

Design for the Environment Program Criteria for Fragrances

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1 Introduction

Purpose

The DfE Criteria for Fragrances identify safer aroma chemicals and fragrance formulations for use in cleaning products that bear the DfE logo. The contents of the Criteria, including definitions and toxicological preferences, were developed to facilitate use of safer chemistry under the DfE Program. These criteria also enhance the transparency of the DfE Program.

The DfE Criteria for Fragrances is a pragmatic approach that uses hazard-based lists, combined with literature review, modeling, and expert judgment. It is appropriately different from methods typically used by EPA to evaluate chemicals in regulatory programs conducted under the Toxic Substances Control Act (TSCA). Two factors make the DfE approach appropriate for screening fragrance materials. First, the Criteria will be used to evaluate fragrances that typically contain large numbers of raw materials in such small quantities that they are not normally reviewed under other ecolabeling programs. Second, the Criteria provide a practical tool for moving the chemical components of fragrances towards safer substitutes. EPA will consider all sources of developing information, such as the Endocrine Disruptor Screening Program or enhancements to estimation models such as EPI Suite™ that occur over time.

Development

The DfE Criteria for Fragrances was developed by the Environmental Protection Agency's Design for Environment (DfE) Program and a group of stakeholders that included the fragrance industry, cleaning product formulators, environmental non-governmental organizations, and others.

Scope

More than 2,000 chemical substances with diverse chemical structures, and therefore diverse human and environmental health profiles, are used in formulation by the fragrance industry. To identify safer chemicals for this diverse set of raw materials, a range of human health and environment attributes serve as the basis for screening out fragrance raw materials of concern. To pass the fragrance criteria, a fragrance must meet all the thresholds and data requirements in this document. The requirements for all attributes in the Criteria apply to all fragrance raw materials and their components present in the fragrance at or above 100 ppm (0.01% by weight). This threshold is a stakeholder-agreed and conservative approach to screening fragrances. All non-aroma fragrance raw materials must meet the DfE Criteria for their ingredient class (e.g., i.e. solvents must meet the DfE Criteria for Solvents).

The DfE Criteria for Fragrances applies to aroma chemicals used in products, including the following: all purpose cleaners, carpet care products, machine warewash detergents, dishwash detergents, floor care products, manual dishwash detergents, hard surface cleaners, washroom cleaners, hand soaps (non-FDA regulated) and laundry detergents. Product categories that were not considered in development of these Criteria include air fresheners, hand soaps regulated by FDA, and personal care products such as lotions.

2 General Requirements

- 2.1** Data for all relevant routes of exposure will be evaluated. Failure to pass an Attribute by any relevant route of exposure results in failure to pass the Criteria.
- 2.2** The GHS criteria and data evaluation approach and EPA risk assessment guidance will inform professional judgment in the review of both no observed adverse effect levels/concentrations (NOAEL/NOAEC) and lowest observed adverse effect levels/concentrations (LOAEL/LOAEC). NOAEL/NOAEC and LOAEL/LOAEC values are preferred over no observed effect levels/concentrations (NOEL/NOEC) and lowest observed effect levels/concentrations (LOEL/LOEC). In reviews that include conflicting data, a weight-of-evidence approach will determine a pass or fail.
- 2.3** Use of existing data should follow the EPA HPV Challenge Program and OECD HPV Programme data adequacy guidelines: <http://www.epa.gov/HPV/pubs/general/datadfin.htm>.
- 2.4** The manufacturer shall fully disclose the fragrance formulation, including all raw materials (either individual chemicals or essential oils) intentionally added or present at a level of 100 ppm (0.01% by weight) or higher. Known impurities and residuals present at greater than 0.1% by weight in the fragrance raw material must be reported.
- 2.5** The Criteria apply to all fragrance raw materials and their components present in the fragrance at or above 100 ppm (0.01% by weight). All non-aroma fragrance raw materials must meet the DfE Criteria for their ingredient class (e.g., solvents must meet the DfE Criteria for Solvents).
- 2.65** The reviewer shall use the IFRA/IOFI Labeling Manual to identify components of essential oils that are potential concerns and therefore must be reviewed.
- 2.7** Fragrances must meet the International Fragrance Association (IFRA) Standards. The cleaning product manufacturer shall demonstrate compliance by supplying a written letter from all suppliers.
- 2.8** EPA will perform an additional in-depth review of a chemical under certain conditions. Conflicting data on a chemical, detection in bio- or environmental monitoring studies, or presence on a flagging list will trigger such a review. The additional review will apply GHS criteria and other criteria explained in this document.

3 Terms

- 3.1 Acute aquatic toxicity** is the intrinsic property of a substance to be injurious to an organism in a short-term exposure to that substance. (GHS)
- 3.2 Acute mammalian toxicity** refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours. (GHS)
- 3.3 Attribute:** The general property of the fragrance or raw material that is being evaluated (i.e. acute mammalian toxicity, biodegradability).
- 3.4 Auxiliary fragrance raw material:** Any ingredient in the fragrance whose primary function is something other than to impart a scent. For the purposes of these Criteria, this may include solvents, surfactants, chelating agents, and anti-oxidants.
- 3.5 Bioaccumulation** is a process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment, i.e., dietary and ambient environment sources. Bioaccumulation is the net result of competing processes of chemical uptake into the organism at the respiratory surface and from the diet and chemical elimination from the organism including respiratory exchange, fecal egestion, metabolic biotransformation of the parent compound and growth dilution. [1]
- 3.6 Biodegradation** is a process in which the destruction of the chemical is accomplished by the action of a living organism. (Handbook of Property Estimation Methods for Chemicals, 2000)
- 3.7 Carcinogen** denotes a chemical substance or mixture of chemical substances which induces cancer or increases its incidence. (GHS)
- 3.8 A chemical** is identified by its Chemical Abstract Service (CAS) number.
- 3.9 Chronic aquatic toxicity** is the potential or actual properties of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life cycle of the organism. (GHS)

3.10 Closed-loop dispensing system: A proportioning device that automatically mixes concentrates with water, and has the ability to dispense into secondary containers such as spray bottles or mop buckers, or equipment such as carpet cleaners. To be considered closed-loop, the dispensing system must prevent contact of concentrated chemical with end users or the environment at every stage in the process - from installing a new refill, to dispensing product, to replacing used refill containers.

3.103.11 A **component of a fragrance raw material** is defined as a chemical constituent of the fragrance raw material (e.g. an essential oil) present in the fragrance at greater than 0.01% by weight.

3.113.12 Criteria: Endpoints and cutoffs for attribute information. Example: oral acute mammalian toxicity LD50 must be > 50 mg/kg. Data quality requirements (including acceptable test methods and information sources) are developed for all criteria.

3.123.13 Degradation products of concern are chemicals formed from degradation of fragrance chemicals with high acute aquatic toxicity ($L/E/IC_{50} \leq 10\text{ppm}$) and which mineralize <60% in 28 days.

3.133.14 Dermal sensitizer: A substance that will induce an allergic response following skin contact (GHS)

- | **3.143.15** **Developmental toxicity:** Adverse effects in the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the lifespan of the organism. The major manifestations of developmental toxicity include: (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. (EPA Risk Assessment Guidelines [2])
- | **3.153.16** An **endocrine disruptor** is an external agent that interferes in some way with the role of natural hormones in the body. An agent might disrupt the endocrine system by affecting any of the various stages of hormone production and activity, such as by preventing the synthesis of hormones, by directly binding to hormone receptors, or by interfering with the natural breakdown of hormones. (EPA) [3]
- | **3.163.17** **Flagging list:** A publicly available list of chemicals that may have potential hazard concerns as identified by the authors of that list.
- | **3.173.18** **Fragrance (or fragrance finished product):** A complex mixture of fragrance raw materials for use in a cleaning product for the primary purpose of imparting a scent and/or masking base odor.
- | **3.183.19** **Fragrance compound:** A blend of fragrance ingredients, representing a specific fragrance formula. (IFRA Code of Practice)
- | **3.193.20** **Fragrance raw material:** Any substance, obtained by chemical synthesis or derived from a natural source, intentionally added or present in a fragrance at greater than 0.01 percent by weight whose primary purpose is to impart scent. In the context of these Criteria, fragrance raw materials include aroma chemicals, ~~fragrant extracts (essential oils)~~, and components of essential oils.
- | **3.203.21** **Genotoxicity:** The more general terms genotoxic and genotoxicity apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects. (GHS)
- | **3.213.22** An **ingredient** may be one chemical or a blend of multiple chemicals that are intentionally added.
- | **3.223.23** **LOAEL:** Lowest Observed Adverse Effect Level
- | **3.233.24** **Mutagen:** The term mutagenic and mutagen will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. (GHS)
- | **3.243.25** **Neurotoxicity:** An adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent. (US EPA Risk Assessment Guidelines)
- | **3.253.26** **NOAEL:** No Observed Adverse Effect Level
- | **3.263.27** **Persistence:** The length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes. (EPA PBT Final Rule [4])
- | **3.273.28** **Photo sensitizer:** A substance that will lead to an allergic response following skin contact under the influence of light exposure.

- | ~~3.283.29~~ **Reproductive toxicity:** The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. (US EPA Risk Assessment Guidelines [5])
- | ~~3.293.30~~ **Respiratory sensitizer:** A substance that will induce hypersensitivity of the airways following inhalation of the substance. (GHS)
- | ~~3.303.31~~ **Suitable analog:** Suitable analogs will be based on a chemically (e.g., based on chemical structure) or biologically (e.g., based on metabolic breakdown, or likely mechanistic/mode of action considerations) similar chemical. Guidance for identifying a suitable analog can be found in OECD *Series on Testing and Assessment No. 80 Guidance on Grouping of Chemicals* [6]. The analog used must be appropriate for the attribute being evaluated
- | ~~3.313.32~~ **Weight-of-evidence:** For the purposes of this document, weight-of-evidence refers to the process of considering the strengths and weaknesses of various pieces of information in reaching and supporting a conclusion concerning a property of the substance. (ECHA [7])

4 Preferences

- 4.1** When data are developed to meet the requirements for Repeated Dose Toxicity, EPA requests that a functional observational battery, such as OPPTS 870.6200: Neurotoxicity Screening Battery [8], be added to the test method to provide neurotoxicity information.
- 4.2** Data for evaluation of chemicals under these criteria are preferred in the following order: 1) measured data on the specific chemical, 2) measured data from a suitable analog, 3) estimated data from appropriate models. Data requirements specific to each attribute are outlined in Section 5. The majority of measured data are expected to be from laboratory experiments. However, any available human data will be considered, e.g. Human Repeat Insult Patch Tests. Human data may require appropriate review for ethical treatment of the subjects.
- 4.3** The links and references in this document are current as of the publication date of these Criteria. The reviewer must use the most recent version of each authoritative list, EPA data interpretation guidance, and test protocol when reviewing a chemical against these Criteria. In the case where a GHS reference in this document is superseded by a more recent version, EPA may choose to update these Criteria to incorporate that newer version. EPA will consider all sources of developing information, such as the Endocrine Disruptor Screening Program¹ [9] or enhancements to estimation models such as EPI SuiteTM [10] that occur over time.

¹ The Agency does not consider endocrine disruption to be an adverse endpoint per se, but as a step that could lead to toxic outcomes, such as cancer or adverse reproductive effects...."[3]. USEPA, *Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis*. , in *Risk Assessment Forum*. 1997: Washington DC.

5 Attributes of Concern for All Fragrances

Each Attribute applies to all fragrance raw materials present at 100 ppm (or 0.01% by weight) or greater in the fragrance. Failure to pass an Attribute results in failure to pass the Fragrance Criteria. All non-aroma fragrance raw materials must meet the DfE Criteria for their ingredient class (i.e.g., solvents must meet the DfE Criteria for Solvents).

5.1 ACUTE MAMMALIAN TOXICITY

Criteria

Fragrance raw materials considered acute toxicants according to the authoritative lists in Table 1a do not pass the Criteria. Chemicals not reviewed in the context of these authoritative lists, but for which data are available, require additional review.

Applying GHS [11], a chemical does not pass the Criteria if the median lethal dose or concentration is less than or equal to those values listed in Table 1. For inhalation studies, exposure duration should be at least four hours; the thresholds for inhalation are the same for exposures greater than four hours. Exposures of less than four hours will be evaluated on a case-by-case basis.

Table 1a – Authoritative Lists

<u>Authoritative Body</u>	<u>Does not pass DfE Criteria</u>
<u>EU Risk Phrases [11]</u>	<u>R26: Very toxic by inhalation; R27: Very toxic in contact with skin; R28: Very toxic if swallowed; and And all combination risk phrases containing one or more of the above.</u>

Table 1— GHS Thresholds

<u>Route of Exposure</u>	<u>Median Lethal Dose/Concentration</u>
<u>Oral LD50 (mg/kg)</u>	<u>50</u>
<u>Dermal LD50 (mg/kg)</u>	<u>200</u>
<u>Inhalation, gas LC50 (ppmV)</u>	<u>500</u>
<u>Inhalation, vapor LC50 (mg/L)</u>	<u>2.0</u>
<u>Inhalation, dust/mist/fumes LC50 (mg/L)</u>	<u>0.5</u>

Supporting Information

The EU Risk Phrases for acute toxicity do not align with the GHS thresholds in Table 1. However, the following EU Risk Phrases [12] can provide data for use in evaluating the acute toxicity of a fragrance raw material:

- R20: Harmful by inhalation;
- R21: Harmful in contact with skin;
- R22: Harmful if swallowed;
- R23: Toxic by inhalation;
- R24: Toxic in contact with skin;
- R25: Toxic if swallowed;
- R26: Very toxic by inhalation;
- R27: Very toxic in contact with skin;
- R28: Very toxic if swallowed; and

~~—All combinations of risk phrases containing one of more of the above.~~

Data Requirements

~~Measured data on the chemical and/or a suitable analog are required for at least one route of exposure and must be generated to fill any data gaps. Data from estimation models may be considered as part of the weight of evidence.~~

Additional Review

~~Where acute toxicity data are available and have not been reviewed in the context of the authoritative lists in Table 1a, an additional review will be performed. When an additional review is performed, GHS criteria [12], cited in Table 1b, will be used. For inhalation studies, exposure duration should be at least four hours; the thresholds for inhalation are the same for exposures greater than four hours. Exposures of less than four hours will be evaluated on a case-by-case basis.~~

~~All available data will be evaluated. Measured and/or modeled data, for the chemical and/or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.~~

Table 1b – GHS Thresholds

Route of Exposure	Median Lethal Dose/Concentration
Oral LD50 (mg/kg)	50
Dermal LD50 (mg/kg)	200
Inhalation, gas LC50 (ppmV)	500
Inhalation, vapor LC50 (mg/L)	2.0
Inhalation, dust/mist/fumes LC50 (mg/L)	0.5

Supporting Information

~~The following EU Risk Phrases for acute toxicity do not align with the GHS thresholds in Table 1b. However, the following EU Risk Phrases [11] can provide data for use in evaluating the acute toxicity of a fragrance raw material:~~

- ~~– R20: Harmful by inhalation;~~
- ~~– R21: Harmful in contact with skin;~~
- ~~– R22: Harmful if swallowed;~~
- ~~– R23: Toxic by inhalation;~~
- ~~– R24: Toxic in contact with skin;~~
- ~~– R25: Toxic if swallowed;~~
- ~~– All combinations of risk phrases containing one of more of the above.~~

Sources for Data Interpretation

- GHS Ch 3.1 Acute Toxicity [12], and
- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then

“DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC”. [13].

5.2 CARCINOGENICITY

Criteria

Fragrance raw materials considered carcinogens according to the authoritative lists in Table 2a do not pass the Criteria. Chemicals not reviewed in the context of these authoritative lists, but for which data are available, require additional review. Chemicals that appear on any of the flagging lists specified below also require additional review.

Table 2a – Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
National Toxicology Program (NTP)	Known to be Human Carcinogen Reasonably Anticipated to be Human Carcinogen
U.S. Environmental Protection Agency (EPA)	(2005/1999) Carcinogenic to humans, Likely to be carcinogenic to humans, or Suggestive evidence of carcinogenic potential (1996) Known/Likely (1986) Group A – Human Carcinogen, Group B – Probable human carcinogen, or Group C – Possible human carcinogen
International Agency for Research on Cancer (IARC)	Group 1 – Carcinogenic to humans Group 2A – Probably carcinogenic to humans Group 2B – Possibly carcinogenic to humans ²
EU CMR List [11]	Category 1 – Known to be carcinogenic to humans Category 2 – Should be regarded as if carcinogenic to humans Category 3 – Cause for concern for humans owing to possible carcinogenic effects
EU Risk Phrases [11]	R45: May cause cancer R49: May cause cancer by inhalation R40: Limited evidence of a carcinogenic effect <i>And all combination risk phrases containing one or more of the above.</i>

Additional Review

In the case where carcinogenicity data are available and have not been reviewed in the context of the authoritative lists in Table 2a, an additional review will be performed. When an additional review is performed, GHS criteria, cited in Table 2b, will be used.

All available data will be evaluated. Measured and/or estimated data, for the chemical and/or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

Flagging Lists

Chemicals that appear on one of the following flagging lists require additional review:

1. Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [14, 15],
2. Substances prioritized for testing for endocrine disruption by the US EPA Endocrine Disruptor Screening Program [9],

² Chemicals listed as “possibly carcinogenic to humans” are evaluated largely on animal studies. DfE will consider appropriate data that show cancer concerns are not relevant to humans, e.g., because of an animal specific tissue effect or mode of action. If the data demonstrate that cancer concerns are not relevant to humans, that chemical can be considered under the DfE Criteria.

3. Substances listed on the State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA) California Proposition 65 (Safe Drinking Water and Toxic Enforcement Act Of 1986) as Known to the State to Cause Cancer [16].

Table 2b – GHS Criteria

Authoritative Body	Does not pass DfE Criteria
Globally Harmonized System (GHS) [17]	Category 1A – Known to have carcinogenic potential for humans Category 1B – Presumed to have carcinogenic potential for humans Category 2 – Suspected human carcinogens

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then “DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC”. [13];
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20];
- GHS Ch 3.6 Carcinogenicity [17];
- Section 2, Hazard Assessment in Guidelines for Carcinogen Risk Assessment (Risk Assessment Forum) (EPA 2005), http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=439797 [21] and
- The following link can be used to identify substances prioritized for testing for endocrine disruption by the European Commission: http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm#priority_list. To download the list of substances, see the zipped file under the heading “Priority List” [14].
- The following report describes the process used to develop the endocrine disruptors priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf [15].
- EPA Endocrine Disruptors Screening Program, available at: <http://www.epa.gov/endo/>. [9]
- Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, available at: http://www.epa.gov/ttn/atw/childrens_supplement_final.pdf [22].

5.3 GENETIC TOXICITY

Criteria

Fragrance raw materials considered mutagens or genetic toxicants according to the authoritative lists in Table 3a do not pass the Criteria. Chemicals not reviewed in the context of these authoritative lists, but for which data are available, require additional review. Effects to be considered include heritable germ cell mutagenicity (including gene mutation and chromosome mutation), germ cell genetic toxicity, and somatic cell mutagenicity or genetic toxicity.

Table 3a – Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU CMR List [11]	Category 1 – Substances known to be mutagenic to humans Category 2 – Substances which should be regarded as if they are mutagenic to humans Category 3 – Substances which cause concern for humans owing to possible mutagenic effects ³
EU Risk Phrases [11]	R46: May cause heritable genetic damage R68: Possible risk of irreversible effects <i>And all combination risk phrases containing one or more of the above.</i>

Additional Review

In the case where mutagenicity or genetic toxicity data are available and have not been reviewed in the context of the authoritative lists in Table 3a, an additional review will be performed. When an additional review is performed, GHS criteria, cited in Table 3b, will be used.

All available data, including *in vivo*, *in vitro*, and epidemiological studies, will be evaluated. Measured and/or estimated data, for the chemical and/or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

Table 3b – GHS Criteria

Authoritative Body	Does not pass DfE Criteria
Globally Harmonized System (GHS) [23]	Category 1A – Chemicals known to induce heritable mutations in germ cells of humans Category 1B – Chemicals which should be regarded as if they induce heritable mutations in the germ cells of humans Category 2 – Chemicals which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans

³ Per EU guidance, chemicals classified as Category 3 substances may be placed in that category based on positive results in assays showing (a) mutagenic effects or (b) other cellular interaction relevant to mutagenicity. If a chemical is classified in Category 3(b) only and that classification appears overly conservative, then the submitter may request EPA expert review. In such as case, if EPA determines the data do not support a concern for possible mutagenic effects, then the chemical will pass the criteria.

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then “DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC”; [13]
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20]; and
- GHS Ch 3.5 Germ Cell Mutagenicity [23].

5.4 REPEATED DOSE AND NEUROTOXICITY

Criteria

If a fragrance contains one or more fragrance raw materials that are considered repeated dose (systemic) or neurotoxicants per GHS, then the compound must be evaluated using the GHS criteria for mixtures [24]. Repeated dose and neurotoxicity are evaluated using the GHS chapter called Specific Target Organ Toxicity Repeated Exposure. To pass the Criteria, the fragrance compound must not be considered a specific target organ toxicant mixture by any route of exposure (i.e., no GHS Category 1 or Category 2 fragrance compounds).

Data Evaluation: Review of Fragrance Raw Materials

Each fragrance raw material that is evaluated using GHS must be reviewed for general systemic toxicity/organ effects and neurotoxicity effects. If a fragrance raw material meets any of the conditions below, it must be reviewed based on GHS criteria, using test data or weight-of-evidence:

- Requiring at least one of the specified EU Risk Phrases [11]
 - R33: Danger of cumulative effects (repeated exposure)
 - R39: Danger of very serious irreversible effects (single exposure)
 - R48: Danger of serious damage to health by prolonged exposure (repeated exposure)
 - R68: Possible risk of irreversible effects (single exposure), or
- Having new data not yet incorporated into the EU Risk Phrases, or
- Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [14, 15]
- Substances prioritized for testing for endocrine disruption by the US EPA Endocrine Disruptor Screening Program [9].

Table 4a – GHS Category 1 – Specific Target Organ Toxicity – Repeated Exposure

Route of Exposure	Guidance values*
Oral (mg/kg-bw/day)	< 10
Dermal (mg/kg-bw/day)	< 20
Inhalation (gas) (ppm/6h/day)	< 50
Inhalation (vapor) (mg/L/6h/day)	< 0.2
Inhalation (dust/mist/fume) (mg/L/6h/day)	< 0.02
<i>*The doses provided are for 90-day studies. Guidance values are tripled for chemicals evaluated in 28-day studies and similarly modified for studies of longer durations.</i>	

Table 4b – GHS Category 2 – Specific Target Organ Toxicity – Repeated Exposure

Route of Exposure	Guidance values*
Oral (mg/kg-bw/day)	10 - 100
Dermal (mg/kg-bw/day)	20 - 200
Inhalation (gas) (ppm/6h/day)	50 - 250
Inhalation (vapor) (mg/L/6h/day)	0.2 - 1.0
Inhalation (dust/mist/fume) (mg/L/6h/day)	0.02 - 0.2
<i>*The doses provided are for 90-day studies. Guidance values are tripled for chemicals evaluated in 28-day studies and similarly modified for studies of longer durations.</i>	

If one or more fragrance raw materials meet GHS criteria for Category 1 or Category 2 Specific Target Organ Toxicity – Repeated Exposure, then the fragrance must be evaluated per GHS mixture rules (see Data Evaluation: Review of Fragrances).

Data Evaluation: Review of Fragrances

Fragrance raw materials that meet GHS criteria for Category 1 or Category 2 Specific Target Organ Toxicity – Repeated Exposure are limited based on percentage as described by the GHS mixture rules. The GHS mixture rules (GHS section 3.9.3.4 – *Classification of mixtures when data are available for all components or only for some components of the mixture* [24]) will be applied. To pass the Criteria, the fragrance compound must not be considered a specific target organ toxicant mixture by any route of exposure (i.e., no GHS Category 1 or Category 2 fragrances).

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then “DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC”. [13]
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.9 Specific Target Organ Toxicity Repeated Exposure [24].
- Section 3, Hazard Characterization in *Guidelines for Neurotoxicity Risk Assessment* [25].
- The following link can be used to identify substances prioritized for testing for endocrine disruption by the European Commission:
http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm#priority_list. To download the list of substances, see the zipped file under the heading “Priority List”. [14]
- The following report describes the process used to develop the endocrine disruptors priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf [15].
- EPA Endocrine Disruptors Screening Program, available at: <http://www.epa.gov/endo/> [9].

5.5 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Criteria

Fragrance raw materials considered reproductive or developmental toxicants according to the authoritative lists in Table 5a do not pass the Criteria. Chemicals not reviewed in the context of these authoritative lists, but for which data are available, require additional review. Chemicals that appear on any of the flagging lists specified below also require additional review.

Table 5a – Reproductive/Developmental Toxicity Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU CMR List [11] ⁴	Category 1 – Known to impair fertility in humans or known to cause developmental toxicity in humans Category 2 – Should be regarded as if they impair fertility in humans or cause developmental toxicity to humans Category 3 – Cause concern for human fertility or possible developmental toxic effects
EU Risk Phrases [11] ⁴	R60: May impair fertility R61: May cause harm to the unborn child R62: Possible risk of impaired fertility R63: Possible risk of harm to the unborn child R64: May cause harm to breastfed babies <i>And all combination risk phrases containing one or more of the above.</i>

Additional Review

In the case where reproductive or developmental toxicity data are available and have not been reviewed in the context of the authoritative lists in Table 5a, an additional review will be performed. When an additional review is performed, GHS criteria and the guidance values in Table 5b will be applied. Chemicals that are considered GHS Category 1 (Known or presumed human reproductive toxicant) or Category 2 (Suspected reproductive toxicant) and demonstrate adverse effects at doses equivalent to or below the guidance values in Table 5b do not pass the Criteria.

All available data will be evaluated. Measured and/or estimated data, for the chemical and/or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

Flagging Lists

Chemicals that appear on one of the following flagging lists require additional review:

1. Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [14, 15]
2. Substances prioritized for testing for endocrine disruption by the US EPA Endocrine Disruptor Screening Program [9]
3. Substances listed on the State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA) California Proposition 65 (Safe Drinking Water and Toxic Enforcement Act Of 1986) as Known to the State to Cause Reproductive Toxicity [16].

⁴ The EU classification criteria do not currently consider a limit dose above which an adverse effect would not trigger classification. EPA will consider evidence demonstrating that a chemical carrying a reproductive/developmental toxicity risk phrase or listed as toxic to reproduction (in Table 5a) did not cause an adverse effect below the TSCA 8(e) Guidance Values listed in Table 5b. Such a chemical may be determined, upon EPA review, to pass the DfE criteria for reproductive/developmental toxicity.

Table 5b – Reproductive/Developmental Toxicity

Route of Administration (units)	Guidance Values
Oral (mg/kg-bw/day)	250
Dermal (mg/kg-bw/day)	200
Inhalation (gas) (ppm/6h/day)	250
Inhalation (vapor) (mg/L/6h/day)	1.0
Inhalation (dust/mist) (mg/L/6h/day)	0.2

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then “DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC” [13].
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.7 Reproductive Toxicity [26].
- Part A, Section 3, Hazard Characterization in *Guidelines for Reproductive Toxicity Risk Assessment* (EPA 1998), <http://www.epa.gov/ncea/raf/pdfs/repro51.pdf> [5].
- Part A, Section 3, Hazard Characterization in *Guidelines for Developmental Toxicity Risk Assessment* (EPA 1991), <http://www.epa.gov/NCEA/raf/pdfs/devtox.pdf> [2].
- The following link can be used to identify substances prioritized for testing for endocrine disruption by the European Commission:
http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm#priority_list. To download the list of substances, see the zipped file under the heading “Priority List” [14].
- The following report describes the process used to develop the endocrine disruptors priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf [15].
- EPA Endocrine Disruptors Screening Program, available at: <http://www.epa.gov/endo/> [9].

5.6 PHOTO SENSITIZATION

No fragrance raw material considered a photo sensitizer according to the International Fragrance Association Standards and Code of Practice (IFRA CoP, current amendment) [27] will pass the Criteria.

5.7 RESPIRATORY SENSITIZATION

Criteria

Fragrance raw materials considered respiratory sensitizers according to the authoritative list in Table 6a do not pass the Criteria. Chemicals not reviewed in the context of this list, but for which data are available, require additional review. Chemicals that appear on the flagging list specified below require additional review.

Table 6a – Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU Risk Phrase [11]	R42: May cause sensitization by inhalation

Additional Review

In the case where respiratory sensitization data are available and have not been reviewed in the context of the authoritative list in Table 6a, an additional review will be performed. When an additional review is performed, GHS criteria in Table 6b will be used.

Acknowledging that recognized animal models for the testing of respiratory hypersensitivity are not available at present, data on respiratory sensitization will normally be based on human evidence; all available data will be reviewed. EPA will search public literature and EPA-confidential data to support the review. Chemicals associated with hypersensitivity after appropriate clinical testing may not pass the criteria. See GHS guidance [28] for further details.

Flagging List

Aroma chemicals designated as ~~sensitizer-induced possible~~ asthmagens (“Rs”, ~~or~~ “Rrs”, “Rr”, or “G”) on the specified flagging list below require additional review using all relevant and available data to support GHS classification:

1. Association of Occupational and Environmental Clinics (AOEC) Exposure Code List [29].⁵

Table 6b – GHS Criteria

Authoritative Body	Does not pass DfE Criteria
Globally Harmonized System (GHS) [28]	Category 1A – high frequency of occurrence or sensitization rate in humans Category 1B – low to moderate frequency of occurrence or sensitization rate in humans

⁵ Note that this list contains many non aroma chemicals. Only aroma chemicals as defined in the Scope section of this document (p. 2) are eligible for review using the DfE Criteria for Fragrances. Chemicals in other functional classes (e.g. solvents) must meet either DfE Criteria for that functional class where available or DfE Master Criteria. All chemicals in a fragrance are subject to the full set of relevant criteria.

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on “CLASSIFICATION-LABELLING”, then “DIRECTIVE 67-548-EEC”, then “ANNEX I OF DIRECTIVE 67-548-EEC”, and then either of the files listed as: “Annex I of Directive 67548EEC” [13].
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.4 Respiratory and Skin Sensitization [28].
- Association of Occupational and Environmental Clinics Exposure Code List, available from: <http://www.aoecdata.org/Default.aspx> [29].

5.8 SKIN SENSITIZATION

Criteria

Each fragrance raw material that is considered a dermal sensitizer under GHS may be present ~~in the cleaning product formulation~~ at a level no greater than 0.01% by weight in the final product as sold. Alternatively, disclosure of skin sensitizers on the label for consumer products, or use of a closed-loop dispensing system for industrial and institutional products is acceptable as described below. A fragrance raw material will be considered a dermal sensitizer if it falls into one of the categories below. (When evaluating dermal sensitization, percutaneous dermal absorption is assumed to be 100%.)

- EU Risk Phrase R43: May cause sensitization by skin contact [11];
- EU 26 Allergens List, found at http://ec.europa.eu/enterprise/sectors/chemicals/files/legislation/allergenic_subst_en.pdf;
- International Fragrance Association Standards and Code of Practice (IFRA CoP) [30]:
Fragrance raw material is listed as a dermal sensitizer; or
- GHS Category 1 – Dermal Sensitizer [28].

Data Requirements

Measured data on the chemical and/or a suitable analog are required, ~~and must be generated to fill any data gaps.~~ Data from estimation models may be considered sufficient evidence along with expert judgment as part of the weight of evidence.

Sensitizers in Consumer Products

DfE will allow manufacturers to use a skin sensitizer, whether component of an essential oil or synthetic compound, at levels above 0.01% in the product in certain, limited situations.

To qualify, the final product manufacturer must disclose such ingredients on the product label and meet the following requirements by providing evidence that the fragrance is:

- used in consumer products only;
- critical to the fragrance essence or product identity;
- otherwise in compliance with the fragrances criteria;
- the subject of good faith explorations of alternatives; and
- included in a monitoring system that would alert DfE if a consumer reports any adverse effects from the product.

Alternatively, the manufacturer may demonstrate that the formulated product does not cause a sensitization response through appropriate testing (see test methods listed in section 6.6) and limit the amount of fragrance chemicals in the product to the level in the test sample.

Sensitizers in Industrial & Institutional (I/I) Products

For I/I concentrates, DfE would allow a skin sensitizer in proportions greater than 0.01% in the final product only if the manufacturer can make the following showing:

- a good faith exploration of alternatives;
- otherwise in compliance with the fragrances criteria;
- the product is part of a closed dilution-controlled dispensing system; and
- skin sensitizers at the most concentrated ready-to-use (RTU) dilution would not exceed 0.01% in the final product.

Alternatively, the manufacturer may demonstrate that the formulated product does not cause a sensitization response through appropriate testing (see test methods listed in section 6.6) and limit the amount of fragrance chemicals in the product to the level in the test sample.

Supplemental Criteria – Quantitative Risk Assessment (QRA) Thresholds

All sensitizers as defined above must not be present at greater than 0.01% by weight in the cleaning product formulation. In addition, any fragrance raw material evaluated under RIFM's QRA must meet its specified QRA threshold. From the International Fragrance Association Standards and Code of Practice, "no fragrance raw materials shall be present in the cleaning product above the QRA threshold specified for the fragrance raw material for the intended category of use" [30]. The stricter of the two requirements will be applied.

Supplemental Criteria – *d*-Limonene

~~When *d*-Limonene is used at a level above the threshold for sensitizers (0.01 percent by weight), it may be present because of its solvent properties. Where this is the case, the DfE screening approach treats *d*-Limonene as a solvent, and no longer considers this chemical under the DfE Criteria for Fragrances.~~

Oxidation products of *d*-Limonene have tested positive for dermal sensitization. *D*-Limonene may be used in a DfE-recognized product in concentrations at which the potential oxidation products may be present at 20 millimoles per liter (mmol/L) or less (corresponding to a *d*-Limonene concentration of 1.36 % or less, as a percent by weight) in an overall formulation. Products that contain *d*-Limonene should not also contain oxidizers, like hydrogen peroxide, which may accelerate the formation of *d*-Limonene oxidation products and harm product integrity.

Sources for Data Interpretation

- EU Dangerous Substances Directive, <http://ecb.jrc.ec.europa.eu/documentation/>. To access the list of substances carrying Risk Phrases, click on "CLASSIFICATION-LABELLING", then "DIRECTIVE 67-548-EEC", then "ANNEX I OF DIRECTIVE 67-548-EEC", and then either of the files listed as: "Annex I of Directive 67548EEC" [13].
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.4 Respiratory or Skin Sensitization [28].
- EU Cosmetics Directive 7th Amendment (76/768/EEC and subsequent updates/amendments) [31].
- International Fragrance Association Standards and Code of Practice (43rd amendment) [30].

5.9 ENVIRONMENTAL TOXICITY AND FATE (ET&F)

Criteria

In general, no persistent, bioaccumulative, and toxic (PBT) substances are allowed, nor very persistent and very bioaccumulative (vPvB) or very persistent and very toxic (vPvT) fragrance materials. Fragrance raw materials that exceed the criteria below for persistence, bioaccumulation, and aquatic toxicity do not pass the Criteria.

- No persistent, bioaccumulative and toxic (PBT) substances as defined below:
 1. Half-life in water, soil, and sediment ≥ 2 months (≥ 60 days); and
 2. Bioconcentration factor (BCF)/Bioaccumulation factor (BAF) $\geq 1,000$; and
 3. Chronic aquatic toxicity (mg/L) ≤ 1 mg/L or acute aquatic toxicity (mg/L) ≤ 10 mg/L.
- No very persistent and very bioaccumulative substances (vPvB) as defined below:
 1. Half-life in water, soil, and sediment > 180 days (or recalcitrant); and
 2. Bioconcentration factor/bioaccumulation factor $> 5,000$.
- No very persistent and very toxic (vPvT) substances as defined below:
 1. Half-life in water, soil, and sediment > 180 days (or recalcitrant); and
 2. Chronic aquatic toxicity (mg/L) < 0.1 mg/L or acute aquatic toxicity (mg/L) < 1 mg/L.

Data Requirements

Aquatic toxicity: Measured data are preferred. Data from a suitable analog are acceptable, and ECOSAR estimations may be used when those data are unavailable. Data, whether measured, estimated or from analogs, are required for each of the following groups of organisms: algae, aquatic invertebrates and fish (all fresh water). Data for marine species may be added when available.

Bioaccumulation potential: Measured data are preferred. Data from a suitable analog are acceptable, and EPI Suite™ estimations (from the most current version) may be used when those data are unavailable. Results from both the BAF and BCF models should be considered. An estimated BAF is preferred to an estimated BCF for compounds where $\log K_{ow} > 5$.

Persistence: Measured data are preferred. Data from a suitable analog are acceptable, and EPI Suite™ estimations (from the most current version) may be used when those data are unavailable. When using modeled data, persistence will be evaluated in the dominant compartment based on the fugacity portion of the latest version of EPI Suite™. Before using those results, consideration should be given to the chemical structure, physical-chemical properties (such as measured log Kow), and the results of other biodegradation models in EPI Suite™.

Supplemental Criteria - Direct Release Products

The criteria in Table 7 address direct release products, which typically by-pass wastewater treatment, and therefore can directly affect aquatic organisms upon discharge.

Table 7 – Direct Release Criteria

	<u>Acute Aquatic Toxicity Value (L/E/IC50)⁶</u>	<u>Persistence</u> (Measured in terms of rate of biodegradation)	<u>Bioaccumulation Potential</u>	<u>Status</u>
<u>1</u>	<u>If ≤10 ppm...</u>			<u>Not acceptable.</u>
<u>2</u>	<u>If >10 ppm and <100 ppm...</u>	<u>...and biodegradation⁷ occurs within a 10-day window without products of concern⁸...</u>	<u>...and BCF/BAF ≤1000.</u>	<u>Acceptable.</u>
<u>3</u>	<u>If ≥100 ppm...</u>	<u>... and biodegradation⁷ occurs within 28 days without products of concern⁸...</u>		<u>Acceptable.</u>

⁶ In general, there is a predictable relationship between acute aquatic toxicity and chronic aquatic toxicity for organic chemicals, i.e., chemicals that have high acute aquatic toxicity also have high chronic aquatic toxicity [Rand, G.M., ed. Fundamentals of Aquatic Toxicology, 2nd ed. 1995, Taylor & Francis: Washington, DC.]. Since acute aquatic toxicity data are more readily available, DfE uses these data to screen chemicals that may be toxic to aquatic life. Where measured chronic toxicity data is available, it will be assessed with other data and applied in the screen based on the relationship between acute and chronic aquatic toxicity.

⁷ Generally, >60% mineralization (to CO₂ and water) in a Ready Biodegradation test.

⁸ Products of concern are compounds with high acute aquatic toxicity (L/E/IC50 ≤ 10ppm) and a slow rate of biodegradation (greater than 28 days).

1. ~~Fragrance raw materials⁹ present at or above 0.01% in the cleaning product as applied¹⁰ must pass:~~
 - a. ~~The environmental toxicity and fate criteria as defined in the DfE Master Criteria (**Appendix 1**), unless the chemical is used as a substantive chemical¹¹ or is an essential oil or a constituent from an essential oil.¹²~~
 - b. ~~If the chemical is used as a substantive chemical or is an essential oil or a constituent from an essential oil¹³ then it must either meet the ET&F criteria in the DfE Master Criteria (**Appendix 1**) or demonstrate a High Capacity for Ultimate Biodegradation in at least one of the standardized test methods in **Appendix 2**.~~
2. ~~Fragrance raw materials present in the cleaning product as applied below 0.01% and in the fragrance above 0.01% will be reviewed using the most recent version of EPI SuiteTM models.¹⁴ Known impurities and residuals present at greater than 0.1% in the fragrance raw material must be reported.¹⁵ No constituent is allowed in the product that is classifiable as P2 (or higher) and B2 (or higher) and T2 (or higher) as defined by the USEPA New Chemicals Program (**Appendix 3**). If the modeled results are disputed, then the submitter may either~~
 - a. ~~Provide data for the disputed endpoint(s) or~~
 - b. ~~Provide data showing that the raw material demonstrates a High Capacity for Ultimate Biodegradation (**Appendix 2**).~~
3. **Exceptions:**
 - a. ~~EPA may choose to evaluate any fragrance ingredient in the cleaning product against the DfE Master Criteria—ET&F (**Appendix 1**) if the ingredient has been flagged as a potential chemical of concern based on fate and effects data by state or federal authoritative bodies.~~
 - b. ~~All fragrance raw materials in products designed for direct release to the environment (e.g. graffiti removers and marine cleaners) must pass the DfE Criteria for Environmental Toxicity and Fate for Chemicals in Direct Release Products (**Appendix 4**)~~

⁹ Section 3.16 of the DfE Criteria for Fragrances defines a Fragrance raw material as “any substance, obtained by chemical synthesis or derived from a natural source, intentionally added or present in a fragrance at greater than 0.01 percent by weight whose primary purpose is to impart scent. In the context of these Criteria, fragrance raw materials include aroma chemicals, fragrant extracts (essential oils), and components of essential oils.” See: http://www.epa.gov/dfe/pubs/projects/gfcp/dfe_screen_for_fragrances_human_health_criteria_version_1.pdf

¹⁰ Concentration as applied refers to the most concentrated application.

¹¹ A substantive chemical is a chemical substance, derived synthetically or present as a constituent of an essential oil, with the function to provide slow release aroma characteristics; typically has an experimental log Kow > 3.0.

¹² An essential oil is any concentrated, hydrophobic liquid containing volatile aroma compounds from plants. The known components of essential oils are evaluated against the criteria.

<http://www.cosmeticsandtoiletries.com/formulating/ingredient/fragrance/9430486.html>

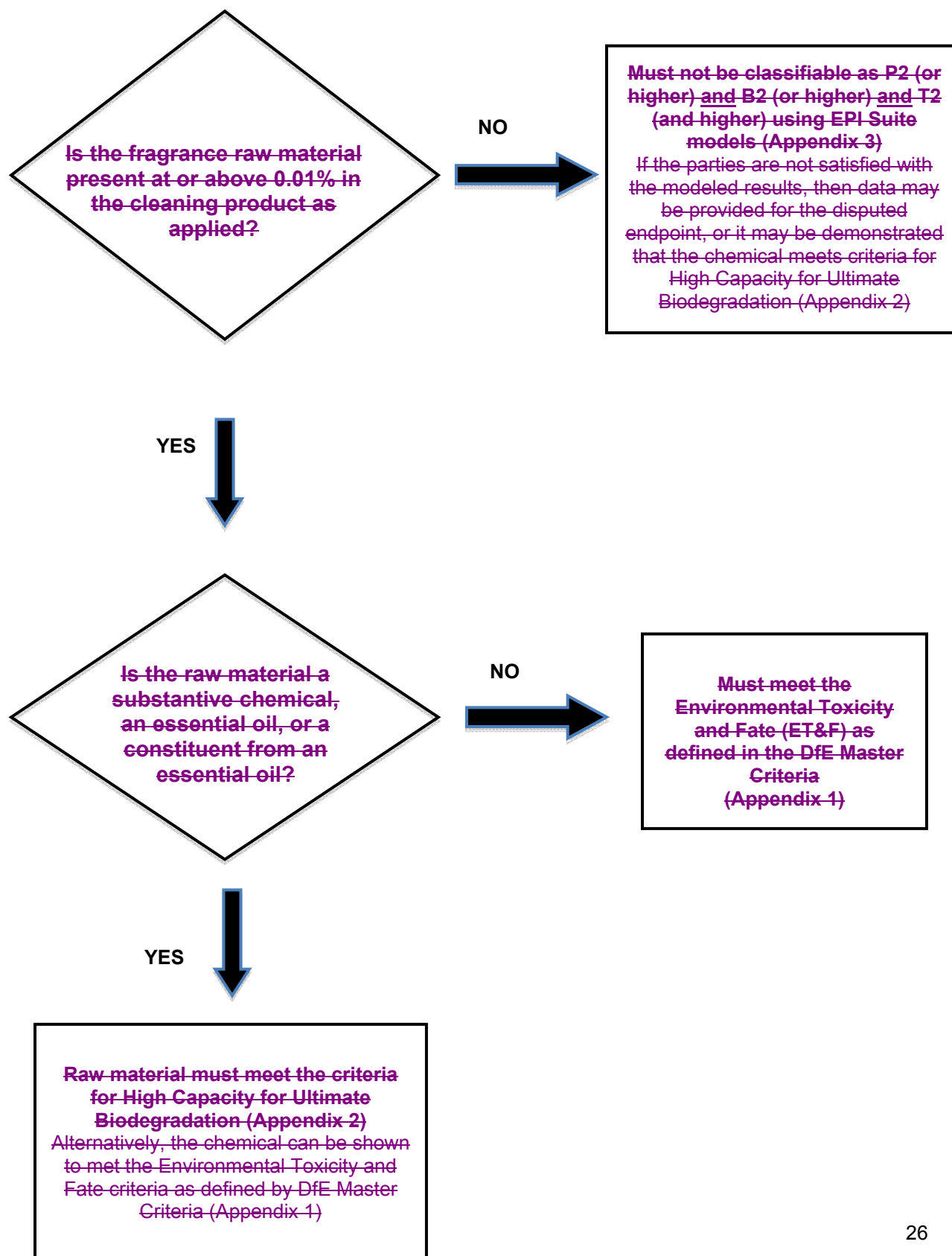
¹³ This does not include “nature identicals”.

¹⁴ As of March 2010, the most recent version is 4.0. The model may be downloaded from <http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm>

¹⁵ For example, constituents (incl. residuals and impurities) A and B are known to be present at > 0.1% in an essential oil raw material. If they are also present at ≥ 0.01% in the fragrance, then they must be screened against the DfE Screen for Fragrances.

Figure 1: Decision Tree for ET&F Criteria

Does not apply to direct release products. See Appendix 4 for direct release product criteria.



6 Test Methods

The test methods in this section should be used to develop data for conducting chemical reviews based on the criteria in Section 5.

6.1 Acute Mammalian Toxicity – Test Methods for GHS Review

- OPPTS Harmonized Guideline 870.1100: Acute oral toxicity [32];
- OPPTS Harmonized Guideline 870.1200: Acute dermal toxicity [33]
- OPPTS Harmonized Guideline 870.1300: Acute inhalation toxicity [34];
- OECD Test Guideline 420: Acute Oral Toxicity-Fixed Dose Method [35];
- OECD Test Guideline 423: Acute Oral Toxicity – Acute Toxic Class Method [36];
- OECD Test Guideline 425: Acute Oral Toxicity – Up-and-Down Procedure [37];
- OECD Test Guideline 402: Acute Dermal Toxicity [38]; and
- OECD Test Guideline 403: Acute Inhalation Toxicity [39].

6.2 Carcinogenicity – Test Methods for GHS Review

- OECD Test Guideline 451: Carcinogenicity Studies [40];
- OECD Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies [41];
- OPPTS Harmonized Guidelines 870.4200: Carcinogenicity [42];
- OPPTS Harmonized Guidelines 870.4300: Combined chronic toxicity/carcinogenicity [43] and
- NTP 2 Year Study Protocol: “Specifications for the conduct of studies to evaluate the toxic and carcinogenic potential of chemical, biological and physical agents in laboratory animals for the National Toxicology Program” [44].

6.3 Genetic Toxicity – Test Methods for GHS Review

Per GHS [23], results from multiple, acceptable test methods must be used in conjunction for evaluation of genetic toxicity.

- OECD Test Guideline 471 (OPPTS 870.5100): Bacterial Reverse Mutation Test [45, 46];
- OECD Test Guideline 473 (OPPTS 870.5375): *In vitro* Mammalian Chromosome Aberration Test [47, 48];
- OECD Test Guideline 474 (OPPTS 870.5395): Mammalian Erythrocyte Micronucleus Test [49, 50];
- OECD Test Guideline 475 (OPPTS 870.5385): Mammalian Bone Marrow Chromosome Aberration Test [51, 52];
- OECD Test Guideline 476 (OPPTS 870.5300): *In vitro* Mammalian Cell Gene Mutation Test [53, 54]; and
- OECD Test Guideline 483 (OPPTS 870.5380): Mammalian Spermatogonial Chromosome Aberration Test [55, 56];
- OECD Test Guideline 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* [57]. This guideline does **NOT** substitute in the necessary minimum set for either the gene mutation or the chromosome aberration test.

6.4 Repeated Dose and Neurotoxicity

Repeated Dose Toxicity – Preferred Test Methods for GHS Review

- OECD Test Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents [58]
- OECD Test Guideline 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents [59]
- OECD Test Guideline 411: Subchronic Dermal Toxicity: 90-day Study [60]
- OECD Test Guideline 413: Subchronic Inhalation Toxicity: 90-day Study [61]
- OPPTS Harmonized Guideline 870.3100: 90-Day oral toxicity in rodents [62]

- OPPTS Harmonized Guideline 870.3150: 90-Day oral toxicity in nonrodents [63]
- OPPTS Harmonized Guideline 870.3250: 90-Day dermal toxicity [64]
- OPPTS Harmonized Guideline 870.3465: 90-Day inhalation toxicity [65]

Repeated Dose Toxicity – Acceptable Test Methods for GHS Review

- OECD Test Guideline 407: Repeated Dose 28-day Oral Toxicity Study in Rodents [66]
- OECD Test Guideline 410: Repeated Dose Dermal Toxicity: 28-day Study [67]
- OECD Test Guideline 412: Repeated Dose Inhalation Toxicity: 28-day Study [68]
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [69]
- OPPTS Harmonized Guideline 870.3050: Repeated dose 28-day oral toxicity study in rodents [70]
- OPPTS Harmonized Guideline 870.3200: 28-Day dermal toxicity [71]

Neurotoxicity – Preferred Test Methods for GHS Review

- OECD Test Guideline 424: Neurotoxicity Study in Rodents [72] and
- OPPTS Harmonized Guideline 870.6200: Neurotoxicity screening battery [8]

Neurotoxicity – Additional Test Methods for GHS Review

Additional evidence from OECD Test Guideline 426: Developmental Neurotoxicity Study [73] and OPPTS Harmonized Guideline: 870.6300 Developmental neurotoxicity study [74] can be used to screen chemicals for neurotoxicity.

6.5 Reproductive and Developmental Toxicity – Test Methods for GHS Review

Fertility test methods, preferred

- OECD Test Guideline 415: One-Generation Reproduction Toxicity Study [75] and
- OECD Test Guideline 416: Two-Generation Reproduction Toxicity Study [76].

Fertility test methods, acceptable

The following test methods may be used to identify reproductive toxicity, per GHS [26]:

- OPPTS Harmonized Guideline 870.3800: Reproduction and fertility effects [77];
- OECD Test Guideline 421: Reproduction/Developmental Toxicity Screening Test [78];
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [69];
- OPPTS Harmonized Guideline 870.3550: Reproduction/developmental toxicity screening test [79]; and
- OPPTS Harmonized Guideline 870.3650: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [80].

Developmental toxicity test methods, preferred

- OECD Test Guideline 414: Prenatal Developmental Toxicity Study [81]

Developmental toxicity test methods, acceptable

The following test methods may be used to identify developmental toxicity, per GHS [26]:

- OPPTS Harmonized Guideline 870.3800: Reproduction and fertility effects [77];
- OECD Test Guideline 421: Reproduction/Developmental Toxicity Screening Test [78];

- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [69];
- OPPTS Harmonized Guideline 870.3550: Reproduction/developmental toxicity screening test [79]; and
- OPPTS Harmonized Guideline 870.3650: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [80].

6.6 Skin Sensitization – Preferred Test Methods for GHS Review

- OECD Test Guideline 406: Skin Sensitization [82]
- OECD Test Guideline 429: Skin Sensitization: Local Lymph Node Assay [83]
- OPPTS Harmonized Guideline 870.2600: Skin Sensitization [84]

6.7 Environmental Toxicity and Fate

The test methods in Section 6.7 apply to Appendix 1 – DfE Master Criteria & Appendix 4 – DfE Master Criteria Environmental Toxicity and Fate (ET&F) Criteria as Modified for Direct Release Products. In addition, they may be used in Appendix 3 in the case where modeled results are disputed.

6.7.1 Test Methods, Acute Aquatic Toxicity

A baseline data set is required that includes test data in algae, aquatic invertebrates and fish. Additional aquatic toxicity data in other species or in marine species will also be reviewed if available.

Preferred Test Methods for Fish

- OECD Test Guideline 203: Fish, Acute Toxicity Test [85] and
- OPPTS Harmonized Guideline 850.1075: Fish acute toxicity test, freshwater and marine [86].

NOTE – EPA may request that the test be carried out using semi-static renewal or a flow-through system with mean measured concentration. Any new testing should be done in consultation with EPA.

Preferred Test Methods for Aquatic Invertebrates

- OECD Test Guideline 202, Part 1, Daphnia sp., Acute Immobilisation Test [87];
- OPPTS Harmonized Guideline 850.1010: Aquatic invertebrate acute toxicity test, freshwater daphnids[88]; and
- OPPTS Harmonized Guideline 850.1035: Mysid acute toxicity test [89].

NOTE – EPA may request that the test be carried out using semi-static renewal or a flow-through system with mean measured concentration. Any new testing should be done in consultation with EPA. A 96-hour Mysid shrimp acute toxicity test can be used in place of a daphnid acute toxicity test when the latter is not available.

Preferred Test Methods for Algae

- OECD Test Guideline 201, Alga, Growth Inhibition Test (and biomass) [90] and
- OPPTS Harmonized Guideline 850.5400: Algal toxicity, Tiers I and II (including growth inhibition and biomass) [91].

Alternative Test Methods, Acute Aquatic Toxicity

The following test methods may be considered, when relevant:

- OPPTS Harmonized Guideline 850.1085: Fish acute toxicity mitigated by humic acid [92];
- OPPTS Harmonized Guideline 850.1025: Oyster acute toxicity test (shell deposition) [93];
- OPPTS Harmonized Guideline 850.1045: Penaeid acute toxicity test [94];
- OPPTS Harmonized Guideline 850.1055: Bivalve acute toxicity test (embryo larval) [95];
- OPPTS Harmonized Guideline 850.4400: Aquatic plant toxicity test using *Lemna spp.* Tiers I and II [96].

6.7.2 Test Methods, Persistence (measured as biodegradation)

Data from experimental methods are generally preferred over estimations of persistence. For the purposes of screening safer chemicals in Appendix 1 (Table A, rows 1-3) and Appendix 4, ready biodegradation tests are preferred. It is noted that simulation tests are likely to better describe the biodegradability of a chemical in specific environmental conditions, and these tests can provide information to evaluate the half-life of a chemical that is aquatically toxic at ≥ 100 ppm. Simulation tests may also contribute useful information in a weight-of-evidence evaluation for chemicals aquatically toxic at < 100 ppm.

Preferred Test Methods for Persistence

- OECD Test Guideline 301: Ready Biodegradability (sections A-F)[97];
- OECD Test Guideline 310: Ready Biodegradability – CO₂ in sealed vessels [98]; and
- OPPTS Harmonized Guideline 835.3110: Ready biodegradability [99].
- For chemicals where acute aquatic toxicity ≥ 100 ppm, if the compound degrades by more than 40% in 28 days during one of the Ready Biodegradability tests specified above or by more than 60% in one of the Inherent Biodegradability tests detailed in OECD Test Guidelines 302 (A-C) [100-102] then the half-life of a chemical is likely to be less than 60 days [103].
- Simulation tests may also be used to determine the half-life of a chemical and may be useful in a weight-of-evidence evaluation for chemicals aquatically toxic at < 100 ppm.
 - OECD Test Guideline 303A (OPPTS 835.3240): Aerobic Sewage Treatment: Activated Sludge Units [104, 105],
 - OECD Test Guideline 309 (OPPTS Harmonized Guideline 835.3190): Aerobic Mineralization in Surface Water - Simulation Biodegradation Test [106, 107],
 - OECD Test Guideline 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater (Note: TG 314 uses elements of OECD TG 301, 303A, 309, 310, and 311) [108],
 - OPPTS Harmonized Guideline 835.3280–Simulation Tests to Assess the Primary and Ultimate Biodegradability of Chemicals Discharged to Wastewater [109],
 - OPPTS Harmonized Guideline 835.3170 - Shake Flask Die-Away Test [110], and
 - OPPTS Harmonized Guideline 835.3180 - Sediment/Water Microcosm Biodegradation Test [111].

Other Methods of Degradation

On a case-by-case basis, DfE will consider other routes of degradation in the environment, such as hydrolysis or photolysis, and degradation in other relevant media, for example, soil or sediment. In evaluating such degradation studies, DfE will consider the relevance of that degradation pathway to the chemical in question as well as the significance of the degradation.

6.7.3 Test Methods, Bioaccumulation

A field-measured BAF (located in the literature) is the most preferred data for indicating bioaccumulation.

Alternative Test Methods for Bioaccumulation

When a field-measured BAF is not available, the following test methods may be used:

- OECD Test Guideline 305: Bioconcentration: Flow-through Fish Test[112];
- OPPTS Harmonized Guideline 850.1710: Oyster BCF[113];
- OPPTS Harmonized Guideline 850.1730: Fish BCF[114];
- Modeled data from sources such as EPI SuiteTM [10] are acceptable when data are unavailable.

Appendix 4
Environmental Toxicity & Fate Criteria from the DfE Master Criteria

Table A. DfE Master Criteria—ET&F

	Acute Aquatic Toxicity Value (L/E/IC50) ^{16,17,18}	Persistence (Measured in terms of level of biodegradation)	Bioaccumulation Potential
1	If ≤1 ppm...	...then may be acceptable if the chemical meets the 10-day window as measured in a ready biodegradation test without degradation products of concern ¹⁶ below...	...and BCF/BAF <1000.
2	If >1 ppm and ≤10 ppm...	...then the chemical must meet the 10-day window as measured in a ready biodegradation test without degradation products of concern ¹⁶ below...	
3	If >10 ppm and <100 ppm...	...then the chemical must reach the pass level within 28 days as measured in a ready biodegradation test without degradation products of concern ¹⁶ below...	
4	If ≥100 ppm...	...then the chemical need not reach the pass level within 28 days as measured in a ready biodegradation test if there are no degradation products of concern ¹⁹ and its half-life < 60 days...	

Data Requirements

Acute aquatic toxicity: Measured data are preferred. ECOSAR estimations may be used along with data from a suitable analog(s). Data, whether measured or from analogs, are required for each of the following groups of organisms: algae, aquatic invertebrates and fish (all fresh water). If only estimated data are available, the use of estimated data may be acceptable in combination with EPA expert review. Data for marine species may be added when available.

BCF/BAF: Measured data are preferred. Data from a suitable analog is acceptable, and EPI Suite™ estimations (from the most current version) may be used when those data are unavailable.

¹⁶ In general, there is a predictable relationship between acute aquatic toxicity and chronic aquatic toxicity for organic chemicals, i.e., chemicals that have high acute aquatic toxicity also have high chronic aquatic toxicity [Rand, G.M., ed. *Fundamentals of Aquatic Toxicology*, 2nd ed. 1995, Taylor & Francis: Washington, DC.]. Since acute aquatic toxicity data are more readily available, DfE uses these data to screen chemicals that may be toxic to aquatic life. Where measured chronic toxicity data is available, it will be assessed with other data and applied in the screen based on the relationship between acute and chronic aquatic toxicity.

¹⁷ A case-by-case approach focusing on rate of biodegradation and degradation products of concern will be implemented for chemicals toxic to aquatic organisms at ≤ 1 ppm.

¹⁸ For determining the aquatic toxicity of substances that are not toxic at their solubility limit, see ECOSAR Technical Reference Manual Figure 9, p 17 (<http://www.epa.gov/oppt/newchems/tools/ecosartechfinal.pdf>); When a chemical may have effects at saturation as determined using the guidance in the ECOSAR manual, a weight of evidence approach in combination with US EPA expert review will be used. EPA may require additional testing including but not limited to solubility testing, chronic aquatic toxicity testing, or acute aquatic toxicity testing of analogs.

¹⁹ Degradation products of concern are compounds with high acute aquatic toxicity (L/E/IC50 ≤ 10 ppm) which mineralize <60% in 28 days.

~~Results from both the BAF and BCF models should be considered. An estimated BAF is preferred to an estimated BCF for compounds where $\log K_{ow} > 5$.~~

Persistence (measured as level of biodegradation):

~~Measured data are preferred. In the case where measured data are unavailable, data from estimation models or a suitable analog will be accepted as follows:~~

- ~~(1) If acute aquatic toxicity ≤ 1 ppm: Biodegradability must be measured for the chemical or for a suitable analog. Biodegradability predictions from estimation models, such as EPI Suite™, will be used only to support the weight of evidence.~~
- ~~(2) If acute aquatic toxicity > 1 ppm and ≤ 10 ppm: Biodegradability must be measured for the chemical or for a suitable analog. Biodegradability predictions from estimation models, such as EPI Suite™, will be used only to support the weight of evidence.~~
- ~~(3) If acute aquatic toxicity > 10 ppm and < 100 ppm: Biodegradability must be measured for the chemical or for a suitable analog. Biodegradability predictions from estimation models, such as EPI Suite™, will be used only to support the weight of evidence.~~
- ~~(4) If acute aquatic toxicity ≥ 100 ppm: Biodegradability for the chemical or for a suitable analog are preferred. Biodegradability predictions from estimation models, such as EPI Suite™ (the most current version), may be acceptable.~~

Appendix 2 Criteria for High Capacity for Ultimate Biodegradation

Any of the test methods and associated criteria in Table B can be used to demonstrate a high capacity for ultimate biodegradation.

Scope: ~~These criteria are intended to apply to substantive chemicals and constituents from essential oils²⁰ present at >0.01% in the cleaning product as applied. They define test methods and pass levels that can be used to demonstrate a high capacity for ultimate biodegradation of each fragrance raw material. These criteria do not apply to fragrances in direct release products. See Appendix 4.~~

Data Requirements: ~~Test data are required. Test data on the exact chemical or essential oil are preferred. Test data from suitable analogs will be accepted. Modeled results, such as from EPI Suite, can supplement a weight of evidence approach, but cannot be used exclusively.~~

Duration: ~~All pass levels apply to tests lasting not longer than 28 days.~~

Accuracy and Validation of Test Results: ~~In order to ensure that test results do support claims of a high capacity for ultimate biodegradation, the guidelines presented in each protocol must be followed. For example, the guidelines describe the type of substances that may be evaluated with a given test protocol, and the conditions of the test that support the conclusion that a substance has actually demonstrated biodegradability (rather than adsorption by activated sludge).~~

Table B.
Test Methods and Associated Criteria that May be Used to Demonstrate a High Capacity for Ultimate Biodegradation

	Method ²⁴	Level of Biodegradation
Ready Biodegradation Tests ²²	301A: DOC Die Away [96]	> 70% DOC removal (with 10-day window) ²³
	301E: Modified OECD Screen [96]	
	301B: CO ₂ Evolution [96]	> 50% ThCO ₂ /ThIC removal ²⁴
	301C: MITI (I) [96]	> 50% ThOD removal ²¹
	301D: Closed Bottle [96]	
Inherent Biodegradation Tests ¹⁹	301F: Manometric Respirometry [96]	> 50% ThIC removal ²⁴
	310: CO ₂ in sealed vessels [97]	
	302A: Modified SCAS Test [99]	> 70% DOC removal ²⁵
	302B: Zahn-Wellens/EVPA Test [101]	> 70% DOC or BOD removal ²²
	302C: Modified MITI Test (II) [114]	> 70% of theoretical (BOD, DOC removal or COD) ²²

²⁰ This does not apply to "nature identicals"

²⁴ When a radiolabel is used, it must be located in the most recalcitrant part of the molecule. An appropriate non-specific parameter, such as CO₂ evolution, must still be measured to ensure degradation is occurring.

²² Equivalent OPPTS harmonized test guidelines are also included.

²³ Standard pass criterion; see reference 96. OECD, *OECD Guidelines for the Testing of Chemicals: Test No. 301: Ready Biodegradability*. 1992.

²⁴ Suggested by Boethling, R., Lynch, D. and G.C. Thom. 2003. Predicting Ready Biodegradability of Premanufacture Notice Chemicals. *Environmental Toxicology and Chemistry*, Vol. 22, No. 4, pp. 837–844.

²⁵ 70% pass level is suggested in OECD. *Revised Introduction to the OECD Guidelines for the Testing of Chemicals, Section 3. Part 1: Principles and Strategies Related to the Testing of Degradation of Organic Chemicals*. 2006, see page 7.

	Method ²⁴	Level of Biodegradation
Simulation Tests ¹⁹	303A: Simulation Test—Aerobic Sewage Treatment: Activated Sludge Units [103]	> 70% in 28 days²⁶
	303B: Simulation Test—Aerobic Sewage Treatment: Biofilms [103]	> 70% in 28 days²³
	307: Aerobic and Anaerobic Transformation in Soil [115]	> 70% in 28 days²³
	308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems [116]	> 70% in 28 days²³
	309: Aerobic Mineralisation in Surface Water—Simulation Biodegradation Test [105]	> 70% in 28 days²³
	314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater [107]	> 70% in 28 days

²⁶ 70% pass level in 28 days is suggested in OECD. Revised Introduction to the OECD Guidelines for the Testing of Chemicals, Section 3. Part 1: Principles and Strategies Related to the Testing of Degradation of Organic Chemicals, 2006, see page 9.

Appendix 3

Criteria for P2B2T2 based on US EPA New Chemicals Program and EPI Suite™ Modeling

Persistence (Biodegradation) Criteria (P2)

1. Half life in water, soil, and sediment ≥ 2 months (≥ 60 days)
2. Persistence will be evaluated in the dominant compartment based on the fugacity portion of the latest version of EPI Suite™. Before using those results, consideration should be given to the chemical structure, physical-chemical properties (such as measured $\log K_{ow}$), the results of other biodegradation models in EPI Suite™, and available biodegradation data.

Bioaccumulation Criteria (B2)

1. Bioconcentration factor (BCF)/Bioaccumulation factor (BAF) $\geq 1,000$

Aquatic Toxicity Criteria (T2)

1. **Chronic** aquatic toxicity (mg/L) < 10 mg/L
2. **Acute** aquatic toxicity (mg/L) < 100 mg/L

Criteria adapted from PBT Profiler²⁷

²⁷ <http://www.pbtprofiler.net/criteria.asp>

Appendix 4

Table C. Environmental Toxicity and Fate (ET&F) Criteria from the DfE Master Criteria as Modified for Direct Release Products

These criteria are modified from Appendix 1: Master Criteria — ET&F in order to address direct release products, which typically by-pass wastewater treatment, and therefore can directly affect aquatic organisms upon discharge.

	Acute Aquatic Toxicity Value (L/E/IC50) ²⁸	Persistence (Measured in terms of rate of biodegradation)	Bioaccumulation Potential	Status
1	≤1 ppm	May be acceptable if biodegradation ²⁹ occurs within a 10-day window without products of concern ³⁰	...and BCF/BAF <1000.	Not acceptable.
2	>1 ppm and ≤10 ppm	Biodegradation ²⁶ occurs within a 10-day window without products of concern ²⁷		Could be improved.
3	>10 ppm and <100 ppm	Biodegradation ²⁶ occurs within 28 days without products of concern ²⁷		Could be improved.
		Biodegradation ²⁶ occurs within a 10-day window without products of concern ²⁷		Acceptable †
4	≥100 ppm	Biodegradation ²⁶ occurs within 28 days without products of concern ²⁷		Acceptable †

²⁸ In general, there is a predictable relationship between acute aquatic toxicity and chronic aquatic toxicity for organic chemicals, i.e., chemicals that have high acute aquatic toxicity also have high chronic aquatic toxicity [Rand, G.M., ed. Fundamentals of Aquatic Toxicology. 2nd ed. 1995, Taylor & Francis: Washington, DC.]. Since acute aquatic toxicity data are more readily available, DfE uses these data to screen chemicals that may be toxic to aquatic life. Where measured chronic toxicity data is available, it will be assessed with other data and applied in the screen based on the relationship between acute and chronic aquatic toxicity.

²⁹ Generally, >60% mineralization (to CO₂ and water) in a Ready Biodegradation test.

³⁰ Products of concern are compounds with high acute aquatic toxicity (L/E/IC50 ≤ 10ppm) and a slow rate of biodegradation (greater than 28 days).

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