Design for the Environment Program
Criteria for Chelating and Sequestering Agents

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1 Introduction

Purpose

These Criteria were developed to identify safer chelating and sequestering agents and are based on a comprehensive set of human and environmental health attributes. The Criteria identify chemicals with preferred human and environmental health profiles. Chelating and sequestering agents that pass these Criteria can be included in cleaning products eligible for recognition under the Design for the Environment (DfE) Safer Product Labeling Program. These criteria also enhance the transparency of the DfE Program.

Development

The DfE Criteria for Chelating and Sequestering Agents were developed by the Environmental Protection Agency's DfE Program and a group of stakeholders that included chelating agent manufacturers, cleaning product formulators, environmental non-governmental organizations, certification groups, industry associations, and others.

Scope

These Criteria can be used to identify chelating and sequestering agents suitable for use in products bearing the DfE logo. Such products include, but are not limited to, glass cleaners, general-purpose cleaners, washroom cleaners, carpet cleaners, floor care, laundry detergents, and drain cleaners. The broader scope of builders is not addressed in these criteria, and polymers used as chelating agents are also outside the scope.

The classes of chelating and sequestering agents reviewed in the development of these Criteria included amino carboxylates, carboxylates, hydroxy acids, inorganic phosphates, and phosphonates. Phosphonates will be evaluated under these Criteria with the exception of phosphonates used for hydrogen peroxide stabilization or prevention of scale build-up on heating elements. Builders, polymers, and the phosphonate functionalities listed above must meet the DfE Master Criteria or other relevant functional class criteria.

Classes of chelating and sequestering agents not addressed in the development of these Criteria may be evaluated under these Criteria, but may be subject to review against additional criteria. All chelating agents in products designed for direct release to the environment (e.g. graffiti removers and marine cleaners) must also pass the DfE Criteria for Environmental Toxicity and Fate for Chemicals in Direct Release Products.

2 General Requirements

2.1 Data for all relevant routes of exposure will be evaluated. For chelating agents in the form of salts, data are preferred for either the oral route of exposure or inhalation by dust, mist, or spray. Dermal toxicity studies on salts may not be relevant, because of low skin absorption potential. Inhalation (vapor) studies on salts may not be relevant because ionic compounds are not volatile. 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.

2.4 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.

2.5 When data are evaluated or developed specifically for review under these Criteria, oral, dermal & inhalation studies on chelating agents in the free acid form will be accepted.

2.6 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.

2.7 Residual NTA may be present in some chelating agents as a result of the synthesis process. Where present, residual NTA shall not exceed 0.1% by weight of the chelating agent as sold. This restriction is applied using the protic form of NTA (molecular weight = 191 g/mol).

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 chelating agent that is being evaluated (i.e., acute mammalian toxicity, biodegradability).

3.4 **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]

3.5 **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)

3.6 **Builder:** A material added to a soap or synthetic detergent formulation that enhances or maintains the cleaning efficiency of the surfactant. Performance capability depends upon the builder compound used. Principal functions include supplying alkalinity, and buffering to maintain alkalinity at effective cleaning levels. Other functions include reducing water hardness either by sequestration or chelation, and helping to remove soil in suspension. (modified from ASTM Standard Terminology Relating to Soaps and Other Detergents)

3.7 **Carcinogen** denotes a chemical substance or mixture of chemical substances which induces cancer or increases its incidence. (GHS)

3.8 **Chelating agent** is defined as an organic chemical that forms two or more coordination bonds with a central metal ion. Heterocyclic rings are formed with the central metal ion as part of each ring. Chelating agents can change the properties of metal ions, help to transport metal ions, and prevent scale formation.

3.9 A **chemical** is identified by its Chemical Abstract Service (CAS) number.

3.10 **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)

3.11 **Complex:** The reaction product formed from a chelating agent or sequestrant, and a metal ion; the chelated or sequestered metal ion. (e.g., $[EDTACU]_2$- is the EDTA complex of copper ion.)

3.12 **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.

3.13 **Degradation products of concern** are chemicals formed from degradation of the chelating agent with high acute aquatic toxicity (L/E/IC50 ≤ 10ppm) and which mineralize <60% in 28 days.

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

3.15 **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])

3.16 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]

3.17 **Flagging list:** A publicly available list of chemicals that may have potential hazard concerns as identified by the authors of that list.

3.18 **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)

3.19 An **ingredient** may be one chemical or a blend of multiple chemicals that are intentionally added.

3.20 **Inorganic phosphate**: This category includes all inorganic soluble forms of phosphates, such as, phosphoric acid [PO_4H_3 or OP(=O)(O)O] and its salts or phosphate salts, pyrophosphates, polyphosphates, and organic and inorganic forms of phosphorous that can be oxidized to phosphates rapidly. Inorganic forms of phosphonic acid (H_2PO_3 or OP(=O)O) are not included in this category because monopotassium phosphonic acid [13977-65-6] has been shown not to be an algal nutrient, not to be a replacement for phosphate in algal growth medium, and not to cause exponential growth of green algae. (US EPA New Chemicals) [Note: Inorganic forms of phosphonic acid are not typically used in cleaning products.]

3.21 **LOAEL**: Lowest Observed Adverse Effect Level

3.22 **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)

3.23 **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)

3.24 **NOAEL:** No observed Adverse Effect Level

3.25 **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])

3.26 **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])

3.27 **Respiratory sensitizer:** A substance that will induce hypersensitivity of the airways following inhalation of the substance. (GHS)

3.28 **Sequestering agent:** Any compound that, in aqueous solution, combines with a metal ion to form a complex in which the ion is substantially inactive. The complex is typically more water soluble than the metal ion. (modified from ASTM Standard Terminology Relating to Soaps and Other Detergents)

3.29 **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.

3.30 **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 Program[1] [9] or enhancements to estimation models such as EPI Suite™ [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 Chelating and Sequestering Agents

Each Attribute applies to all chelating and sequestering agents as defined in the Scope in Section 1. Failure to pass an Attribute results in failure to pass the Chelating and Sequestering Agents Criteria.

5.1 ACUTE MAMMALIAN TOXICITY

Criteria

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 1a. 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 – GHS Thresholds

Route (units)	Median Lethal Dose/Concentration
Oral LD50 (mg/kg bw)	2,000
Dermal LD50 (mg/kg bw)	2,000
Inhalation, gas LC50 (ppmV)	20,000
Inhalation, vapor LC50 (mg/L)	20
Inhalation, dust/mist/fumes LC50 (mg/L)	5

Additionally, a chemical does not pass the Criteria if it carries one of the following EU Risk Phrases (Table 1b), which align with the GHS thresholds in Table 1a:

Table 1b – Acute Toxicity Risk Phrases

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 combination risk phrases containing one or 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.

Sources for Data Interpretation

- GHS Ch 3.1 Acute Toxicity [11].
- 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” [12].

5.2 CARCINOGENICITY

Criteria

Chemicals considered carcinogens according to the authoritative lists in Table 2 do not pass the Criteria. Chemicals not on those authoritative lists, but that are known or presumed human carcinogens (Category 1), or suspected human carcinogens (Category 2) under GHS [13], do not pass the Criteria.

Table 2 – Authoritative Lists and GHS Criteria

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 [14]	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 [14]	R45: May cause cancer R49: May cause cancer by inhalation R40: Limited evidence of a carcinogenic effect *And all combination risk phrases containing one or more of the above.*
Globally Harmonized System (GHS) [13]	Category 1A – Known to have carcinogenic potential for humans Category 1B – Presumed to have carcinogenic potential for humans Category 2 – Suspected human carcinogens

Data Requirements

All available data will be evaluated. Measured and/or estimated data, for the chemical and/or a suitable analog will be reviewed against the GHS criteria using a weight-of-evidence approach. All aminocarboxylate chelating agents will be placed in one of three structural subgroups by EPA, namely EDTA and related substances, NTA and related substances, or aspartic acid derivatives. Chemicals in either of the aminocarboxylate subgroups specified below will be subject to additional review.

1. NTA and related substances
2. Aspartic acid derivatives

Flagging Lists

All relevant data and information used to place a chemical on the following flagging lists will be considered when reviewing a listed chemical against the carcinogenicity criteria.

1. Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [15, 16],

[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.

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 Cancer [17].

Additional Review

Chemicals in one of the aminocarboxylate subgroups specified below require review under GHS.

1. NTA and related substances
2. Aspartic acid derivatives

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.

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" [12].
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.6 Carcinogenicity [13].
- 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" [15].
- The following report describes the process used to develop the endocrine disrupters priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf [16].
- 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://cfpub.epa.gov/ncea/CFM/recordisplay.cfm?deid=160003 [22].

5.3 GENETIC TOXICITY

Criteria

Chemicals considered mutagens or genetic toxicants according to the authoritative lists below in Table 3a do not pass the Criteria. Chemicals not reviewed in the context of these authoritative lists, but for which data are available, may 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 [14]	Category 1 – Substances known to be mutagenic to man Category 2 – Substances which should be regarded as if they are mutagenic to man Category 3 – Substances which cause concern for man owing to possible mutagenic effects[3]
EU Risk Phrases [14]	R46: May cause heritable genetic damage R68: Possible risk of irreversible effects *And all combination risk phrases containing one or more of the above.*

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 above, an additional review may be performed. When an additional review is performed, GHS criteria, cited in Table 3b, will be used.

Under additional review, an acceptable data set includes at least one test (*in vitro* or *in vivo*) for gene mutation and at least one test (*in vitro or in vivo*) for chromosomal aberration. 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 as part of the weight-of-evidence. All available data, including *in vivo*, *in vitro*, and epidemiological studies, will be evaluated.

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” [12].
- EU Dangerous Preparations Directive Article 6 and Annex II (1999/45/EC and subsequent updates/amendments) [18-20].
- GHS Ch 3.5 Germ Cell Mutagenicity [23].

5.4 NEUROTOXICITY

Criteria

Chemicals that are considered neurotoxicants under GHS [24] (see GHS guidance values in Table 4) do not pass the Criteria. Neurotoxicity is covered under Specific Target Organ Toxicity Repeated Exposure in GHS.

Table 4 – GHS Guidance Values

Route of Exposure	Guidance Value*
Oral (mg/kg-bw/day)	100
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
**The doses provided are for 90-day studies. Guidance values are tripled for chemicals evaluated in 28-day studies.*	

Data Requirements

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

Sources for Data Interpretation

- Section 3, Hazard Characterization in *Guidelines for Neurotoxicity Risk Assessmen*t [25].
- GHS Ch. 3.9 Specific Target Organ Toxicity Repeated Exposure [24].

5.5 REPEATED DOSE TOXICITY

Criteria

Chemicals that are considered repeated dose (systemic) toxicants under GHS [24] (see GHS guidance values in Table 5a) do not pass the Criteria. Repeated dose toxicity is evaluated using the GHS chapter called Specific Target Organ Toxicity Repeated Exposure.

Table 5a – GHS Guidance Values

Route of Exposure	Guidance Value*
Oral (mg/kg-bw/day)	100
Dermal (mg/kg-bw/day)	200
Inhalation (gas) (ppm/6h/day)	250
Inhalation (vapor) (mg/L/6h/day)	1.0
Inhalation (dust/mist/fume) (mg/L/6h/day)	0.2
**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.*	

Additionally, a chemical does not pass the Criteria if it carries one of the following EU Risk Phrases:

Table 5b – Repeated Dose Toxicity Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU Risk Phrases [14]	R33: Danger of cumulative effects R39: Danger of very serious irreversible effects R48: Danger of serious damage to health by prolonged exposure R68: Possible risk of irreversible effects *And all combination risk phrases containing one or 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. Should testing be pursued to meet the data requirement, a functional observational battery (FOB) should be added to the test method to provide neurotoxicity information.

Flagging Lists

All relevant data and information used to place a chemical on the following flagging lists will be considered when reviewing a listed chemical against the repeated dose toxicity criteria.

1. Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [15, 16]
2. Substances prioritized for testing for endocrine disruption by the US EPA Endocrine Disruptor Screening Program [9].

Sources for Data Interpretation

– GHS Ch 3.9 Specific Target Organ Toxicity Repeated Exposure [24].

- 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". [15]
- The following report describes the process used to develop the endocrine disrupters priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf[16].
- EPA Endocrine Disruptors Screening Program, available at: http://www.epa.gov/endo/ [9].

5.6 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Criteria

Chemicals that are considered reproductive or developmental toxicants under GHS criteria (either Category 1 or 2) [26] and demonstrate adverse effects at doses equivalent to or below the values in Table 6a do not pass the Criteria.

Table 6a – TSCA 8(e) Guidance Values [27]

Route of Administration (units)	Guidance Value
Oral (mg/kg-bw/day)	250
Dermal (mg/kg-bw/day)	500
Inhalation (gas) (ppm/6h/day)	250
Inhalation (vapor) (mg/L/6h/day)	2.5
Inhalation (dust/mist) (mg/L/6h/day)	0.5

Additionally, a chemical does not pass the Criteria if it carries one of the following EU Risk Phrases:

Table 6b – Reproductive and Developmental Toxicity Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU CMR List [14]	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 [14] [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 *And all combination risk phrases containing one or 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. Following the approach in the SIDS Dossier [28], all chemicals must be reviewed for both fertility and developmental effects.

[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.

Flagging Lists

All relevant data and information used to place a chemical on the following flagging lists will be considered when reviewing a listed chemical against the reproductive and developmental toxicity criteria.

1. Substances prioritized for testing for endocrine disruption by the European Commission as Category 1 or 2 [15, 16]
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 [17].

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" [12].
- 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 Assessmen*t (EPA 1998), http://www.epa.gov/ncea/raf/pdfs/repro51.pdf [5].
- Part A, Section 3, Hazard Characterization in *Guidelines for Developmental Toxicity Risk Assessmen*t (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". [15]
- The following report describes the process used to develop the endocrine disrupters priority list: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf [16]
- EPA Endocrine Disruptors Screening Program, available at: http://www.epa.gov/endo/ [9].

5.7 RESPIRATORY SENSITIZATION

Criteria

Chemicals considered respiratory sensitizers according to the authoritative list in Table 7a do not pass the Criteria. Chemicals not reviewed in the context of this list, but for which data are available, may be subject to additional review. Chemicals that appear on the flagging list specified below require additional review.

Table 7a – Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU Risk Phrase [14]	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 7a, an additional review may be performed. When an additional review is performed, GHS criteria in Table 7b 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 [29] for further details.

Flagging Lists

Chemicals designated as sensitizer-induced asthmagens ("Rs" or "Rrs") 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 [30].

Table 7b – GHS Criteria

Authoritative Body	Does not pass DfE Criteria
Globally Harmonized System (GHS) [29]	Category 1A – high frequency of occurrence or sensitization rate in humans Category 1B – low to moderate frequency of occurrence or sensitization rate in humans

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" [12].
- 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 [29].
- Association of Occupational and Environmental Clinics Exposure Code List, available from: http://www.aoecdata.org/Default.aspx [30].

5.8 SKIN SENSITIZATION

Criteria

Chemicals considered skin sensitizers according to the authoritative list in Table 8a do not pass the Criteria. Chemicals not reviewed in the context of this list, but for which data are available, may be subject to additional review.

Table 8a – Authoritative Lists

Authoritative Body	Does not pass DfE Criteria
EU Risk Phrase [14]	R43: May cause sensitization by skin contact

Additional Review

In the case where skin sensitization data are available and have not been reviewed in the context of the authoritative list in Table 8a, an additional review may be performed. When an additional review is performed, GHS criteria in Table 8b will be used.

All available data, including *in vivo*, *in vitro*, and epidemiological studies, will be evaluated. Data from estimation models may be considered as part of the weight-of-evidence.

Table 8b – GHS Criteria

Authoritative Body	Does not pass DfE Criteria
Globally Harmonized System (GHS) [29]	Category 1A – high frequency of occurrence in humans and/or a high potency in animals Category 1B – low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals

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” [12].
- 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 [29].

5.9 ENVIRONMENTAL TOXICITY AND FATE (ET&F)

Criteria

If a chemical is an acute aquatic toxicant (i.e., L/E/IC50 < 100 ppm), then it must biodegrade rapidly and not be bioaccumulative (see Table 9, lines 1-3). If a component has low aquatic toxicity (Table 9, line 4), then its half-life must be less than 60 days.

Table 9 – Environmental Toxicity and Fate

	Acute Aquatic Toxicity Value (L/E/IC50)[5,6,7]	**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[8]…	…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 …	
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…	
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 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.

[5] 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.

[6] 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 ≤ 1ppm.

[7] 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.

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

Bioaccumulation potential: 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. 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 ≤ 1ppm: 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 > 1ppm and ≤ 10ppm: 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 > 10ppm and < 100ppm: 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 ≥ 100pm: 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.

5.10 EUTROPHICATION

Criteria

The total level of phosphorus in the cleaning product will be limited to a maximum level of 0.5 weight % in the cleaning product as sold (measured as elemental phosphorus). Inorganic phosphates, as defined by the US EPA New Chemicals Program [31], cannot make up any portion of the 0.5 weight % of phosphorus.

Note: Inorganic phosphates as defined by the US EPA New Chemicals Program [31] will not be allowed in DfE-labeled products. Eutrophication is a priority concern for EPA scientists, and inorganic phosphates can contribute to eutrophication of fresh water and estuarial ecosystems [31]. Given that the majority of DfE-labeled cleaning products are disposed of down the drain, the likelihood of release to wastewater streams and eventually, water bodies, is high. EPA acknowledges contributions to the phosphorus load from cleaners are relatively small in comparison with other sources[9] and that phosphorus overload may not be an issue in all regions. The EPA policy is consistent with a commitment to reduce contributions of phosphorus, particularly inorganic phosphates, regardless of concentration.

[9] See, for example, 32. *Detailed Assessment of Phosphorus Sources in Minnesota Watersheds*. 2004, Prepared by Barr Engineering Company for Minnesota Pollution Control Agency.
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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 4.

6.1 Acute Mammalian Toxicity – Test Methods for GHS Review

- OPPTS Harmonized Guideline 870.1100: Acute oral toxicity [33];
- OPPTS Harmonized Guideline 870.1200: Acute dermal toxicity [34]
- OPPTS Harmonized Guideline 870.1300: Acute inhalation toxicity [35];
- OECD Test Guideline 420: Acute Oral Toxicity-Fixed Dose Method [36];
- OECD Test Guideline 423: Acute Oral Toxicity – Acute Toxic Class Method [37];
- OECD Test Guideline 425: Acute Oral Toxicity – Up-and-Down Procedure [38];
- OECD Test Guideline 402: Acute Dermal Toxicity [39]; and
- OECD Test Guideline 403: Acute Inhalation Toxicity [40].

6.2 Carcinogenicity – Test Methods for GHS Review

- OECD Test Guideline 451: Carcinogenicity Studies [41];
- OECD Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies [42];
- OPPTS Harmonized Guidelines 870.4200: Carcinogenicity [43];
- OPPTS Harmonized Guidelines 870.4300: Combined chronic toxicity/carcinogenicity [44] 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” [45].

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 [46, 47];
- OECD Test Guideline 473 (OPPTS 870.5375): *In vitro* Mammalian Chromosome Aberration Test [48, 49];
- OECD Test Guideline 474 (OPPTS 870.5395): Mammalian Erythrocyte Micronucleus Test [50, 51];
- OECD Test Guideline 475 (OPPTS 870.5385): Mammalian Bone Marrow Chromosome Aberration Test [52, 53];
- OECD Test Guideline 476 (OPPTS 870.5300): *In vitro* Mammalian Cell Gene Mutation Test [54, 55]; and
- OECD Test Guideline 483 (OPPTS 870.5380): Mammalian Spermatogonial Chromosome Aberration Test [56, 57];
- OECD Test Guideline 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* [58]. This guideline does **NOT** substitute in the necessary minimum set for either the gene mutation or the chromosome aberration test.

6.4 Neurotoxicity – Preferred Test Methods for GHS Review

- OECD Test Guideline 424: Neurotoxicity Study in Rodents [59] 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 [60] and OPPTS Harmonized Guideline: 870.6300 Developmental neurotoxicity study [61] can be used to screen chemicals for neurotoxicity.

6.5 Repeated Dose Toxicity – Preferred Test Methods for GHS Review

- OECD Test Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents [62]
- OECD Test Guideline 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents [63]
- OECD Test Guideline 411: Subchronic Dermal Toxicity: 90-day Study [64]
- OECD Test Guideline 413: Subchronic Inhalation Toxicity: 90-day Study [65]
- OPPTS Harmonized Guideline 870.3100: 90-Day oral toxicity in rodents [66]
- OPPTS Harmonized Guideline 870.3150: 90-Day oral toxicity in nonrodents [67]
- OPPTS Harmonized Guideline 870.3250: 90-Day dermal toxicity [68]
- OPPTS Harmonized Guideline 870.3465: 90-Day inhalation toxicity [69]

Repeated Dose Toxicity – Acceptable Test Methods for GHS Review

- OECD Test Guideline 407: Repeated Dose 28-day Oral Toxicity Study in Rodents [70]
- OECD Test Guideline 410: Repeated Dose Dermal Toxicity: 28-day Study [71]
- OECD Test Guideline 412: Repeated Dose Inhalation Toxicity: 28-day Study [72]
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [73]
- OPPTS Harmonized Guideline 870.3050: Repeated dose 28-day oral toxicity study in rodents [74]
- OPPTS Harmonized Guideline 870.3200: 28-Day dermal toxicity [75]

6.6 Reproductive and Developmental Toxicity – Test Methods for GHS Review

Fertility test methods, preferred

- OECD Test Guideline 415: One-Generation Reproduction Toxicity Study [76] and
- OECD Test Guideline 416: Two-Generation Reproduction Toxicity Study [77].

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 [78];
- OECD Test Guideline 421: Reproduction/Developmental Toxicity Screening Test [79];
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [73];
- OPPTS Harmonized Guideline 870.3550: Reproduction/developmental toxicity screening test [80]
- OPPTS Harmonized Guideline 870.3650: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [81].

Developmental toxicity test methods, preferred

- OECD Test Guideline 414: Prenatal Developmental Toxicity Study [82]

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 [78];
- OECD Test Guideline 421: Reproduction/Developmental Toxicity Screening Test [79];
- OECD Test Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [73];

- OPPTS Harmonized Guideline 870.3550: Reproduction/developmental toxicity screening test [80]; and
- OPPTS Harmonized Guideline 870.3650: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [81].

6.7 Skin Sensitization – Preferred Test Methods for GHS Review

- OECD Test Guideline 406: Skin Sensitization [83]
- OECD Test Guideline 429: Skin Sensitization: Local Lymph Node Assay [84]
- OPPTS Harmonized Guideline 870.2600: Skin Sensitization [85]

6.8 Environmental Toxicity and Fate

6.8.1 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 [86] and
- OPPTS Harmonized Guideline 850.1075: Fish acute toxicity test, freshwater and marine[87].

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 [88];
- OPPTS Harmonized Guideline 850.1010: Aquatic invertebrate acute toxicity test, freshwater daphnids[89]; and
- OPPTS Harmonized Guideline 850.1035: Mysid acute toxicity test [90].

NOTE – EPA may request that the test be carried out using semi-static renewal or flow-through system with a 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) [91] and
- OPPTS Harmonized Guideline 850.5400: Algal toxicity, Tiers I and II (including growth inhibition and biomass) [92].

NOTE – The OECD Test Guideline 201 allows for modification of the growth media in the case where the test substance may form complexes with nutrients essential to algal growth. Per OECD guidance on this modification (see Annex 4 of the OECD publication Number 23: *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures* [89]), algal growth medium may be modified with a hardness of approximately 150 mg/l as $CaCO_3$. All available data will be evaluated, including algal toxicity tests without modification of the growth media. Preference will be given to data with modified growth media. For chemicals where algal toxicity data are only available from tests without modification to the growth media, a manufacturer may generate and submit results with the modified test media.

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 [93];
- OPPTS Harmonized Guideline 850.1025: Oyster acute toxicity test (shell deposition) [94];
- OPPTS Harmonized Guideline 850.1045: Penaeid acute toxicity test [95];
- OPPTS Harmonized Guideline 850.1055: Bivalve acute toxicity test (embryo larval) [96];
- OPPTS Harmonized Guideline 850.4400: Aquatic plant toxicity test using *Lemna spp.* Tiers I and II [97].

6.8.2 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 Table 9, rows 1-3, 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)[98];
- OECD Test Guideline 310: Ready Biodegradability – CO2 in sealed vessels [99]; and
- OPPTS Harmonized Guideline 835.3110: Ready biodegradability [100].
- For chemicals where acute aquatic toxicity ≥100 ppm (i.e., line 4, Table 9), 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) [16-18] then the half-life of a chemical is likely to be less than 60 days [101].
- 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 < 100ppm.
  - OECD Test Guideline 303A (OPPTS 835.3240): Aerobic Sewage Treatment: Activated Sludge Units [102, 103],
  - OECD Test Guideline 309 (OPPTS Harmonized Guideline 835.3190): Aerobic Mineralization in Surface Water - Simulation Biodegradation Test [104, 105],
  - 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) [106],
  - OPPTS Harmonized Guideline 835.3280–Simulation Tests to Assess the Primary and Ultimate Biodegradability of Chemicals Discharged to Wastewater [107],
  - OPPTS Harmonized Guideline 835.3170 - Shake Flask Die-Away Test [108], and
  - OPPTS Harmonized Guideline 835.3180 - Sediment/Water Microcosm Biodegradation Test [109].

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.

6.8.3 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[110];
- OPPTS Harmonized Guideline 850.1710: Oyster BCF[111];
- OPPTS Harmonized Guideline 850.1730: Fish BCF[112];
- Modeled data from sources such as EPI Suite™ [10] are acceptable when data are unavailable.

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