication on the MMO web pages in association with the approved tracers table produced under Objective 1.

The desk note guidance and website text prepared under Objective 2 have been reported separately, but are consistent with this report.

The outputs from the study provide a list of tracers that are considered suitable for exemption, together with lists of criteria that will apply to those exemptions. Any party, hereafter referred to as 'the user', seeking to make use of an exemption will need to satisfy themselves that their activity meets all of the criteria relevant to their proposed use of a tracer. The user must also notify the MMO of their intention to make use of an exemption. If a proposed tracer activity does not meet the requirements for an exemption, it may still be possible to undertake the activity subject to obtaining a marine licence from the MMO.

The work has been undertaken by ABP Marine Environmental Research Ltd (ABPmer), WRc and Partrac under contract to the MMO.

1.1 Tracers

Materials and substances may be released into the marine environment to allow scientists and engineers to directly track the movement of water and water-transported media. Collectively, these materials are known as tracers and can vary from inert particles and soluble fluorescent dyes to radioactive/biocidal substances and bacterial/microbial cells. Their deployment allows for the investigation of water and sediment movement and transport including issues such as dispersion, dilution, stratification and mixing. Tracers are used in a wide range of marine based projects including construction/engineering, dredging, flood/coastal defence, leak detection and water quality studies (Cefas, 2011a).

There are four main types of tracer that are commonly used in the marine environment. These include:

- Chemical dyes
- Microbes (bacteria and bacteriophages)
- Particles (including natural sediment particles, synthetic beads, pebbles and fruit)
- Radioactive substances.

Depending on their characteristics, tracers may pose risks within the marine environment as a result of their persistence, bioaccumulation, toxicity, pathogenicity or risks to human health. Particle tracers may also pose a risk of smothering. In addition, activities associated with the release or subsequent monitoring of the tracer in the marine environment have the potential to cause damage and disturbance within the marine environment or to interfere with other marine activities.

Previous work undertaken by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) has primarily focused on risks associated with the direct ecotoxicity of tracers (Cefas, 2010; 2011a; 2011b). Through this study, the MMO is seeking to ensure that the exemption process takes account of wider environmental risks to the marine environment including, for example, risks associated with persistence and bioaccumulation of tracers, potential toxicological effects in humans and wildlife

(including pathogenicity), smothering, methods of introduction and subsequent monitoring of tracers, together with potential social (including human health) and economic risks such as potential impacts to shellfisheries.

1.2 When would a tracer exemption apply?

The MMO is responsible for regulating marine activity in English inshore and offshore waters and for Welsh and Northern Irish offshore waters. This is referred to as the marine licensing area for the remainder of this report.

The MMO may grant exemptions from marine licensing for certain activities, in accordance with the Marine and Coastal Access Act 2009 (Section 66) and The Marine Licensing (Exempted Activities) Order 2011 (as amended) (Section 17). These provisions apply to the deposit of tracers within the marine licensing area (any area submerged at mean high water spring (MHWS) tide and the waters of every estuary, river or channel so far as the tide flows at MHWS), either in the sea or on or under the sea bed, from any vehicle, vessel, aircraft or marine structure, any container floating in the sea, or any structure on land constructed or adapted wholly or mainly for the purpose of depositing solids in the sea. For example, tracer studies using a boat (vessel) or hovercraft (vehicle) within the marine licensing area are licensable activities by the MMO and, therefore, are considered for exemption in this report.

Tracer releases from a land-based discharge pipe should be carried out within the conditions of an existing Environmental Permit, or through variation or application for a new permit from the Environment Agency. Similarly, in planning tracer releases from land by hand (e.g. from a river bank or beach), the user must consult the Environment Agency to determine whether an Environmental Permit is required. In both circumstances, the activity is not licensable by the MMO (i.e. marine licences are not applicable) and, therefore, the exemption would not apply.

Where a tracer survey is within the MMO's licensing remit but considered suitable for exemption, the user must inform the MMO of their proposed tracer activity by completing the *Notification of an exempt activity form*[1] and emailing it to exemptions@marinemanagement.org.uk prior to commencing the works. It should be noted that activities that are exempt from marine licensing may still require other consents from either the MMO or from other bodies. For example, if the activity is planned to occur within or adjacent to a Site of Special Scientific Interest (SSSI), consent from Natural England may still be needed. Similarly, intrusive works on the seabed (e.g. sediment removal for monitoring) may require a marine licence.

In planning to carry out an exempted activity, the user will need to ensure that they are aware of the criteria attached to them and are able to fully comply with them. Anyone may notify the MMO of a tracer exemption provided they meet the specified criteria. Where exemption criteria cannot be met, the user would have to apply to the MMO for a Marine Licence[2].

[1] https://www.gov.uk/marine-licensing-application-process#exemptions
[2] https://www.gov.uk/marine-licensing-application-process

2. Methodology for Identifying Tracer Exemptions and Future Additions to the Approved List

2.1 Outline

The methodology for identifying tracers that are potentially suitable for exemption involved the following steps:

- Developing a prioritised list of tracers
- Assessing potential risks posed by tracer characteristics
- Developing criteria based on tracer characteristics.

In general, the methodology is described in terms of the process used by the project team to assess the potential environmental risk(s) posed by tracers and develop an approved list of tracers suitable for exemption. However, the methodology should also be used in the future to determine the suitability of adding new tracers to the approved list and, where necessary, further guidance is provided in the relevant sections. A summary of the process that should be used to consider adding new tracers to the approved list is available in Section 2.5.

2.2 Developing a prioritised list of tracers

An initial list of 70 tracers, including dyes, microbiological tracers, particle tracers, radiological tracers and other well documented tracer types was identified (see Annex A) based on a review of existing licences for use of tracers, previous reports and wider literature searches. Following consultation with the MMO, it was decided to prioritise this list of tracers in order to streamline the project team's efforts. Building on the results of previous reports (Cefas, 2010; 2011a; 2011b), a review of tracers licensed over the last few years on the MMO public register and expert judgement, the initial list was prioritised to include only those tracers which are most commonly thought to be used. In addition, the prioritised list included those tracers that were considered likely to be suitable for exemption based on the criteria that are adopted in the project.

A final prioritised list of 24 tracers was identified to include in the project review (Table 1). This included three types of tracer as described in Cefas (2010):

- Chemical dye
- Microbial tracer
- Particle tracer.

However, it should be noted that other tracers (i.e. those not included on the prioritised list) are not necessarily unsuitable for exemption and may be assessed further down the line using the methodology developed in this project.

Table 1: Prioritised list of tracers.

Chemical dyes		
Diphenyl methane	Leucophor CK	Triphenyl methane
Erioglaucine	Malachite green	Uvitex CF
Fluorescein	Pyranine	Uvitex WGS
Leucophor BS	Rhodamine WT	Xanthene
Leucophor C	Sulforhodamine G	
Microbes		
Bacillus globigii spores	Bacteriophage for *Serattia marcens*	PRD1 phage
Bacillus subtlis var *Niger*	MS2 coliphage	
Bacteriophage for *Enterobacter cloacae*	Phi-X174 coliphage	
Particles		
Fluorescent coated natural particles	Fluorescent (painted) pebbles	Fruit (e.g. Oranges and Apples) and seeds

Radioactive tracers have been excluded from the prioritised list on the basis that Cefas (2011b) indicated they may pose a long-term toxicological risk to marine organisms and human health. The risks associated with their use and the public perception of those risks are thought to be the reason why radioactive tracers are infrequently used (Cefas, 2010). It is likely that these forms of tracers, if/when they are used, will require a marine licence and have thus been excluded from the prioritised list of tracers considered for exemption within this work.

In addition, uncertainty surrounding the appropriateness of introducing synthetic particles (e.g. fluorescent microspheres and nano-particles) into the marine environment has led to their exclusion from the prioritised list. This is also related to Marine Strategy Framework Directive (MSFD) targets to reduce marine litter which these types of particle could constitute.

In terms of determining new tracers suitable for exemptions in the future, the process described within this report only considers chemical dyes, microbial tracers and particle tracers. It should be noted that microbial tracers derived from recombinant DNA manipulation should not be considered for exemption to avoid the introduction of genetically modified organisms to the marine environment without formal consideration within a licensing process. Other types of tracer (e.g. radioactive tracers and synthetic particles) should not be considered for exemption unless a specific additional review is undertaken and approved by the MMO.

2.3 Assessing potential risks posed by tracer characteristics

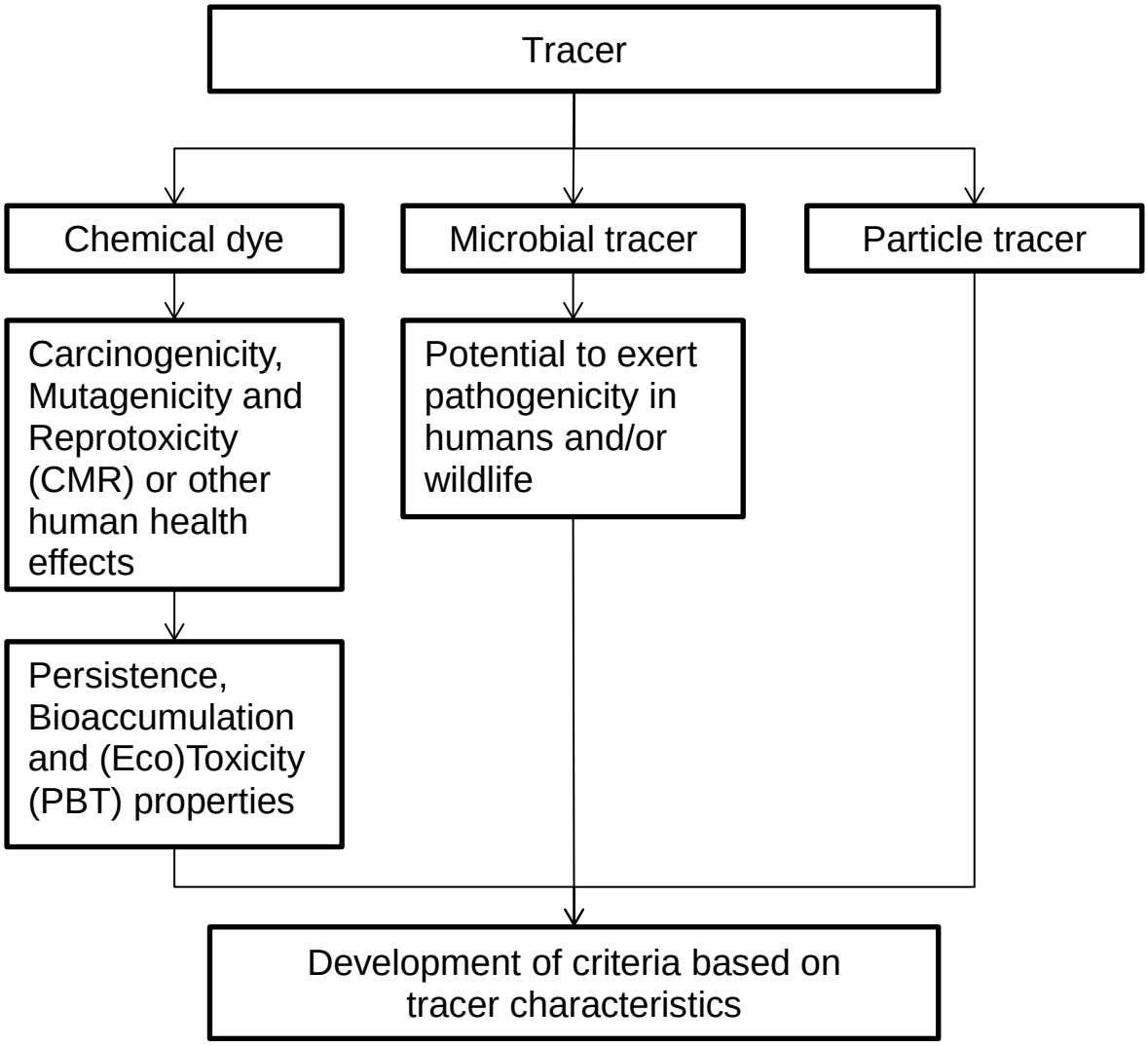
An evaluation of the hazard and potential environmental risk posed by physical characteristics of tracers on the prioritised list was carried out. The type of hazard

posed by tracers varies depending on the type of tracer (Figure 1). The following hazards were identified for specific types of tracer:

- Chemical dyes
  - Carcinogenicity, Mutagenicity and Reprotoxicity (CMR) and other human health effects
  - Persistence, Bioaccumulation and (Eco)Toxicity (PBT).
- Microbial tracers
  - Pathogenicity.

For particle tracers, a simple distinction has been made between naturally occurring and man-made (synthetic) particles. Naturally occurring particles (with fluorescent coating) are considered suitable for exemption, whereas synthetic particles are not. Unlike chemical dyes and microbial tracers, naturally occurring particle tracers do not require further consideration of innate hazardous properties and, therefore, the criteria simply relate to the maximum amount that can be released (Section 2.4).

Figure 1: Information requirements to assess the potential risks posed by tracer characteristics.



2.3.1 Chemical dyes

For chemical dyes, a robust and scientifically defensible evaluation of their toxicity was necessary as part of the hazard and risk assessment. However, available toxicological data for many substances was limited and therefore a pragmatic approach was necessary. The approach taken has been sufficiently precautionary that it does not exempt tracers that present a significant risk of adverse effects to humans or wildlife. Conversely, the criteria are not so overly precautionary that the use of tracers that would not present a hazard to humans or the environment are excluded (i.e. a proportionate approach).

Carcinogenicity, Mutagenicity and Reprotoxicity (CMR) and other human health effects

Chemical dye tracers classified as a carcinogenic, mutagenic and/or reproductively toxic (CMR) substance under the Regulation (EC) N^{o} 1272/2008 on classification, labelling and packaging of substances and mixtures and/or those substances for which there is evidence of chronic toxicity in mammals were not considered suitable for exemption. This included substances with the following classifications:

- Carcinogenicity
- Germ cell mutagenicity
- Reproductive toxicity.

The risks associated with CMR substances are potentially highly significant and, thus, attempting to manage these risks was considered inappropriate within the scope of an exemption process. In addition, tracers for which evidence suggested the following other human health effects, described under Regulation (EC) N^{o} 1272/2008, were not considered suitable for exemption:

- Specific target organ toxicity, single exposure
- Specific target organ toxicity, repeated exposure
- Aspiration hazard
- Sensitisation, respiratory
- Acute toxicity, oral
- Acute toxicity, dermal
- Acute toxicity, inhalation.

A substance classified as a skin or eye irritant was still considered for exemption as the potential risk(s) could be mitigated through standard procedures (e.g. the use of eye protection and gloves). However, if a tracer was considered capable of causing acute (harmful, toxic or fatal) effects to humans through ingestion, exposure via the skin or inhalation then it was not considered suitable for exemption. The same logic was applied to tracers that are classified for aspiration hazard, respiratory sensitisation and/or specific target organ toxicity (single or repeated exposure) since the potential risks associated with their use as tracers would be considered unacceptable.

This information, where available, was provided within the substance's Material Safety Data Sheet (MSDS) and/or the manufacturer's product guidelines under the Regulation (EC) N^{o} 1272/2008 on classification, labelling and packaging of

substances and mixtures. Where this information is not available, the tracer should not be considered for exemption.

Persistence, Bioaccumulation and (Eco)Toxicity
If a chemical dye was not found to pose any CMR or other human health risk, it was then considered in relation to persistence, bioaccumulation and (eco)toxicity (PBT) criteria. Toxicity here is termed 'Ecotoxicity' in reference to aquatic organisms and should be differentiated from mammalian toxicity as previously described (e.g. CMR). A series of 'threshold' values for each of the PBT properties were developed against which tracers could be compared to determine whether they could be made exempt. A review of criteria currently adopted by regulatory frameworks and further information outlining how the PBT thresholds used in this work were derived is available in Annex B.

In summary, the following PBT threshold values were used to consider chemical dye tracers for exemption:

- **Persistence**
  - Upper threshold - Half-life in water of ≤60 days
  - Lower threshold - Half-life in water of ≤40 days or evidence of ready biodegradability in a standard test procedure.
- **Bioaccumulation**
  - Upper threshold - Octanol-water partition (log K_{ow}) coefficient of ≤4.5 or a Bioconcentration Factor of ≤2,000
  - Lower threshold - Octanol-water partition (log K_{ow}) coefficient of ≤3.0 or a Bioconcentration Factor of ≤100.
- **Ecotoxicity**
  - Upper threshold - Lowest short-term[3] LC_{50}/EC_{50} of ≥0.1 mg/l for representative algal, invertebrate and fish species
  - Lower threshold - Lowest short-term LC_{50}/EC_{50} of ≥1.0 mg/l for representative algal, invertebrate and fish species.

In considering these threshold values, it should be appreciated that an equal weighting was not placed on each of the PBT properties. For example, if a chemical tracer did not meet the upper threshold criteria for bioaccumulation and/or ecotoxicity, it was not considered for exemption. However, if a tracer did meet the upper threshold criteria for bioaccumulation and ecotoxicity, it was considered suitable for exemption irrespective of its persistence properties. Chemical tracers that are not toxic and do not bioaccumulate are unlikely to present a significant risk to the receiving environment, even if they have the potential to persist. This places a greater emphasis on the bioaccumulation and ecotoxicity properties of the chemical tracer. Should the tracer be considered suitable for exemption, the PBT threshold criteria were then used to determine the maximum amount that could be released for that specific tracer (explained further in Section 2.4).

Information on the PBT properties of the tracers was identified using targeted internet searches of publically available data. Ecotoxicological data for chemical dyes was principally derived from the United States Environmental Protection

[3] Short-term (acute) and long-term (chronic) tests are defined here as <7 and >7 days, respectively.

Agency (US EPA) Ecotox database using the chemical name or Chemical Abstract Service (CAS) number of the substance. However, information from other sources was also used (e.g. Cefas, 2011a; 2011b). The available short-term experimental (and estimated) ecotoxicity data for chemical dyes is typically expressed as an LC_{50} (lethal concentration, 50% of population) or EC_{50} (effect concentration, 50% of population). If experimental data were not available for certain required endpoints, predicted values were generated using appropriate tools such as those present in the Estimation Programs Interface Suite (EPI Suite™).

In considering future exemptions, it is likely that some literature information will be available on the persistence and bioaccumulation potential of tracers; however, the availability of ecotoxicology data could be relatively limited. Ecotoxicology data is required relating to at least one representative algal, invertebrate and fish species (i.e. one from each group). This should be obtained according to appropriate Organisation for Economic Co-operation and Development (OECD) or International Organization for Standardization (ISO) methods and ideally Good Laboratory Practice (GLP). If insufficient information is available in the literature, supplementary information should be sought on the basis of modelling (e.g. EPI Suite™). If there is insufficient confidence in modelled ecotoxicity data, additional short-term laboratory ecotoxicity testing should be conducted. If sufficient data for a tracer is not readily available and cannot be obtained, the tracer should not be considered for exemption.

The key requirement for the assessment of the hazards and risks posed by chemical dye tracers is that reliable ecotoxicity data is available for the taxonomic group that is expected to be most sensitive to the tracer. In the first instance experimental data should be used where available. If such data is not available, it may then be appropriate to use modelled/estimated data. However, approving a tracer for exemption based solely on modelled data is not considered ideal given the inherent uncertainty in the data. Therefore, where only modelled/estimated data for the most sensitive taxonomic group (i.e. the species with the lowest LC_{50}/EC_{50}) is available a tracer should only be considered for exemption where the data has been reviewed by an expert panel. If the tracer is considered suitable for exemption, the maximum amount that can be released should then be determined (see Section 2.4).

2.3.2 Microbial tracers

Pathogenicity

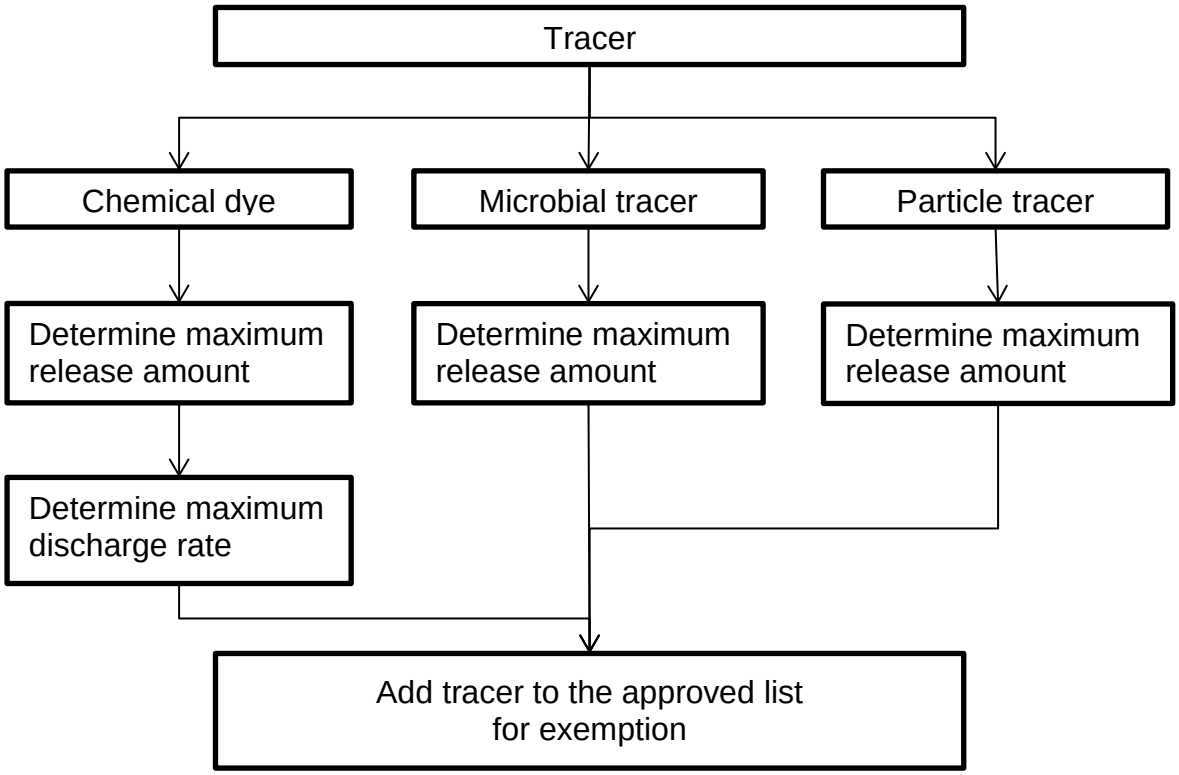
For microbial tracers, adverse effects are considered in terms of pathogenicity to exposed aquatic biota (e.g. shellfish, fish, birds and marine mammals) and humans, rather than endpoints such as survival, development, growth and reproduction. A distinction has been drawn between bacteria and bacteriophage (phage) tracers. Phage tracers only present a threat to their bacterial hosts (i.e. high specificity) and pose no harm to human health; therefore, phage tracers are considered suitable for exemption and consideration has been made regarding the maximum amount that could be released (Section 2.4). However, the risk posed by bacteria depends on the species concerned. In the case of *Bacillus* spp., the genus has been implicated in potential infections and food poisoning (Granum and Lund, 1997; Kamar *et al*. 2013); therefore, following a precautionary approach, it is not considered suitable for exemption.

In terms of future exemptions, a microbial tracer should not be considered for exemption if robust evidence is found from a search of relevant sources (e.g. the Public Health England website)[4] that the microbe can exert pathogenicity in humans and/or wildlife at the concentrations that are likely to be experienced in the receiving environment. As outlined above, bacteriophage tracers should be considered suitable for exemption. If a bacterial tracer does not pose a pathogenic risk to human health and or wildlife, the tracer should also be considered for exemption. Subsequently, the maximum amount of tracer that can be released should then be determined (Section 2.4). It should be noted that the potential risk associated with other types of microbe (e.g. non-specific viruses) is beyond the scope of this exemptions process and, in such circumstances, exemptions would not be possible at present.

2.4 Developing criteria based on tracer characteristics

If a tracer is not considered to pose a significant hazard or environmental risk based on the categories described in Section 2.3, the tracer could potentially be released into the receiving environment under an exemption. However, the amount of tracer that can be added still needs to be limited to avoid excessive use, whilst still enabling the tracer study to yield results in a tidal environment. In addition, it is also considered appropriate to limit the discharge rate of chemical dyes to the receiving environment to avoid concentrations above the No Observed Effect Concentration (NOEC) (also see Figure 2)

Figure 2: Process to develop criteria based on tracer characteristics.



[4] http://www.hpa.org.uk or http://www.gov.uk/government/organisation /public-health-england

2.4.1 Amount

The amount of tracer that can be added to the receiving environment has been established based on previous licence documents obtained from the MMO public register and consideration of potential uses of tracers. The following amounts are considered appropriate:

- **Chemical dyes** - ≤2kg (as sold, i.e. not diluted)
- **Microbes** - ≤10^{14} cells/ml (5l max)
- **Particles** - ≤250kg.

In addition, it is considered necessary to further restrict the amount of chemical dye that can be released if the tracer meets the upper threshold criteria, but does not meet the lower threshold criteria for ecotoxicity. In such circumstances, the maximum amount of chemical dye that can be released depends on the persistence and bioaccumulation properties.

Table 2 summarises the possible outcomes of the PBT assessment of chemical dye tracers and the subsequent criteria relating to the release of the tracer. If the PBT property of the tracer meets the threshold criteria, the tracer is deemed to have 'Passed' the threshold. However, if the PBT property does not meet the threshold criteria, the tracer is deemed to have 'Failed' the threshold. Situations where a PBT property is described to have 'Passed or Failed' suggest the outcome (i.e. whether the tracer is suitable for exemption and, if so, the amount that can be released) will not be influenced by this property. For example, if a tracer does not meet the upper threshold criteria for ecotoxicity, the persistence and bioaccumulation properties are irrelevant as the tracer is not considered suitable for exemption. It should also be noted that when a property is described to have 'Passed (Upper threshold)', it is considered to have met the upper threshold criteria, but not met the lower threshold criteria.

In considering future exemptions, the same amounts should apply for microbial tracers which do not exert pathogenicity in humans and/or wildlife (≤10^{14} cells/ml (5l max)) and particle tracers which are naturally occurring (i.e. non-synthetic) (≤250kg). With regards to chemical dyes, Table 2 should be used to determine the maximum release amount based on PBT properties (assuming the tracer does not pose a CMR or other human health risk).

Table 2: Possible outcomes of the assessment of chemical dye tracers against human health and environmental risk.

Human Health Risk (incl. CMR)	Persistence (P), Bioaccumulation (B) and (Eco)Toxicity (T)			Outcome	Criteria
	P	B	T		
Failed	Passed or Failed	Passed (Upper or Lower threshold)	Passed (Upper or Lower threshold)	No exemption	N/A

Human Health Risk (incl. CMR)	Persistence (P), Bioaccumulation (B) and (Eco)Toxicity (T)			Outcome	Criteria
	P	B	T		
Passed	Passed or Failed	Passed (Upper or Lower threshold)	Failed	No exemption	N/A
Passed	Passed or Failed	Failed	Passed (Upper or Lower threshold)	No exemption	N/A
Passed	Passed (Upper threshold) or Failed	Passed (Upper threshold)	Passed (Upper threshold)	Consider for exemption with more stringent criteria	≤0.2kg
Passed	Passed (Lower threshold)	Passed (Upper threshold)	Passed (Upper threshold)	Consider for exemption with more stringent criteria	≤0.5kg
Passed	Failed	Passed (Lower threshold)	Passed (Upper threshold)	Consider for exemption with more stringent criteria	≤0.5kg
Passed	Passed (Upper or Lower threshold)	Passed (Lower threshold)	Passed (Upper threshold)	Consider for exemption with more stringent criteria	≤1kg
Passed	Passed or Failed	Passed (Upper or Lower threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg

2.4.2 Discharge rate

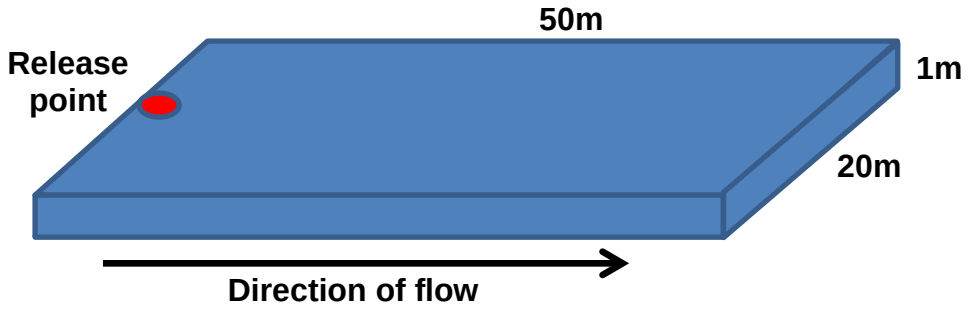
Chemical dyes

In order to avoid releases of chemical dye which would result in concentrations greater than the NOEC of the most sensitive taxonomic group outside of an initial mixing zone, a maximum discharge rate has been established depending on the physical characteristics of the tracer.

Ideally, the ecotoxicity criterion for chemical dye tracers would be based on the short-term No Observed Effect Concentration (NOEC), which is appropriate given that the aim of this assessment is to protect against adverse effects on resident organisms and communities. However, the available short-term experimental (and estimated) data for chemical dyes is typically expressed as an LC_{50} or EC_{50} (previously established to determine the ecotoxicity risk, see Section 2.3) and, therefore, a simple conversion step is required to derive an estimate of the NOEC. Ecotoxicity values for the most sensitive taxonomic group that use an EC_{50} or LC_{50} should be divided by 5 and 10, respectively (see Section B.6 for more information). It should be noted that the conversion of LC_{50}/EC_{50} values to an NOEC is only applicable for the purposes of this report when determining the discharge rate, as opposed to the determination of whether a tracer is suitable for exemption which directly considers the LC_{50}/EC_{50} value (i.e. Section 2.3.1).

In order to evaluate the ecotoxicological risk of releasing chemical dyes into the receiving environment, it was necessary to consider the potential concentration within a 'mixing zone' in the vicinity of the release point. To ensure the process was relatively simple, but suitably precautionary, a mixing zone of 1,000m^3 was considered appropriate to represent the immediate surrounding area with a shallow mixing depth (e.g. equivalent to a volume approximately 50m x 20m x 1m, see Figure 3).

Figure 3: Initial mixing zone used to determine maximum discharge rate of chemical dye tracers suitable for exemption.



To estimate a discharge rate that would not exceed the NOEC for each tracer outside of this mixing zone, a dispersion rate of 2.4m^3/s was used as a conservative flow rate based on the Environment Agency's Horizontal Guidance Note H1 (Environment Agency, 2010). Therefore, the estimated time it would take to fill an initial mixing zone volume of 1,000m^3 is 417 seconds (i.e. 1,000m^3 divided by 2.4m^3/s), which equates to approximately 7 minutes.

The total amount of tracer required within the mixing zone to reach the NOEC is calculated by multiplying the lowest short-term NOEC (in mg/l) by the mixing zone volume (i.e. 1,000,000l) and subsequently dividing by 1,000,000 to convert milligrams to kilograms (i.e. this leads to the same value as the NOEC, but the units are now in kg; Equation 1). This provides the maximum single "gulp" release amount that can be discharged every seven minutes for that specific tracer.

- Equation 1

$$\text{Maximum Single "Gulp" Release (kg)} = \text{Lowest short-term NOEC (mg/l)} \times \frac{1{,}000{,}000\ (\text{l})}{1{,}000{,}000}$$

Next, the total amount of tracer required to reach the NOEC value is divided by 7 (minutes) to calculate the average discharge rate per minute (kg/min) by which the NOEC will not be exceeded outside of the mixing zone (Equation 2).

- Equation 2

$$\text{Average discharge rate (kg/min)} = \frac{\text{Maximum Single "Gulp" Release (kg)}}{7\ (\text{minutes})}$$

Therefore, should a chemical dye tracer be considered suitable for exemption (based on its PBT properties), the maximum amount and discharge rate must be adhered to in order to protect the environment from excessive concentrations.

Microbial tracers

As microbial tracers considered suitable for exemption are not thought to exert pathogenicity in humans or wildlife, they are not subject to a maximum discharge rate. Therefore, exempt microbial tracers can be released in a single “gulp” event providing the amount released is not greater than 5 l at a maximum concentration of 10^{14} cells/ml.

Particle tracers

The key potential impacts of particle tracers are the risk of smothering and elevated suspended sediment concentration. These risks are primarily managed through limiting the amount of particle tracer that can be released. In total, 250kg of particle tracer would equate to less than 0.2m^3 of material, assuming a conservative material density of 1,300kg/m^3. It is considered that the smothering risk can be effectively managed through application of the following criteria:

- Where material is placed directly on the seabed/intertidal zone, the tracer must not be placed on an area greater than 10m^2
- The median particle diameter of the particle tracer must not be more than 1 phi class smaller than the prevailing sediment[5].

It is considered that the risks associated with elevated water column suspended sediment concentration will also be effectively managed through application of the above criteria. For example, should all of the maximum 250kg of tracer become rapidly entrained within the water column, this would equate to an average

[5] The phi (Ø) scale (Krumbein, 1934) is a logarithmic transformation of the Wentworth (1922) grade scale based on the negative logarithm to the base 2 of the particle size. The following is an example of the normal progression of sizes in mm and phi units from the Wentworth scale: Fine sand (125-250µm, 3 to 2 Ø); Medium sand (0.25-0.5mm, 2 to 1 Ø); Coarse sand (0.5-1.0mm, 1 to 0 Ø); Very coarse sand (1.0-2.0mm, 0 to -1 Ø); Very fine gravel (2.0-4.0mm , -1 to -2 Ø); Fine gravel (4.0-8.0mm, -2 to -3 Ø).

suspended sediment concentration of 250mg/l within an indicative mixing zone of 1,000m^3. Even if such an event should occur, it would not persist for any significant time. Information on the impact of elevated suspended sediment on ecological features indicates an increase in suspended sediment concentration of up to 300mg/l for a few hours would not be expected to give rise to significant effects on any ecological features (Tillin *et al*. 2010).

2.5 Methodology for adding new tracers to the approved exemptions list

The methodology for adding new tracers to the approved exemptions list follows the methodology described in Sections 2.1 to 2.4. For chemical dyes (Section 2.5.1), consideration of potential CMR risks, other human health effects and PBT risks will determine whether a tracer is suitable for exemption and, if appropriate, the maximum amount to be released and the discharge rate to the receiving environment can be established. Consideration of potential pathogenicity risks will determine whether microbial tracers (Section 2.5.2) are suitable for exemption and, if so, the maximum amount that can be released into the receiving environment can be established. Finally, consideration of particle tracer type (i.e. naturally occurring or synthetic) will determine whether a tracer is suitable for exemption and how much can be added to the receiving environment (Section 2.5.3). The following types of tracer are currently not considered suitable for exemption:

- Radioactive substances
- Synthetic particles
- Microbial tracers which have been derived from recombinant DNA manipulation.

2.5.1 Chemical dyes

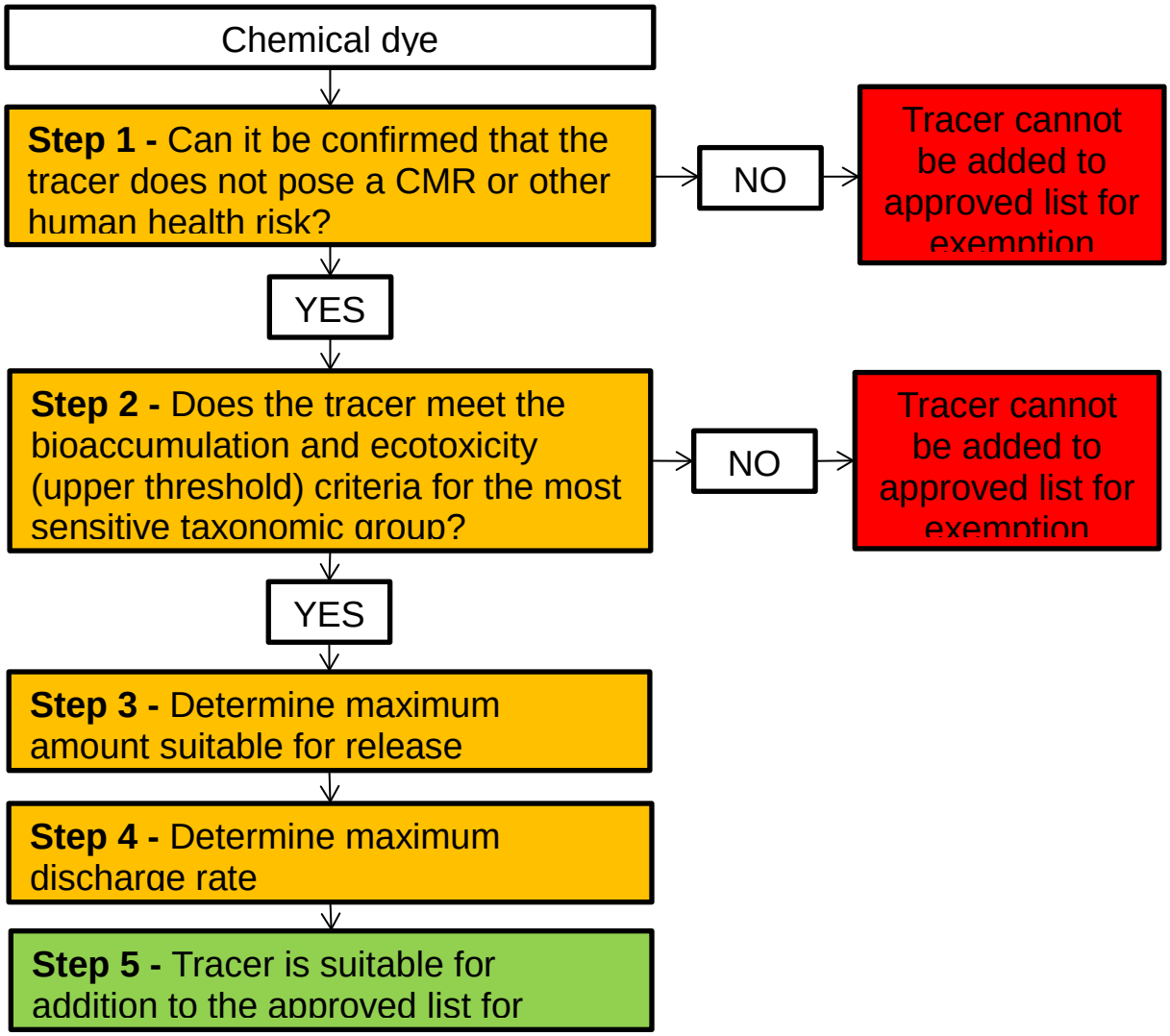
In order to determine whether a new chemical dye can be added to the approved list of exempt tracers, the process outlined below should be followed (also see Figure 4).

Step 1. Can it be confirmed that the tracer does not pose a CMR or other human health risk?

The first consideration regarding whether a chemical dye is suitable for inclusion on the approved list of exempt tracers relates to potential CMR risks and other human health effects. If evidence is available to suggest a tracer presents one or more of the 10 hazard classes outlined in Section 2.3.1, the tracer should not be considered suitable for exemption.

Information relating to CMR and human health effects should be readily available within the substance's MSDS and/or the manufacturer's product guidelines under the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. If a tracer does not pose a CMR or other human health risk, the assessment should progress to Step 2.

Figure 4: Flow diagram illustrating process to add new chemical dye tracers to approved exemption list. CMR = Carcinogenicity, Mutagenicity and Reprotoxicity



Step 2. Does the tracer meet the bioaccumulation and ecotoxicity (upper threshold) criteria for the most sensitive taxonomic group?
It is necessary to assess potential ecological effects of the tracer through consideration of its PBT properties. Values for PBT can be obtained from the literature, or generated from experimental studies or modelled using computer simulations. In the first instance, the assessment of the hazard posed by a tracer should be based on reliable experimental data obtained from literature searches.

It is likely that some literature information will be available on the persistence and bioaccumulation potential of tracers, but the available ecotoxicology data could be relatively limited. Ecotoxicity data is required relating to representative algal, invertebrate and fish species. If sufficient experimental information is available in the literature, the values should be compared to the PBT thresholds (Step 3). If insufficient information is available in the literature, supplementary information should be sought on the basis of modelling. If there is insufficient confidence in

modelled ecotoxicity data, additional short-term laboratory toxicity testing could be conducted, followed by comparison of PBT thresholds (Step 3). If sufficient data for a tracer is not readily available and cannot be obtained, the tracer should not be considered for exemption.

Using the PBT thresholds criteria described in Section 2.3.1, the tracer should be compared to the possible outcomes defined in Table 2. If the tracer meets the upper threshold criteria for ecotoxicity for the most sensitive taxonomic group (irrespective of persistence), the tracer should be considered suitable for exemption and the maximum release amount should be determined (Step 3).

Step 3. Determine maximum amount suitable for release
Should the tracer be considered suitable for exemption, this step defines the maximum amount that can be released. This should be determined based on the outcome of the PBT assessment in Step 2 (Table 2).

Step 4. Determine maximum discharge rate
The method that should be used to calculate the maximum discharge rate of a tracer to the receiving environment is described in Section 2.4.2. The method entails calculating a release rate that will not lead to concentrations greater than the lowest NOEC (estimated from the lowest short-term LC_{50}/EC_{50}) outside of a mixing zone of 1,000m^3 (approximately 50m x 20m x 1m), based on the ecotoxicity of the tracer and a conservative dispersion rate.

In summary, if the maximum amount permissible for release is lower than the amount required to lead to concentrations greater than the lowest short-term NOEC outside of the mixing zone, a single “gulp” release of the whole amount would be possible. However, if the maximum amount permissible for release is greater than the amount required to lead to concentrations greater than the lowest short-term NOEC outside of the mixing zone, it will be necessary to establish a maximum discharge rate for the tracer. Releases of new tracers added to the approved exemptions list would not be allowed to exceed this maximum discharge rate.

Step 5. Tracer is suitable for addition to the approved list for exemption
The suitability of the tracer for exemption is considered using information as described in Steps 1 to 4. However, the final decision on whether a tracer will be added to the approved list for exemption rests with the MMO.

2.5.2 Microbial tracers

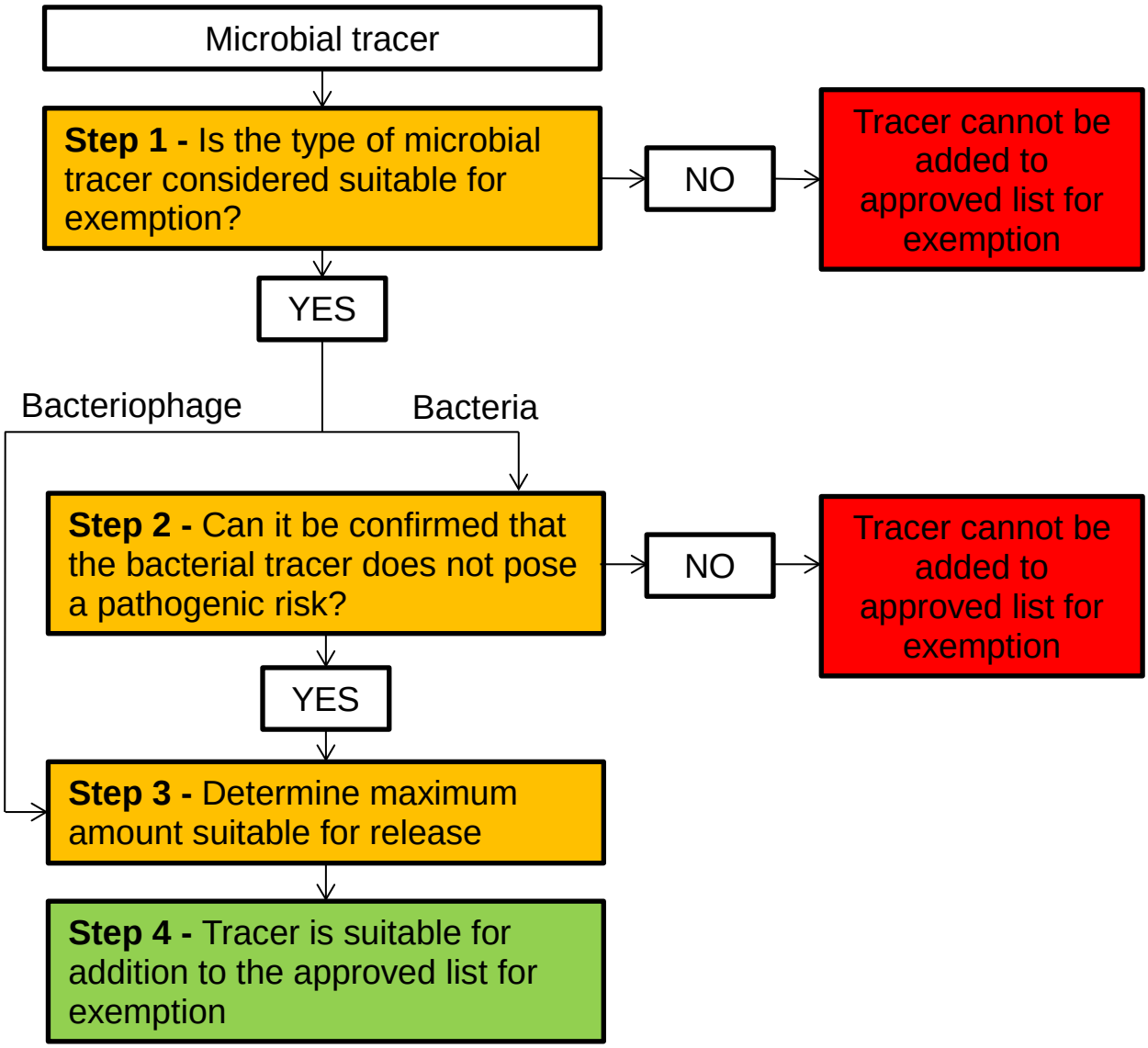
In order to determine whether a new microbial tracer can be added to the approved list of exempt tracers, a process to follow has been developed, as described below and illustrated in Figure 5.

Step 1. Is the type of microbial tracer considered suitable for exemption?
The primary concern regarding the use of microbial tracers relates to potential pathogenic risks. A distinction should be made between the following two types of microbial tracers:

- Bacteria
- Bacteriophages (Phage).

As described in Section 2.3.2, bacteriophages will only pose a risk to the host bacteria and do not pose a risk to the environment or human health. Therefore, bacteriophage tracers should be considered for exemption providing their release does not exceed the maximum amount permissible (skip to Step 3). In contrast, bacterial tracers require further consideration as to their suitability for exemption (Step 2). Other types of microbial tracer (including those derived from recombinant DNA manipulation) should not be considered for exemption as the potential ecological impacts on human health and the environment are unknown and beyond the scope of an exemption process.

Figure 5: Flow diagram illustrating process to add new microbial tracers to approved exemption list.



Step 2. Can it be confirmed that the bacterial tracer does not pose a pathogenic risk?

For microbial tracers, toxicity is more appropriately expressed as pathogenicity which relates to evidence of disease in important food species (e.g. molluscs and fish), birds, marine mammals (e.g. seals, dolphins and porpoises) and humans. *Bacillus* spp. has not been considered suitable for exemption based on evidence suggesting the bacteria could be associated with a significant risk to human health (e.g. Granum and Lund, 1997; Kamar *et al*. 2013).

A microbial tracer should not be considered for exemption if robust evidence is found from a search of relevant sources (e.g. the Public Health England website)[6] that the microbe can exert pathogenicity in humans and/or wildlife at the concentrations that

[6] Available at http://www.hpa.org.uk and http://www.gov.uk/government/organisation

are likely to be experienced in the receiving environment. If, based on robust evidence from the literature, a microbe is not thought to pose a pathogenic risk to humans and/or wildlife, it should be considered for exemption providing the release does not exceed the maximum amount permissible (Step 3).

Step 3. Determine maximum amount suitable for release
If a microbial tracer is not considered to exert pathogenicity in humans and/or wildlife, as defined in Steps 1 and 2, up to 10^{14} cells/ml (5l max) could be released in a single “gulp” event.

Step 4. Tracer is suitable for addition to the approved list for exemption
The suitability of the tracer for exemption is considered using the information as described in Steps 1 to 3. However, the final decision on whether a tracer will be added to the approved list for exemption rests with the MMO.

2.5.3 Particle tracers

With regard to the addition of new particle tracers to the approved exemptions list, the primary concern relates to long-term persistence in the marine environment and the potential to smother sensitive seabed features. Synthetic particles were not considered suitable for exemption in this report as the potential impacts of their introduction into the marine environment are still largely unknown. Therefore, it is suggested that future exemptions do not include synthetic particles unless sufficient evidence is developed to suggest their release is suitable. Should other classes of particle tracer be identified, these should be considered on their own merits having regard to the principles applied to existing particle tracers.

Unlike the chemical dyes and microbial tracers for which assessment of the innate properties of the tracer is required, particle tracers should be considered suitable for exemptions providing they are naturally occurring (with/without fluorescent coating). The maximum release amount for particle tracers added to the approved list is 250kg, as described in Section 2.4.1.

3. Results of Applying the Methodology to the Prioritised List of Tracers

The methodology for considering tracers suitable for exemption, as summarised in Section 2, has been used to consider the tracers proposed on the prioritised list (Table 1). A summary of the data used to consider chemical dyes on the prioritised list is available in Annex B.

3.1 Chemical dyes

Table 3 presents the outcome of applying the methodology to chemical dyes on the prioritised list, highlighting which tracers are considered suitable for exemption and the criteria (amount) imposed to manage the risk.

Table 3: Outcomes of the assessment of chemical dye tracers on the prioritised list in relation to human health and environmental risk.

Tracer	Human Health Risk (incl. CMR)	Persistence (P), Bioaccumulation (B) and (Eco)Toxicity (T)			Outcome	Criteria
		P	B	T		
Diphenyl methane	Passed	Passed (Lower threshold)	Passed (Upper threshold)	Passed (Upper threshold)	Consider for exemption with stringent criteria	≤0.5kg
Triphenyl methane	Passed	Passed (Lower threshold)	Failed	Failed	No exemption	N/A
Erioglaucine	Passed	Failed	Passed (Lower threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg
Fluorescein	Passed	Passed (Lower threshold)	Passed (Lower threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg
Leucophor BS	No Data	Failed	Passed (Lower threshold)	Passed (Lower threshold)	No exemption	N/A
Leucophor C	No Data	No Data	No Data	No Data	No exemption	N/A
Leucophor CK	No Data	No Data	No Data	No Data	No exemption	N/A

Tracer	Human Health Risk (incl. CMR)	Persistence (P), Bioaccumulation (B) and (Eco)Toxicity (T)			Outcome	Criteria
		P	B	T		
Malachite green	Failed	Passed (Upper threshold)	Passed (Lower threshold)	Failed	No exemption	N/A
Pyranine	Failed	Passed (Upper threshold)	Passed (Lower threshold)	Passed (Lower threshold)	No exemption	N/A
Rhodamine WT	Passed	Failed	Passed (Lower threshold)	Passed (Upper threshold)	Consider for exemption with stringent criteria	≤0.5kg
Sulforhod-amine G	Passed	Passed (Upper threshold)	Passed (Lower threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg
Uvitex CF	Passed	Failed	Passed (Upper threshold)	Failed	No exemption	N/A
Uvitex WGS	Passed	Passed (Lower threshold)	Passed (Upper threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg
Xanthene	Passed	Passed (Lower threshold)	Passed (Upper threshold)	Passed (Lower threshold)	Consider for exemption with criteria	≤2kg

Leucophor BS, Leucophor C and Leucophor CK have not been considered for exemption as no data was found regarding these substances (particularly regarding CMR risk and other human health effects) and, thus, it would not be appropriate to exempt the use of these tracers (see Annex B). Malachite green and Pyranine have not been considered for exemption as both are considered to pose a potential risk to human health. Triphenyl methane and Uvitex CF did not meet the PBT threshold criteria and, therefore, have not been considered suitable for exemption. A very low ecotoxicity (invertebrate) value was estimated for Rhodamine WT (0.94µg/l). However, it was considered appropriate to disregard this value on the basis that it does not appear to be consistent with the reported experimental invertebrate data (>100mg/l). Therefore, Rhodamine WT has been considered suitable for exemption, albeit at a reduced release amount (0.5kg) due to the potential toxic effects on algal species.

After determining which chemical dye tracers were suitable for exemption, the maximum discharge rate into the receiving environment was also calculated for each tracer. Table 4 presents the results of the maximum discharge rate calculations (see Section 2.4.2). On the basis of these calculations, for Erioglaucine and Sulforhodamine G, the entire 2kg could be added in a single “gulp” release. For the remaining tracers, releases will need to occur over a longer time period to avoid exceeding the lowest short-term NOEC outside of the mixing zone. An average discharge rate has also been presented in Table 4 to demonstrate the amount of these tracers that can be released per minute and the total duration in order to release the maximum permissible amount.

Table 4: Summary of maximum rates of delivery (single release and average release rate) for chemical dye tracers on the prioritised list. NOEC are estimated values.

Tracer	NOEC (mg/l)	Total amount (kg)	Maximum single release (kg)	Average rate of delivery for releases exceeding maximum single release	
				Amount per minute (kg/min)	Total duration (minutes)
Erioglaucine	29.0	2.00	2.00	-	-
Sulforhodamine G	7.94	2.00	2.00	-	-
Uvitex WGS	0.91	2.00	0.91	0.130	15.4
Fluorescein	0.89	2.00	0.89	0.128	15.7
Xanthene	0.32	2.00	0.32	0.046	43.8
Diphenyl methane	0.10	0.50	0.10	0.015	33.7
Rhodamine WT	0.05	0.50	0.05	0.007	70.0

3.2 Microbial tracers

Table 5 presents the outcome of the exemption process for microbial tracers, highlighting which tracers are considered suitable for exemption and the criteria (concentration/amount) imposed to manage the risk. As previously discussed, *Bacillus* spp. has been considered unsuitable for exemption based on potential pathogenic risk.

Table 5: Outcomes of the assessment of microbial tracers on the prioritised list in relation to pathogenicity risk.

Tracer	Pathogenicity	Outcome	Criteria
Bacillus globigii spores	Failed	No exemption	N/A
Bacillus subtlis var *Niger*	Failed	No exemption	N/A
Phi-X174 coliphage	Passed	Consider for exemption with criteria	≤10^{14} cells/ml (5l max)
MS2 coliphage	Passed	Consider for exemption with criteria	≤10^{14} cells/ml (5l max)
PRD1 phage	Passed	Consider for exemption with criteria	≤10^{14} cells/ml (5l max)
Bacteriophage for *Enterobacter cloacae*	Passed	Consider for exemption with criteria	≤10^{14} cells/ml (5l max)
Bacteriophage for *Serattia marcens*	Passed	Consider for exemption with criteria	≤10^{14} cells/ml (5l max)

3.3 Particle tracers

Table 6 presents the outcome of the exemption process for particle tracers, highlighting which tracers are considered suitable for exemption and the criteria (amount) imposed to manage the risk.

Table 6: Outcomes of the assessment of particle tracers on the prioritised list in relation to the risk of smothering.

Tracer	Smothering	Outcome	Criteria
Fluorescent coated natural particles	Passed	Consider for exemption with criteria	≤250kg
Fruit (e.g. Oranges and Apples) and seeds	Passed	Consider for exemption with criteria	≤250kg
Fluorescent (painted) pebbles	Passed	Consider for exemption with criteria	≤250kg

3.4 Approved list of exempt tracers

Table 7 presents the full approved list of exempt tracers, summarising the tracers from the prioritised list which are considered suitable for exemption. In total, seven chemical dyes, five microbial tracers and three types of particle tracers have been included.

Table 7: Approved list of exempt tracers.

Tracer	Total release amount	Maximum single release	Average rate of delivery for releases exceeding maximum single release
Chemical dyes			
Erioglaucine	2.00kg	2.00kg	-
Sulforhodamine G	2.00kg	2.00kg	-
Uvitex WGS	2.00kg	0.91kg	0.130kg/min
Fluorescein	2.00kg	0.89kg	0.128kg/min
Xanthene	2.00kg	0.32kg	0.046kg/min
Diphenyl methane	0.50kg	0.10kg	0.015kg/min
Rhodamine WT	0.50kg	0.05kg	0.007kg/min
Microbial tracers			
Phi-X174 coliphage	$\leq 10^{14}$ per ml (5l max)	-	-
MS2 coliphage	$\leq 10^{14}$ per ml (5l max)	-	-
PRD1 phage	$\leq 10^{14}$ per ml (5l max)	-	-
Bacteriophage for *Enterobacter cloacae*	$\leq 10^{14}$ per ml (5l max)	-	-
Bacteriophage for *Serattia marcens*	$\leq 10^{14}$ per ml (5l max)	-	-
Particle tracers			
Fluorescent coated natural particles	≤250kg	-	-
Fruit (e.g. Oranges and Apples) and seeds	≤250kg	-	-

Tracer	Total release amount	Maximum single release	Average rate of delivery for releases exceeding maximum single release
Fluorescent (painted) pebbles	≤250kg	-	-

For chemical dyes, it should be noted that the project team has utilised both experimental and estimated ecotoxicological data to assess the most sensitive taxonomic groups and establish an approved list of exempt tracers. This was undertaken through expert judgement and consideration of the small quantities of tracer permissible for release using this methodology. Furthermore, a pragmatic approach was necessary in order to generate an approved list based on the limited data available. However, as stated in the methodology (Section 2), future additions to the approved list of exempt tracers should in the first instance consider experimental data for the most sensitive taxonomic groups. Where only modelled/estimated data is available for the most sensitive taxonomic group (i.e. the species with the lowest LC50/EC50) a tracer should only be considered for exemption where the data has been reviewed by an expert panel.

In order to apply an exemption using a tracer on the approved list (Table 7), the tracer must be used in accordance with the maximum release amount stated, as well as the maximum discharge rate (chemical dyes only). However, it is also necessary to consider the activity of releasing and monitoring the tracer and the potential risks these activities may pose on the environment. Section 4 provides an assessment of these risks and the criteria to mitigate those risks.

4. Risk Assessment for Application of Exempt Tracers

4.1 Outline

The activities associated with the release and subsequent monitoring of tracers in the marine environment may give rise to environmental risks associated with damage or disturbance to ecological receptors and/or interfere with existing marine activities.

The methods of tracer introduction and requirements for subsequent monitoring can vary significantly between different tracer releases on a project-specific basis and different methods and approaches may be used for the same tracer on different projects. The duration of such activities may also vary between projects. The environmental risks associated with such activities are also likely to be very site specific, depending on the distribution and sensitivity of particular receptors. For example, a small amount of additional boat activity in a busy waterway may pose little additional risk to seabirds, whereas such activity in a more remote location adjacent to a seabird breeding colony in the breeding season could have more significant impacts.

If a tracer survey is to be exempted, criteria need to be established that can effectively manage such risks across a wide range of different possible activities and take account of the differing sensitivity of the receiving environment to such activities. To facilitate the identification of potential risks to different receptors, a risk assessment has been undertaken, involving the following steps:

- Define the range of activities associated with different types of tracer release and monitoring activity
- Identify the hazards associated with these activities
- Assess the environmental risks associated with the hazards
- Define criteria to manage the risks.

4.2 Define the range of activities

To support the risk assessment approach, it was necessary to understand the activities relating to the release of tracers into the receiving environment and their monitoring once they have been released. Such information facilitated the consideration of changes that would be introduced into the marine environment. This could include the presence of people, vehicles and vessels in and around the marine environment which may present a hazard to wildlife or navigation. However, the activities are not inherently hazardous and the degree of environmental risk is, therefore, very much dependent on the exposure of receptors to the change.

Information contained in recently approved licences on the MMO public register relating to activities undertaken during tracer studies has been used to categorise the method by which tracers are typically released into the environment and subsequently monitored. Based on these licences, the following two categories were considered to best summarise tracer activities:

- From a vehicle (including hovercraft) or vessel
- Other (e.g. aeroplane or marine structure).

The first category is relatively specific in that it considers tracer release and monitoring activities from a vehicle or vessel only. In contrast, the second category has been left purposefully open to enable the capture of all alternative, and perhaps less common, licensable methods of tracer activity (e.g. from an aeroplane). Any proposed tracer survey using release methods in the 'Other' category are not considered suitable for an exemption as such activities could pose novel risks to the marine environment which merit more detailed consideration. It should be noted that tracer releases from land by hand are not licensable by the MMO and, therefore, an exemption would not apply. It is advised that the user consults the Environment Agency in order to consider undertaking such an activity.

4.3 Assess the risks

Understanding the methods (or locations) used to release and monitor a tracer has enabled consideration of potential impact pathways for a risk assessment. For instance, knowing a tracer is to be released from a vessel allows appropriate impact pathways to be considered; in this case, potential impact pathways may include, amongst others, underwater noise, anchoring and visual disturbance. To identify the potential risks to the environment and other marine activities, a risk assessment has been undertaken which takes account of the different nature of possible release and monitoring activities. This has been undertaken by identifying linkages between potential impact pathways and environmental and human activity receptors.

To support the risk assessment, the following list of standard impact pathways have been used for environmental receptors (based on the Natura 2000 Regulation 35 impact categories), together with the identification of relevant social and economic receptors:

- Environmental receptors
  - Physical damage of habitats and species
    - Direct removal of seabed
    - Smothering
    - Trampling
    - Damage/disturbance to habitat
    - Damage/disturbance to species.
  - Non-physical disturbance
    - Airborne noise
    - Underwater noise
    - Barrier to species movement
    - Death or injury by collision
    - Vessel collision (incl. release of pollutants)
    - Visual disturbance
    - Introduction or spread of non-indigenous species (NIS).
- Social receptors
    - Visual disturbance
    - Airborne noise

- Economic receptors
  - Tourism/recreation impacts
    - Vessel collision (incl. release of pollutants)
    - Barrier to vessel navigation
    - Visual disturbance
    - Airborne noise.
  - Commercial navigation
    - Vessel collision (incl. release of pollutants)
    - Barrier to vessel navigation.

To avoid duplication, the above impact pathways have been grouped to form a set of pressures which could result in a significant impact. In addition, ‘direct removal of seabed’ and ‘smothering’ have not been assessed. This is because an MMO licence for sediment removal and the criteria (amount) established for particle tracers are thought to mitigate these respective impact pathways.

All impact pathways identified were considered applicable to activities from a vehicle and vessel. Similarly, all impact pathways have been marked for ‘Other’ activities as it is not possible to define the activity. However, as previously suggested, such activities are not suitable for exemption due to this uncertainty. Therefore, the primary consideration of this risk assessment process relates to activities from a vehicle or vessel. The relevant receptors of the impact pathways considered are as follows:

- Benthic habitats and species
- Fish
- Birds
- Marine mammals
- Protected sites
- Fisheries/Shellfisheries
- Tourism/Recreation
- Shipping.

Table 8 presents a simple matrix of receptors against potential impact pathways. However, receptors were only marked (ticked) if a significant impact could be anticipated. For example, airborne noise associated with a tracer survey could cause significant disturbance to bird or marine mammal (e.g. seal) populations, but it is unlikely to significantly affect tourism/recreation receptors. To mitigate the risks posed by tracer activities on these receptors, Section 4.4 outlines a series of criteria which must be complied with in order for an exemption to apply, as well as several recommendations for best practice.

Table 8: Matrix of receptors against potential impact pathways of deployment methodologies for tracers.

Receptor	Impact pathway										
	Trampling	Damage/disturbance to habitat	Damage/disturbance to species	Airborne noise	Underwater noise	Barrier to species movement	Death or injury by collision	Visual disturbance	Introduction or spread of non-indigenous species	Vessel collision (incl. release of pollutants)	Barrier to vessel navigation
Benthic Habitats/ Species		✓	✓						✓	✓	
Fish					✓				✓	✓	
Birds	✓			✓			✓	✓		✓	
Marine Mammals	✓			✓	✓	✓	✓	✓		✓	
Protected Sites		✓	✓	✓	✓		✓	✓	✓	✓	
Fisheries/ Shellfisheries		✓	✓						✓	✓	✓
Tourism/ Recreation								✓		✓	✓
Shipping										✓	✓

4.4 Developing criteria for application of exempt tracers

To support the risk assessment process and manage any potential environmental risk attributed to tracer applications, a series of criteria needed to be developed (Section 4.4.1). In addition, it was considered appropriate to recommend best practice measures relating to tracer activities in the marine environment (Section 4.4.2), although these are not licensable by the MMO.

4.4.1 Criteria

Given the nature of the exemption process, all exemptions need to be underpinned by some criteria which the user must adhere to in order to adequately manage environmental risk. Therefore, it was necessary to establish a series of general criteria which, under all circumstances, the user must comply with for the proposed tracer study to be considered for exemption. A list of criteria have been developed through consideration of previous licence documents relating to the use of tracers on the MMO public register and input received from the MMO and its primary advisors (Table 9). This includes strict direction for the user as to the permissible nature of tracer activities, such as not using the exemption process as a means for disposal (Article 17 of The Marine Licensing (Exempted Activities) Order 2011 (as amended)). In addition, the criteria have been established to enable the frequency of exempt

tracer activities to be regulated, limiting the number of exemptions permissible per user.

Table 9: Criteria for application of exempt tracers.

Criteria
An exemption does not apply should the tracer activity be likely to cause obstruction or danger to navigation.
An exemption does not apply should the tracer release be made for the purpose of disposal.
In applying the exemption, the user must not release the same approved tracer in the same location (at least 1km difference) on more than twelve occasions in a twelve month period, with a maximum of four releases in any given month and with at least one day between each release.
If applying the exemption within an area under the jurisdiction of a port or harbour authority, the user must consult with the responsible local navigation authority prior to any activities commencing.
If applying the exemption within 1km of a bathing water during the bathing season (15 May to 30 September), the user must consult the Environmental Agency prior to any activities commencing.
The user must report and record any oil, fuel or chemical spill in accordance with the advice at https://www.gov.uk/marine-pollution-incident-response.
If applying the exemption through the use of a hovercraft within a Special Area of Conservation (SAC), Special Protection Area (SPA) or Site of Special Scientific Interest (SSSI), a wildlife license is likely to be required and various controls may apply. In such circumstances, the user must also consult Natural England.
If applying an exemption using a particle tracer placed directly on the seabed/ intertidal zone, the tracer must not be placed on an area greater than $10m^2$.
If applying an exemption using a particle tracer, the median particle diameter of the tracer must not be more than 1 phi class smaller than the prevailing sediment.
The user must apply for and obtain any other relevant licences to conduct the proposed work prior to any activities commencing.

It could be argued that it is necessary to impose a restriction on tracer release location based on proximity to shellfish harvesting areas or protected sites. For instance, The Marine Licensing (Exempted Activities) Order 2011 (as amended) suggests an exemption should not apply if the tracer activity is likely to have a significant effect on a Ramsar site or capable of affecting protected features of Marine Conservation Zones (MCZs). However, considering this is a process by which to exempt tracers which are not deemed to pose any significant environmental risk, as demonstrated in Section 3, these additional criteria are not considered necessary. A distinction for ‘bathing waters’ was retained due to the potential direct overlap between tracer and human receptors and the impact on public perception (hence, consultation with the Environment Agency is required).

It is recognised that the exemptions process also needs to take account of potential cumulative effects. However, those notifying an exemption will not have information on other projects or plans and it is not realistic to expect those notifying exemptions to undertake detailed assessments of potential cumulative impacts. To minimise the risk of potential cumulative effects, a criterion has been proposed that limits the

number of tracer releases that an individual may make under exemptions within a given time period. In addition, the general approach to defining exemptions has adopted a conservative approach, thus minimising the risk of cumulative effects.

The number of applications for tracer releases currently applied for from the MMO indicates that this assumption is robust. The MMO runs a regular check on exemption numbers before reporting exemption figures which should allow a sense check for tracer exemptions to ensure this situation does not change.

If these criteria cannot be met, an exemption cannot be used and the user would need to apply for and obtain a marine licence if they wish to proceed with the activity. It is not anticipated that these criteria will need modification in the future. However, if a tracer activity currently considered 'Other' (see Section 4.2) is frequently used and considered not to pose a significant environmental risk, new criteria may be required if the activity is to be considered suitable for use with exempt tracers.

4.4.2 Recommendations for best practice

The criteria established in Section 4.4.1 mitigate many of the potential impacts considered as a result of the risk assessment. However, in considering other potentially significant risks to receptors, Table 10 identifies a series of recommendations for best practice.

Table 10: Recommendations for best practice regarding tracer activities.

Recommendations for best practice
When anchoring is necessary to complete the activity, the user should avoid seagrass beds and reef habitats.
Activities should avoid seal colonies and seal haul outs throughout the year.
Activities should avoid seabird breeding colonies during the breeding season.
Ballast water releases should be managed as part of the activity.
Good practice for operating vessels in the marine environment should be followed.
The user should ensure that any equipment, temporary works and/or debris (including litter) associated with the activity are removed upon completion of the activity.

While these recommendations for best practice are not enforceable by the MMO, users should be encouraged to adopt them to ensure that impacts to the marine environment are minimised.

5. Conclusions

This study has developed and applied a process for defining exemptions for existing tracers and a process for determining exemptions for potential new tracers in the future. The method has been used to produce an approved list of tracers considered suitable for exemption (Table 11). In addition, a series of criteria associated with tracer exemptions have been established (Table 12), as well as recommendations for best practice (Table 13). While the recommendations for best practice are not enforceable by the MMO, users should be encouraged to adopt them to ensure that impacts to the marine environment are minimised.

It is suggested that the MMO undertake consultation on the proposals prior to their formal implementation. The MMO should review any subsequently adopted exemptions within two years of implementation and obtain feedback from users.

Table 11: Approved list of exempt tracers.

Tracer	Total release amount	Maximum single release	Average rate of delivery
Chemical dyes			
Erioglaucine	2.00kg	2.00kg	-
Sulforhodamine G	2.00kg	2.00kg	-
Uvitex WGS	2.00kg	0.91kg	0.130kg/min
Fluorescein	2.00kg	0.89kg	0.128kg/min
Xanthene	2.00kg	0.32kg	0.046kg/min
Diphenyl methane	0.50kg	0.10kg	0.015kg/min
Rhodamine WT	0.50kg	0.05kg	0.007kg/min
Microbial tracers			
Phi-X174 coliphage	$\leq 10^{14}$ per ml (5l max)	-	-
MS2 coliphage	$\leq 10^{14}$ per ml (5l max)	-	-
PRD1 phage	$\leq 10^{14}$ per ml (5l max)	-	-
Bacteriophage for *Enterobacter cloacae*	$\leq 10^{14}$ per ml (5l max)	-	-

Tracer	Total release amount	Maximum single release	Average rate of delivery
Bacteriophage for *Serattia marcens*	≤10^{14} per ml (5l max)	-	-
Particle tracers			
Fluorescent coated natural particles	≤250kg	-	-
Fruit (e.g. Oranges and Apples) and seeds	≤250kg	-	-
Fluorescent (painted) pebbles	≤250kg	-	-

Table 12: Criteria for application of exempt tracers.

Criteria
An exemption does not apply should the tracer activity be likely to cause obstruction or danger to navigation.
An exemption does not apply should the tracer release be made for the purpose of disposal.
In applying the exemption, the user must not release the same approved tracer in the same location (at least 1km difference) on more than twelve occasions in a twelve month period, with a maximum of four releases in any given month and with at least one day between each release.
If applying the exemption within an area under the jurisdiction of a port or harbour authority, the user must consult with the responsible local navigation authority prior to any activities commencing.
If applying the exemption within 1km of a bathing water during the bathing season (15 May to 30 September), the user must consult the Environmental Agency prior to any activities commencing.
The user must report and record any oil, fuel or chemical spill in accordance with the advice at https://www.gov.uk/marine-pollution-incident-response.
If applying the exemption through the use of a hovercraft within a Special Area of Conservation (SAC), Special Protection Area (SPA) or Site of Special Scientific Interest (SSSI), a wildlife license is likely to be required and various controls may apply. In such circumstances, the user must also consult Natural England.
If applying an exemption using a particle tracer placed directly on the seabed/ intertidal zone, the tracer must not be placed on an area greater than 10m^2.
If applying an exemption using a particle tracer, the median particle diameter of the tracer must not be more than 1 phi class smaller than the prevailing sediment.
The user must apply for and obtain any other relevant licences to conduct the proposed work prior to any activities commencing.

Table 13: Recommendations for best practice regarding tracer activities.

Recommendations for best practice
When anchoring is necessary to complete the activity, the user should avoid seagrass beds and reef habitats.
Activities should avoid seal colonies and seal haul outs throughout the year.
Activities should avoid seabird breeding colonies during the breeding season.
Ballast water releases should be managed as part of the activity.
Good practice for operating vessels in the marine environment should be followed.
The user should ensure that any equipment, temporary works and/or debris (including litter) associated with the activity are removed upon completion of the activity.

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Annex A: Initial List of Tracers

Tracers included in the prioritised list are highlighted (bold).

Table A.1: Initial list of tracers identified.

Initial list of tracers		
Chemical Dyes		
Amino Acid G	**Leucophor C**	Rhodamine B
Auramine	**Leucophor CK**	**Rhodamine WT**
Calcophor White ST	Lissamine	Safranine
Diphenyl Brilliant Flavine	Lissamine FF	Sodium Napthionate
Diphenyl Methane	**Malachite Green**	**Sulforhodamine G**
Disazo	Methyl Violet/Paris Violet	Sulphorhodamine B
Eosin Yellow	Methylene Blue	Tinopal
Erioglaucine	Photine CU	**Triphenyl Methane**
Erythrosin B	Primuline	Uranine
Fluorescein	**Pyranine**	**Uvitex CF**
Fluorescent Brightener 28	Rhodamine 123	**Uvitex WGS**
Fuchsine Acid	Rhodamine 123	**Xanthene**
Leucophor BS	Rhodamine 6G	
Mircobial Tracers		
***Bacillus globigii* spores**	**Bacteriophage for *Serattia marcens***	**PRD1 phage**
Bacillus subtlis* var *Niger	**MS2 coliphage**	*Serratia indica*
Bacteriophage for *Enterobacter cloacae*	**Phi-X174 coliphage**	

Initial list of tracers		
Radionuclides		
Barium-133	Radium-224	Thorium-234
Carbon-14	Scandium-46	Tritium (3H)
Lanthanum-140	Selenium-75	
Lead-210	Silver-110m	
Particle Tracers		
Club moss (*Lycopodium clavatum*) spores, sometimes coloured with fluorescent dyes such as acridine orange (particularly adhesive) (Behrens *et al*. 2001)	Fluorescent polymer particles	Magnetic tracers
Fluorescent formaldehyde resin plastic	**Fluorescent coated natural particles**	Manufactured particles designed to mimic natural particles
Fluorescent (painted) pebbles	**Fruit (such as Oranges and Apples) and seeds**	Molecular (plant DNA coded) polymer particles
Other well documented tracer types		
Sulphur Hexafluoride (SF6)	EDTA complexes with metals	Perfluorodecalin (PFD) or Perfluoromethyldecalin (PMD)
Chlorofluorocarbons (CFC)	Benzoates and Fluorobenzoates	

Annex B: Assessing the Relative Environmental Hazards and Risks Posed by Individual Tracers

B.1 Background

The physico-chemical properties of the tracers have been characterised to establish a set of criteria that can be used to assess the relative environmental hazards and risk posed by these substances and to establish thresholds of acceptability for an exemption. The tracers that are of greatest potential concern with regard to the health of humans and environmental (wildlife) species are those that exhibit:

- Persistence (P), whereby they remain intact (chemical dyes or particles) or viable (microbes) for exceptionally long periods of time. In the case of synthetic compounds or particles, persistence can be years and even decades.
- Bioaccumulation (B) in the fatty tissue of living organisms, including humans, and are found at higher concentrations at higher levels in the food chain when compared to background levels in the corresponding environment (e.g. air or water), leading to adverse effects.
- Short-term toxicity (T) to humans and/or environmental (wildlife) species with consideration of endpoints such as growth, reproduction and survival (less than seven days exposure). For microbial tracers, toxicity is more appropriately expressed as pathogenicity which relates to evidence of disease in important food species (such as molluscs and fish), birds, marine mammals (such as seals, dolphins and porpoises) and humans.

Substances showing persistence, bioaccumulation and/or toxicity (PBT) properties may also become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air. The European Union Technical Guidance Document on Risk Assessment (TGD)[7] indicates additional issues for PBT chemicals that may not be adequately addressed by the traditional risk assessment methodologies, including:

- The concern that such substances may accumulate in parts of the environment.
    - The effects of such accumulation are unpredictable in the long-term.
    - Accumulation would be practically difficult to reverse.
- The concern that remote areas of the world's oceans should remain untouched by hazardous substances resulting from human activity, and that the intrinsic value of pristine environments should be protected.

Internationally, the principles of persistence, bioaccumulation potential and toxicity as indicators of hazard came to form part of various regulatory initiatives in the late 1990s. Subsequently, these principles have been developed at both national and international levels.

[7] Available at: http://ihcp.jrc.ec.europa.eu

B.2 Review of PBT criteria adopted by regulatory frameworks

A review of the PBT criteria adopted by a number of relevant regulatory bodies under differing legislative frameworks was carried out to assess the variability in the approach adopted. The legislative frameworks considered were the following:

- The United Nations Economic Commission for Europe (UN-ECE) Aarhus Protocol.
- The United Nations Environment Programme (UNEP) Stockholm Convention.
- The OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic.
- The European Union Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (REACH) (relevant to the coastal waters of EU Member States).

The criteria given in the Offshore Chemicals Notification Scheme (OCNS) were also considered, though a specified PBT approach is not adopted in the scheme.

The PBT criteria for the different legislative frameworks will generally have initially been derived by a Technical Committee, but the final values adopted may have included an element of consensus where large numbers of countries are involved in the decision-making process.

B.3 Comparison of different PBT criteria used in regulatory frameworks

The legislative frameworks shown in Table B.1 have agreed threshold criteria by which PBT substances can be identified as part of their regulatory programmes. However, at present, there is little coordination or consistency between the approaches and the criteria defined by different authorities. This is illustrated by the data given in Table B.1, which highlight the following points:

- For the persistence criterion, the ranges in half-life values vary significantly
    - Half-life ≥2 days to >2 months in air
    - Half-life >40 days to >6 months in water
    - Half-life >120 days to >12 months in sediment
    - Half-life >120 days to >6 months in soil.
- For the bioaccumulation criterion, the threshold log K_{ow} value ranges from >3 to >5, whilst the Bioconcentration Factor (BCF) (and related Bioaccumulation Factor) threshold value ranges from >100 to >5000 (i.e. a 50 fold difference).
- For the toxicity criterion (human health), the substance is classified as Carcinogenic, mutagenic or toxic for reproduction (CMR) under the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures and/or there is evidence of a chronic toxicity in mammals.
- For short-term toxicity data (ecotoxicity), the lowest LC_{50} and EC_{50} value ranges from <1.0mg/l to <10mg/l.

The legislative frameworks given in Table B.1 relate specifically to chemicals and, therefore, there are no specific criteria for pathogenicity.

Table B.1: Overview of PBT criteria applied under different legislative frameworks.

Regulation	Criteria		
	Persistence	**Bioaccumulation**	**Toxicity**
United Nations Economic Commission for Europe (UN-ECE) Aarhus Protocol[1]	Half-life in water >2 months. **or** Half-life in sediment >6 months. **or** Half-life in soils >6 months.	Octanol-water partition coefficient value (log K_{ow}) >5. **or** Bioconcentration Factor (BCF) or Bioaccumulation Factor (BAF) >5000.	Potential to adversely affect human health or the environment[2].
United Nations Environment Programme (UNEP) Stockholm Convention[3]	Half-life in water >2 months **or** Half-life in sediment >6 months. **or** Half-life in soils >6 months.	log K_{ow} >5 **or** BCF or BAF >5000	Evidence of adverse effect on human health or the environment or toxicity characteristics indicating potential damage to human health or environment[2].
OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic on the Marine Environment (as defined in Annex 4 of OSPAR Publication No. 256/2006)	Not readily biodegradable. **or** Half-life in water >50 days.	log K_{ow} ≥4 **or** BCF ≥500 **or** Monitoring data in biota.	***Acute aquatic toxicity*** LC_{50} or EC_{50} ≤1mg/l and long-term No Observed Effect Concentration (NOEC) ≤0.1mg/l. **or** ***Mammalian toxicity*** Carcinogenic, mutagenic or toxic for reproduction (CMR) or chronic toxicity.

Regulation	Criteria		
	Persistence	**Bioaccumulation**	**Toxicity**
European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	Half-life in marine water is >60 days. **or** Half-life in fresh or estuarine water is >40 days. **or** Half-life in marine sediment is >180 days. **or** Half-life of fresh or estuarine sediment is >120 days. **or** Half-life in soil is >120 days.	BCF >2000	The long-term NOEC for marine or freshwater organisms is <0.01mg/l. **or** The substance is classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2) or toxic for reproduction (category 1, 2 or 3). **or** There is evidence of chronic toxicity, as identified by the classifications T, R48, or Xn, R48 according to Directive 67/548/EEC.
UK Department of Trade and Industry (DTI) Offshore Chemical Notification Scheme (OCNS)	<70% degradation after 28 days in OECD TG 301A or 301E. **or** <60% degradation after 28 days in OECD TG 301B. 301C, 301D, 301F or 306.	log K_{ow} ≥3. **or** BCF ≥100.	Lowest short-term LC_{50} or EC_{50} value from a range of aquatic taxonomic groups (namely algae, invertebrates and fish) <10mg/L.

[1] Criteria are applied for long range transport potential, namely: Vapour pressure <1000 Pa and half-life in air >2 days or Monitoring data in remote area.
[2] Exact criteria are not defined, but for the context of this assessment, the potential to adversely affect human health or the environment is considered to be satisfied if the substance is a CMR or if there is demonstrable long-term chronic toxicity data (i.e. a chronic NOEC) at <0.1mg/l.

Regulation	Criteria		
	Persistence	Bioaccumulation	Toxicity
[3] Criteria are applied for long range transport potential, namely: Measured levels far from source or monitoring data in remote area or multimedia modelling evidence and half-life in air >2 days.			

B.4 Adoption of PBT criteria for the assessment of the hazards and risk posed by a tracer

For the identification of tracers which can be exempted because they pose no or a limited hazard and risk to humans or wildlife, the PBT criteria applied need to be proportionate, scientifically defensible and precautionary. However, the criteria should not be overly precautionary such that tracers that would not present a hazard or risk to humans and the environment are excluded. Having reviewed the criteria for the legislative frameworks in Table B.1, the following PBT criteria have been used to consider tracers suitable for exemption:

- Persistence
  - Upper threshold - Half-life in water of ≤60 days
  - Lower threshold - Half-life in water of ≤40 days or evidence of ready biodegradability in a standard test procedure.
- Bioaccumulation
  - Upper threshold - Octanol-water partition (log K_{ow}) coefficient of ≤4.5 or a Bioconcentration Factor of ≤2,000
  - Lower threshold - Octanol-water partition (log K_{ow}) coefficient of ≤3.0 or a Bioconcentration Factor of ≤100.
- Toxicity (Ecotoxicity)
  - Upper threshold - Lowest short-term LC_{50}/EC_{50} of ≥0.1 mg/l for representative algal, invertebrate and fish species
  - Lower threshold - Lowest short-term LC_{50}/EC_{50} of ≥1.0 mg/l for representative algal, invertebrate and fish species.
- Toxicity (Human health)
  - Not classified as a CMR substance under the Regulation (EC) N^{o} 1272/2008 on classification, labelling and packaging of substances and mixtures
  - No evidence of chronic toxicity in mammals.

B.5 Collation and generation of the data required for the assessment of the hazards posed by tracers

B.5.1 Collation of experimental data

The assessment of the hazard posed by tracers in terms of their PBT properties is ideally based on reliable experimental data. It is expected that some literature information will be available on the persistence and bioaccumulation potential of tracers, but that the available toxicological data for these substances may be more limited. It is important that a robust and scientifically defensible evaluation of the toxicity of tracers is carried out as part of the hazard and risk assessment. Therefore, the approach taken to data availability needs to be sufficiently precautionary that it will not exempt tracers that present a risk of adverse effects to humans or wildlife, whilst only requiring a “reasonable” dataset to carry out the assessment.

A “reasonable” dataset would comprise the following experimental toxicological information for chemical dyes (to address the ecotoxicity criterion):

- Ecotoxicity data for a representative range of marine species, including at least one algal, invertebrate and fish species
- Data on potential adverse effects on humans (in terms of classification as Carcinogenic, mutagenic or toxic for reproduction (CMR) under the Regulation (EC) N^{o} 1272/2008 on classification, labelling and packaging of substances and mixtures and/or evidence of chronic toxicity in mammals).

In terms of chemical dye tracers on the prioritised list, information on the PBT properties was identified using targeted internet searches of publically available data. Ecotoxicological data was principally derived from the United States Environmental Protection Agency (US EPA) Ecotox database which is searchable based on chemical name or Chemical Abstract Service (CAS) number. However, information from other sources was also used (e.g. Cefas, 2011a; 2011b). Information on the effects of chemical dyes on human health in terms of CMR classification and/or chronic toxic effects in mammals was primarily derived from MSDSs available from the websites of manufacturers.

For microbial tracers, pathogenicity should be assessed by information on whether the microbes had the potential to cause disease in humans (and potentially other mammals).

B.5.2 Generation of estimated data

If experimental data were not available for certain required endpoints, predicted values were generated using appropriate tools such as those present in the Estimation Programs Interface Suite (EPI Suite™). The tool is a freely available Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by the US EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC)[8]. EPI Suite™ has undergone detailed review by a panel of the US EPA's independent Science Advisory Board (SAB). Furthermore, EPI Suite is included in the OECD QSAR Toolbox, which is identified for use under REACH.

EPI Suite™ comprises the following estimation programs: KOWWIN™, AOPWIN™, HENRYWIN™, MPBPWIN™, BIOWIN™, BioHCwin, KOCWIN™, WSKOWWIN™, WATERNT™, BCFBAF™, HYDROWIN™, KOAWIN and AEROWIN™ and the fate models WVOLWIN™, STPWIN™ and LEV3EPI™. The Ecological Structure Activity Relationships (ECOSAR™), which estimates ecotoxicity, is also included in EPI Suite™.

The BIOWIN™ and BCFBAF™ tools can be used to generate information on the biodegradability and bioaccumulation of substances. The ECOSAR™ Class Program predicts the potential toxicity of chemicals to organisms living in the water body to which the chemicals are discharged (i.e. fresh or marine waters). The model, which has been developed by the US EPA Office of Pollution Prevention and Toxics (OPPT), uses measured data to predict the toxicity of chemicals lacking experimental data by using Structure Activity Relationships (SARs) and Quantitative Structure Activity Relationships (QSARs). These estimate a chemical's acute (short-term) toxicity and, when data are available, chronic (long-term or delayed) toxicity.

[8] http://www.epa.gov/ oppt/exposure/pubs/episuite.htm

ECOSAR™ contains a library of chemical class-based (Q)SARs for predicting aquatic toxicity, along with an expert decision tree for selecting the appropriate chemical class for a new substance. (Q)SARs include acute and chronic toxicity endpoints for fish (both fresh and marine species), aquatic invertebrates (including the freshwater cladoceran *Daphnia* and marine mysids) and aquatic plants (principally freshwater green algae). In the ECOSAR™ tools, predictions for chemical dyes are generated based on a neutral organics mode of toxic action (which represents baseline toxicity) and, where appropriate, based on the chemical structure of the chemical dye substance classes with specific modes of toxic action (such as esters and triazines).

B.5.3 Outcome of the data evaluation exercise for chemical dyes

Tables B.2 and B.3 summarise the experimental and estimated persistence, bioaccumulation potential and toxicity (environmental and human health) data that has been collated for the chemical dye tracers identified in the prioritised list agreed with MMO. In the tables, estimated data for different criteria is highlighted to discriminate from experimental data. Where possible, ecotoxicological data were generated for marine species, but in certain instances data could only be generated for freshwater species. No data could be generated for the chemical dyes Leucophor C and Leucophor CK. The issue with Leucophor C was identifying an appropriate CAS Number to be used for literature searching. Since a CAS Number was not identified, searching was based on the substance name but did not result in the required data being identified. A CAS Number was identified for Leucophor CK, but the data searching exercise did not identify any of the required data. For Leucophor BS, a CAS Number was not identified, but data on persistence, bioaccumulation and toxicity was identified; however, no information on CMR status could be located.

A comparison of the experimental short-term toxicity data for marine copepods and the estimated data for marine mysids from the ECOSAR tool for prioritised chemical dyes (see Table B.4) shows that:

- Where the estimated values for marine mysids were based on specific chemical classes (esters for fluorescein, phenols-acid for pyranine and schiff bases-azomethine acid for sulforhodamine), the ratios between experimental and estimated values only differed by between 2.6 and 31.4 times.
- Where the estimated values for marine mysids were based on neutral organics, larger differences in the ratios between experimental and estimated values were evident (i.e. >690 times).

On the basis of this limited dataset, the ecotoxicological results estimated from the ECOSAR tool can be considered to be appropriate where they are generated from SARs/QSARs developed for specific chemical classes. This is because the extent of the differences between the experimental and estimated toxicity values are not markedly greater than may be found between laboratories for tests on the same substance. However, there is evidently greater uncertainty when baseline toxicity for chemical dyes is estimated using the neutral organics SARs/QSARs. Therefore, it is recommended that short-term toxicity values for specific acting chemicals are not estimated using these types of QSARs since they may provide marked under- or over-estimates of the likely toxicity from experimental studies.

Table B.2: Experimental and estimated persistence and bioaccumulation potential data collated for the chemical dye tracers identified in the prioritised list.

Substance	Formula	CAS Number	Persistence		Bioaccumulation	
			Half-life in water	Ready biogradability prediction	Octanol water partition coefficient (log K_{ow})	Bioconcentration Factor (l/kg)
Diphenyl methane	$C_{13}H_{12}$	101-81-5	15 days	Not readily biodegradable	4.02*	250*
Triphenyl methane	$C_{19}H_{16}$	519-73-3	38 days	Not readily biodegradable	5.37*	1610*
Erioglaucine	$C_{37}H_{45}N_5O_9S_3$	2650-18-2	180 days	Not readily biodegradable	-1.50*	3.16*
Fluorescein	$C_{20}H_{12}O_5$	153954	38 days	Not readily biodegradable	0.047	75.9*
Leucophor BS	$C_{32}H_{26}N_{12}O_6S_2Na_2$	Not identified	180 days	Not readily biodegradable	1.95*	3.16*
Leucophor C	Not identified	Not identified	No data	No data	No data	No data
Leucophor CK	Not identified	115469-37-9	No data	No data	No data	No data
Malachite green	$C_{23}H_{26}N_2O$	569-64-2	60 days	Not readily biodegradable	0.80*	3.16*

Substance	Formula	CAS Number	Persistence		Bioaccumulation	
			Half-life in water	Ready biogradability prediction	Octanol water partition coefficient (log K_{ow})	Bioconcentration Factor (l/kg)
Pyranine	$C_{16}H_{10}O_{10}S_3$	6358-69-6	60 days	Not readily biodegradable	-3.97*	3.16*
Rhodamine WT	$C_{29}H_{29}ClN_2Na_2O_5$	37299-86-8	180 days	Not readily biodegradable	0.047	5.62*
Sulforhodamine G	$C_{25}H_{25}N_2NaO_7S_2$	5873-16-5	60 days	Not readily biodegradable	-1.98*	3.16*
Uvitex CF	$C_{34}H_{28}N_{10}Na_2O_8S_2$	3426-43-5	180 days	Not readily biodegradable	4.03*	56.2*
Uvitex WGS	$C_{14}H_{17}NO_2$	91-44-1	38 days	Not readily biodegradable	3.22*	61.9*
Xanthene	$C_{13}H_{10}O$	92-83-1	38 days	Not readily biodegradable	4.23*	287*
Note: The majority of persistence and bioaccumulation data collated was estimated. *Values for which estimated data was obtained.						

Table B.3: Experimental and estimated toxicity (human health and environmental) data collated for the chemical dye tracers identified in the prioritised list.

Substance	Toxicity (human health)		Toxicity (Ecotoxicity) (mg/l)				
	CMR status	Evidence of chronic toxicity	Algal toxicity (EC_{50} value)	Invertebrate (copepod) toxicity (EC_{50} value)	Invertebrate (Oyster) toxicity (EC_{50} value)	Invertebrate toxicity (LC_{50} or EC_{50} value)	Fish toxicity (LC_{50} value)
Diphenyl Methane	Not classified	No data	>1.0	No data available	No data available	0.528	7.5
Triphenyl Methane	Not classified	No data	0.34*	No data available	No data available	0.019*	0.21*
Erioglaucine	Not classified	No data	74000*	145.1	No data available	>100000*	>100000*
Fluorescein	Not classified	No data	4.47*	160.7	28.0	337	997
Leucophor BS	No data	No data	4.56*	No data available	No data available	2.33*	3.25*
Leucophor C	No data	No data	No data available	No data available	No data available	No data available	No data available
Leucophor CK	No data	No data	No data available	No data available	No data available	No data available	No data available

Substance	Toxicity (human health)		Toxicity (Ecotoxicity) (mg/l)				
	CMR status	Evidence of chronic toxicity	Algal toxicity (EC_{50} value)	Invertebrate (copepod) toxicity (EC_{50} value)	Invertebrate (Oyster) toxicity (EC_{50} value)	Invertebrate toxicity (LC_{50} or EC_{50} value)	Fish toxicity (LC_{50} value)
Malachite Green	Not classified	Reproduction toxicity (Cat 2), H361d	10.0	No data available	No data available	0.012	0.28
Pyranine	Not classified	Specific target organ toxicity - single exposure (Cat 3), H335	42229*	2,703	485.4	7131*	54819*
Rhodamine WT	Not classified	No data	0.25*	422.3	118.2	0.00094*	10.0
Sulforhodamine G	Not classified	No data	63.4*	171.4	44.7	39.7*	298.3*
Uvitex CF	Not classified	No data	0.058*	No data available	No data available	0.21*	1.60*
Uvitex WGS	Not classified	No data	5.84*	No data available	No data available	4.56*	8.46*
Xanthene	Not classified	No data	1.60*	No data available	No data available	20.3	1.62*
Note: *Values for which estimated data was obtained.							

Table B.4: Comparison of the experimental short-term data for marine copepods and the estimated data for marine mysids generated from the ECOSAR tool for prioritised chemical dyes.

Chemical dye	Experimental short-term toxicity data for marine copepod (*Tisbe battagliai*) (48-hr EC_{50}) (mg/l)	Estimated short-term toxicity data for marine mysids (96-hr LC_{50}) (mg/l)	Ratio of short-term marine copepod to marine mysid toxicity data
Erioglaucine	145.1	>100000	0.00145
Fluorescein	160.7	5.12	31.4
Pyranine	2703	7131	0.38
Rhodamine WT	422.3	0.00094	449255
Sulforhodamine G	171.4	39.7	4.32

B.6 Conversion of EC_{50} and LC_{50} values to NOEC

B.6.1 EC_{50}

To gain a better understanding of the likely ratios between NOEC and EC_{50} values for chemical dyes, a review of the data reported by Cefas for the short-term *Crassostrea gigas* and *Tisbe battagliai* tests (Cefas, 2011) was conducted. The data indicated that the ratios between NOEC and EC_{50} values were between 1.1 and 6.2 (average of 3.0) for a range of chemical dyes (see Table B.5). On the basis of the available data, a conversion factor of 5 has been considered appropriate for EC_{50} values (i.e. divide EC_{50} values by 5 to estimate the NOEC).

Table B.5: Summary of the toxicity data for chemical dyes reported for short-term EC_{50} *Crassostrea gigas* and *Tisbe battagliai* tests (Cefas, 2011) and short-term LC_{50} tests on fish species.

Test Organism	Chemical Dye	NOEC (mg/l)	EC_{50} (mg/l)	EC_{50} to NOEC ratio
Crassostrea gigas (Oyster)	Eosin Yellow	0.22	0.31	1.4
	Fluorescein	20.9	28.1	1.3
	Pyranine	80	118	1.5
	Rhodamine WT	97	485.4	5.0
Tisbe battagliai (Copepod)	Eosin Yellow	9.9	28.3	2.9
	Erioglaucine	65	145.1	2.2
	Erythrosin B	4.4	14.6	3.3
	Fluorescein	43.7	160.7	3.7
	Fluorescent Brightener 28 (Tinopol)	22	63.8	2.9
	Lissamine	0.6	2.0	3.3
	Pyranine	2425	2703	1.1
	Rhodamine 6G	0.095	0.11	1.2

Test Organism	Chemical Dye	NOEC (mg/l)	EC_{50} (mg/l)	EC_{50} to NOEC ratio
	Rhodamine B	0.95	1.9	2.0
	Rhodamine WT	80	422.3	5.3
	Sulforhodamine B	7.5	32.8	4.4
	Sulforhodamine G	27.6	171.4	6.2

B.6.2 LC_{50}

Information on the relationship between NOEC and LC_{50} values for chemical dyes was obtained from the United States Environmental Protection Agency (US EPA) Ecotox database. Only limited relevant data is available for the identified chemical dyes which is summarised in Table B.6. The data indicated values between 1.4 and 2.8 but because of the greater uncertainty associated with the limited data that is available it is proposed that a conversion factor of 10 is used to estimate NOEC values from LC_{50} values (i.e. divide LC_{50} values by 10 to estimate the NOEC).

Table B.6: Summary of the toxicity data for chemical dyes reported for short-term LC_{50} tests on fish species.

Test organism	Chemical dye	NOEC (mg/l)	LC_{50} (mg/l)	LC_{50} to NOEC ratio
Leuciscus spp. *melanotis* (Carp)	Diphenyl methyl	5.0	7.5	1.5
Morone saxatilis (Striped bass)	Malachite green	25	71	2.8
Psetta maximus (Left eyed flounder)	Fluorescein	700	997.1	1.4