

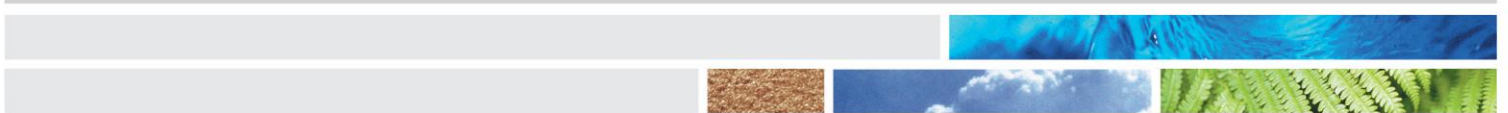


Environmental
Protection Authority
Te Mana Rauhi Taiao

Thresholds and Classifications

Under the Hazardous Substances and New Organisms Act 1996

JANUARY 2012 (CONTENT AS ORIGINALLY PUBLISHED MARCH 2008)



USER GUIDE

Record of Amendments

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Disclaimer

This user guide provides only general advice on the thresholds and classifications for substances under the HSNO Act. It is not a definitive interpretation of the HSNO Act or its regulations. We suggest you carefully consider the HSNO Act and its regulations, and obtain your own professional advice.

Preface

The Hazardous Substances and New Organisms Act 1996 (HSNO Act) reforms and restates the law relating to the management of hazardous substances and new organisms in New Zealand. With particular reference to hazardous substances, the HSNO Act replaced the Dangerous Goods Act 1974, Explosives Act 1957, Pesticides Act 1979, and Toxic Substances Act 1979 from 2 July 2001. A series of hazardous substances regulations made under the HSNO Act replaced the regulations made under the earlier legislation.

A key feature to managing hazardous substances under the HSNO Act is determining what substances are classed as 'hazardous substances'. The initial responsibility for making this judgement rests with the importer or manufacturer of the substance. To assist you in making this decision, the EPA has prepared this user guide.

The determination of whether a substance is 'hazardous' is a technical and a legal determination. The manufacture or importation of a hazardous substance without an approval is an offence under section 25(1) of the HSNO Act. If a company is manufacturing or importing a hazardous substance otherwise than in accordance with a HSNO Act approval, a HSNO Act enforcement agency such as the Department of Labour could prosecute that company.

We strongly recommend that if, after completing an evaluation, you decide a substance is not hazardous, you thoroughly document your reasons for this decision. It is also a condition of Group Standard approvals that you retain a record of the classification determination for the purposes of assignment to a particular Group Standard.

You may wish to obtain expert advice to support your decision. The EPA provides a Status of Substance service to provide informal advice about whether a substance is hazardous and/or covered by an existing approval (see our website for more information, www.epa.govt.nz). The EPA will make formal determinations only in special circumstances. (These circumstances include the determination of whether or not a substance is a hazardous substance under section 26 of the HSNO Act and regulations made under section 75(1)(g) of the HSNO Act, declaring a substance not to be hazardous for the purposes of the Act.)

If you conclude that your substance is hazardous you need to get an approval from the EPA, unless your substance is covered by an existing Group Standard or other existing HSNO Act approval. The Status of Substance service also says whether the substance is covered by an existing Group Standard or a HSNO Act approval. If you are considering making an application to import or manufacture the substance, our staff are happy to advise you. You may obtain more information about the HSNO Act and EPA procedures from our website (www.epa.govt.nz).

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1. Introduction to the Hazardous Substances and New Organisms Act 1996 and to Using this Guide

1.1. Introduction to the HSNO Act

1.1.1. Purpose of the HSNO Act

The purpose of the Hazardous Substances and New Organisms Act 1996 (HSNO Act) is to protect the environment and the health and safety of people by preventing or managing the adverse effects of hazardous substances.

The HSNO Act replaced the Dangerous Goods Act 1974, Explosives Act 1957, Pesticides Act 1979, Toxic Substances Act 1979, and the regulations associated with these Acts.

Section 25(1)(a) of the HSNO Act states:

No hazardous substance shall be imported or manufactured ... otherwise than in accordance with an approval issued under this Act or in accordance with Parts XI to XVI of this Act.

Parts XI to XV of the HSNO Act were the transitional provisions of the Act for substances that had approvals under the predecessor legislation. That is, pesticides, toxic substances, dangerous goods, and explosives. These parts of the Act have now expired.

The HSNO Act provides for a series of regulations to be developed. These regulations enable hazardous substances to be defined, and for the level of hazard to be classified and then managed to minimise adverse effects.

When an application is made to the EPA to import or manufacture a hazardous substance, a classification for each hazardous property of the substance is determined. This classification triggers a set of controls (called default controls) from the controls regulations. The EPA may also, in some circumstances, vary the default controls.

1.1.2. Definition of a hazardous substance

Section 2 of the HSNO Act defines a 'substance' as:

- (a) *Any element, defined mixture of elements, compounds, or defined mixture of compounds, either naturally occurring or produced synthetically, or any mixtures thereof:*
- (b) *Any isotope, allotrope, isomer, congener, radical, or ion of an element or compound which has been declared by the Authority, by notice in the Gazette, to be a different substance from that element or compound:*
- (c) *Any mixtures of combinations of any of the above:*
- (d) *Any manufactured article containing, incorporating or including any hazardous substance with explosive properties:*

A substance is considered a 'hazardous substance' when it has an effect more hazardous than any one or more of the regulated threshold levels for any of the intrinsic properties of:

- explosiveness;
- flammability;
- oxidising capacity;
- corrosiveness;
- toxicity; and
- ecotoxicity.

1.1.3. What is a threshold?

A threshold is the amount or concentration of a substance that is likely to cause an adverse effect on people or the environment. It is a trigger level for an effect that the EPA may consider requires controls on the substance to meet the purpose of the HSNO Act.

The threshold level is the bottom 'rung' on the classification 'ladder'. As you move up the ladder, the substance becomes more hazardous and requires greater controls to protect people and/or the environment.

The thresholds and classification categories reflect the international harmonisation of classification systems for hazardous substances and mixtures under the auspices of the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007a).

1.1.4. Description of thresholds and classification systems

Thresholds for hazardous properties

The thresholds for the HSNO Act hazardous properties are set out in Schedules 1 to 6 of the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. Regulation 4 of those regulations states that a substance is not hazardous for the purposes of the HSNO Act unless data indicates it meets the minimum degrees of hazard for at least one of the intrinsic hazardous substance properties specified.

In those regulations, data includes 'values that are directly measured, calculated, or estimated for any of the measures given'. This means it is not necessary to rely only on directly measured data to determine whether a substance exceeds any of the hazardous property threshold criteria. It may be possible to calculate a relevant parameter for a substance based on the directly measured values available on its components by making use of 'mixture rules' (see the relevant chapters for details). Alternatively, a relevant parameter for a substance may be estimated based on the similarity of that substance to another substance for which the hazardous properties are known.

Classification criteria for hazardous properties

The classification criteria for the HSNO Act hazardous properties are set out in Schedules 1 to 6 of the Hazardous Substances (Classification) Regulations 2001.

The classification systems comprise:

- numbered classes (for example, class 6), indicating the intrinsic hazardous property;
- numbered subclasses (for example, subclass 6.1), indicating the type of hazard; and
- lettered categories (for example, category A) indicating the degree of hazard.

Exceptions to this are explosive substances, which are classified into a subclass (indicating the type of explosive hazard) and a category (indicating compatibility groupings) in the combinations permitted by the United Nations Recommendations on the Transport of Dangerous Goods Model Regulations (United Nations, 2007b). Categories for explosive substances do not indicate the degree of hazard. Other exceptions are the two separate classifications for sensitisation, where a substance can be classified as both 6.5A (respiratory sensitisation) and 6.5B (contact sensitisation). Likewise, the 6.8C (causes developmental effects via lactation) category is independent of the other 6.8A and 6.8B categories. Further guidance is provided in the relevant chapters for these properties.

The combination of numbers and letters used in the classification system (eg, 6.1A) constitutes a hazard classification of a substance.

Classes for the hazardous properties

The nine classes for the hazardous properties are:

- class 1: explosiveness (see chapter 2 [below](#));
- class 2: flammability, gases (see chapters 3 [below](#) and 4 [below](#));
- class 3: flammability, liquids (see chapters 3 [below](#) and 5 [below](#));
- class 4: flammability, solids (see chapters 3 [below](#) and 6 [below](#));
- class 5: oxidising capacity (see chapter 7 [below](#));
- class 6: toxicity (see chapters 9–17 [below](#));
- class 8: corrosiveness (see chapter 8 for metal corrosivity [below](#) and chapter 11 for corrosion of biological tissues [below](#)); and
- class 9: ecotoxicity (see chapters 18–23 [below](#)).

Class 7 is unallocated in the HSNO Act classification system, because it is reserved for radioactivity, which is outside the scope of the HSNO Act. Class 7 is used in the United Nations classification system for the transport of dangerous goods for radioactive materials. In New Zealand, these substances are covered by the Radiation Protection Act 1965, which is administered by the National Radiation Laboratory of the Ministry of Health.

Similarly, subclass 6.2 is unallocated in the HSNO Act classification system for toxicity, because it is reserved in the United Nations classification system for the transport of dangerous goods for infectious substances. These are also outside the scope of the hazardous substances part of the HSNO Act.

1.1.5. Exemptions from the HSNO Act

Some human medicines and food are categories of substance that are exempt from requiring approval under the HSNO Act even if they have properties that exceed the hazardous property thresholds. These exemptions are set out in sections 5 and 6 of the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.

Human medicines

Human medicines in their finished dose form are excluded from the HSNO Act unless the substance is a gas contained at a pressure greater than 170 kPa in a container larger than 100 mL, before the gas is administered to a person for a therapeutic purpose.

However, new medicines (as defined in the Medicines Act 1981) are not excluded from the HSNO Act if they meet any of the threshold criteria and are either a substance to which section 3(1)(b) of the Medicines Act 1981 applies (that is, they are an ingredient of a medicine) or the substance is registered as a veterinary medicine under the Agricultural Compounds and Veterinary Medicines Act 1997.

Food

Food in a ready-to-consume form, which may meet the hazardous property thresholds, is excluded from the HSNO Act.

Food additives are not excluded from the HSNO Act if they meet any of the threshold criteria and if they have not been mixed with or added to any other food or drink that is in a ready-to-consume form.

1.1.6. Manufactured articles

Manufactured articles containing or incorporating hazardous substances with properties other than explosiveness are not substances under the HSNO Act.

Manufactured articles with explosive properties (such as flares or detonators) are hazardous substances under the HSNO Act.

Other manufactured articles containing, incorporating, or including a hazardous substance may be regulated under the HSNO Act through the provisions for Group Standards (Part 6A of the HSNO Act). However, in these instances, the article itself would not be subject to hazard classification.

Manufactured products such as glues, paints, and pesticides are also considered substances under the HSNO Act.

The EPA has adopted the following as a working definition of 'manufactured article':

A manufactured article is something for which its intended use is primarily to do with its physical shape, rather than its chemical composition.

However, because this distinction is not always clear, we have expanded the definition and established that an item is a manufactured article if it satisfies all of the following criteria.

- The item is deliberately formed to a specific shape or design during manufacture.
- The item has an end use function wholly or partly dependent on its shape or design.

- The item undergoes no change of chemical composition during end use, except as an intrinsic part of that end use.
- The item is not a particle or fluid.

Fluids or particles contained within a vessel serving simply to store, transport, and dispense its contents are considered to be substances. In general, all fluids and particles such as cleaners, solvents, fuels, glues, sealants, inks, paints, and other coatings are substances if they are merely contained in some form of packaging. That is, the contents of containers such as bottles, jars, cans, aerosol cans, drums, barrels, tanks, bags, tubes, and sachets are chemical substances or mixtures of chemical substances.

More detailed information on manufactured articles is provided in the information sheet Manufactured Articles (ERMA New Zealand, 2001).

1.1.7. Definitions

The chapters on each hazardous property list the key definitions relevant to that property.

1.1.8. Application forms and related publications

If a substance is assessed as having properties that are above one or more of the hazardous property thresholds discussed in this document, then an approval for the substance is required under the HSNO Act.

Several EPA publications and application forms will help applicants with their application for an approval. For more information on the HSNO Act and EPA procedures, see our website (www.epa.govt.nz).

1.2. How to use this guide

1.2.1. Aim of this guide

This guide is to help you to interpret the:

- threshold regulations, which determine whether a substance is hazardous and subject to the requirements of the HSNO Act; and
- classification regulations, which assign levels of hazard to hazardous substances.

1.2.2. Responsibility for deciding whether a substance is ‘hazardous’

The initial responsibility for deciding whether a substance is ‘hazardous’ rests with the importer or manufacturer of the substance.

1.2.3. Hazardous properties

Each substance must be assessed for each of six hazardous properties before a conclusion can be reached.

The threshold regulations set the level of hazard below which a substance is not considered hazardous.

1.2.4. How to determine whether a substance is ‘hazardous’

The determination as to whether a substance is ‘hazardous’ is not only a technical determination but also a legal one. The manufacture or importation of a hazardous substance without an approval is an offence.

[Figure 1.1](#) overviews the process for determining whether a substance is hazardous and requires a HSNO Act approval to be imported or manufactured.

This guide has separate sections for each hazardous property (see 'Classes for the hazardous properties' in section 1.1.4 [above](#)).

While many substances trigger only one threshold, other substances trigger more than one. Therefore, it is necessary to evaluate each substance against the thresholds in each section. This evaluation is a moderately complex technical task.

We have developed this guide assuming you have sufficient scientific and technical knowledge and experience to determine whether a substance is hazardous. If you do not have the ability to address the technical issues, seek advice from people who do.

To evaluate a substance collect as much relevant information about the characteristics of the substance as you reasonably can. Then compare this information with the criteria within each property that may trigger the threshold.

For advice about evaluating the quality of data, see section 1.1.4 [above](#).

Note that an inability to access the information does not necessarily mean there is no information. If you do not have adequate information, use your technical judgement, including answering the following questions.

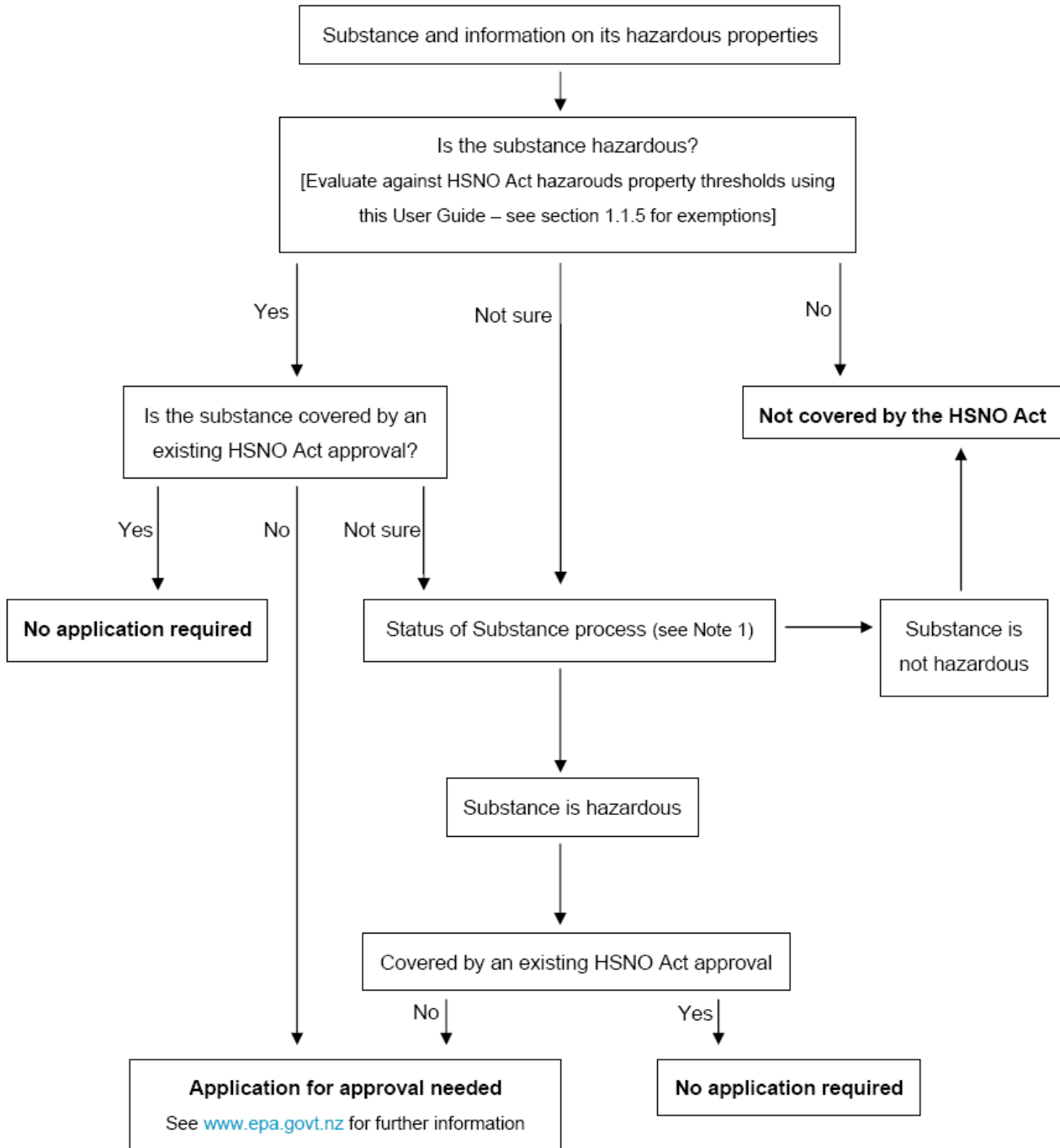
- Do similar substances have properties that would give reliable guidance?
- Is it plainly unreasonable to expect the substance to have such a property?
- Should this gap be referred to an expert in the field?

If a substance does not trigger a threshold, then it is not 'hazardous' and does not need an approval under the HSNO Act. However, if a substance does trigger a threshold, it cannot be imported or manufactured in New Zealand without an approval.

If the substance is not covered by an existing approval, including Group Standards, then you need to make an application before importing or manufacturing it (see [Figure 1.1](#)).

The EPA provides a Status of Substance service if you wish to obtain informal advice about whether a substance is hazardous and/or covered by an existing approval.

Figure 1.1: Process for determining whether a substance is hazardous and requires a HSNO Act approval



Note 1: The Status of Substance process can be used to seek informal advice from the EPA about whether a substance is hazardous and/or covered by an existing approval.

1.3. Evaluating the quality of data

1.3.1. Reliability, relevance, and adequacy

In general, the three aspects to assessing the quality of data from studies are reliability, relevance, and adequacy. Klimisch et al (1997) defined these terms in the following way.

- Reliability – the inherent quality of a test report or publication evaluated in relation to a standard test methodology. This includes considering the clarity in how experimental procedures are described and the plausibility of the results.
- Relevance – the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation.
- Adequacy – the usefulness of data for hazard or risk assessment purposes. Studies may be undertaken for many purposes, and while the research may be scientifically valid, it may not always be adequate for use in a hazard assessment. When there is more than one study for each element, attach the greatest weight to the study that is the most reliable and relevant.

Evaluate carefully the quality of the study, its method, the report of the results, and the conclusions drawn.

Data may vary in quality because studies:

- use outdated test guidelines;
- fail to characterise the test substance properly (for example, in terms of purity and physical characteristics);
- use techniques and procedures that have since been refined; or
- have not recorded or measured certain endpoint information that is now recognised as important.

Determine whether a study is reliable, before determining its relevance and adequacy.

1.3.2. Reliability considerations

Undertake an initial, quick assessment to filter out unreliable studies, and then focus further resources on the most reliable studies. It is critical you know how the study was carried out, because without this information, all other considerations are likely to be irrelevant.

Klimisch et al (1997) developed a scoring system for reliability, particularly for ecotoxicology and health studies, that may be extended to physicochemical and environmental fate and pathway studies.

- 1 = reliable without restrictions:
studies or data ... generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to Good Laboratory Practice (GLP)) or in which the test parameters documented are based on a specific (national) testing guideline ... or in which all parameters described are closely related/comparable to a guideline method.
- 2 = reliable with restrictions
studies or data ... (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which

investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.

- 3 = not reliable
studies or data ... in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.
- 4 = not assignable
studies or data ... which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).

Klimisch codes are a useful tool for discarding unreliable studies, organising studies for further review, focusing on the most reliable studies, and allowing additional time to be devoted to considerations of relevance and adequacy of only reliable studies.

The best studies are those that give a precise description of the nature of the effect, the number of subjects or the percentage of animals affected by the observed effects, and the exposure conditions (duration and concentration).

Evaluate reliability using international standard test guidelines as references. The classification should not exclude all unreliable data from further consideration by experts. In general, data with lower reliability may be used as supporting data.

Use the criteria listed in Table 1.1 to screen test results for reliability. These criteria address the overall scientific integrity and validity of the information in a study; that is, reliability. Any study not meeting the criteria in the table would be assigned a Klimisch score of 4 (not assignable). Such studies could provide only supplementary information.

Table 1.1: Key reliability criteria for screening data

| Criteria | Required for specific tests | | |
|--|-------------------------------------|--------------------|--------------------------|
| | Physical and/or chemical properties | Environmental fate | Toxicity and ecotoxicity |
| Test substance identification (adequate description of test substance, including chemical purity and identification or quantification of impurities to the extent available] | x | x | x |
| Temperature | x ¹ | x | x |
| Controls ² | | x | x |
| Species, strain, number, gender, and age of organisms | | | x |

| | | | |
|---|---|---|---|
| Dose or concentration levels | | x | x |
| Route or type of exposure ³ | | | x |
| Duration of exposure | | x | x |
| Statistics (with some exceptions, eg, the <i>Salmonella</i> /Ames assays) | | | x |
| Full citation or reference | x | x | x |

Notes

- For vapour pressure, octanol or water partition coefficient, and water solubility values.
- All studies must have negative controls and some studies (eg, biodegradation, *Salmonella*/Ames assay) must also have positive controls. If a vehicle is used in the administration of the test substance, vehicle controls must also be established and reported. Exceptions may be allowed for acute mammalian toxicity studies.
- The route or type of exposure (eg, oral or inhalation for mammalian studies) or test system (eg, static, flow-through for ecotoxicity) must be reported.

1.3.3. Relevance and adequacy considerations

The use of sound scientific judgment is the most important principle in considering relevance and adequacy. The chapters on specific hazard properties provide more information on which studies are considered relevant and adequate for assessing each property.

The EPA assigns Klimisch scores to the data used to classify substances. It uses a weight-of-evidence approach (see section 1.3.4) to evaluate all the available data for a particular hazard classification, including bridging principles from the GHS (United Nations, 2007a).

Each hazardous property chapter states the data required for classification purposes. The quality and type of additional data required vary with different types of substance and different HSNO Act approval categories.

Further information is included in the user guide for each application form, and the EPA website (www.epa.govt.nz).

1.3.4. Weight of evidence

In the GHS a weight-of-evidence approach is given prominence for classification (United Nations, 2007a). All available information that bears on the determination of classification for an endpoint is considered together. Include information such as epidemiological studies and case reports in humans and specific studies along with subchronic, chronic and special study results in animals that provide relevant information. You may also include evaluations of substances chemically related to the material under study, particularly when information on the material is scarce.

The weight given to the available evidence is influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans, and freedom from bias. Collate both positive and negative results into a weight-of-evidence determination. However, a single,

positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.

Each hazardous property chapter contains further information on specific approaches that may be used in reaching a classification decision based on the weight of evidence.

1.3.5. Data sources

Data sources are highly variable in terms of quality, reliability, accuracy, extent of peer review, the time spans covered, the number of chemicals addressed, and the extent of detail. Experienced searchers will know which sources have been most useful to them in the past.

There are a large number of other potentially useful data sources, and many require specific searching skills in order to ensure all relevant information is retrieved. The person classifying a substance should be looking to optimise the value of the searches carried out. As is the case for many specialised activities, possibly the most efficient mechanism is to use the services of people who have expertise in searching the data sources. While it might be possible in the future to define a stepwise approach to data searching (or a minimal acceptable search strategy), it is not considered appropriate at present to recommend any specific strategy as being sufficient for purpose. A critical aspect is that the search strategy is clearly recorded to allow transparency in relation to the depth and width of searching that has been undertaken, the dates on which searches were carried out, and details of the coverage (for example, topics, relevance, size, and years) of the data sources that are examined.

When no data are found, other types of information (such as Quantitative Structure Activity Relationships (QSARs)) might be valuable. When validated, QSARs are available for specific endpoints. This is indicated in the relevant chapters (for example, chapter 19 on aquatic toxicity).

1.3.6. Examples of information sources

Information sources include the following.

- Company files may include studies generated in-house, commissioned studies carried out on contract, information about experience with using the material, reports from downstream companies and customers, purchased reports from other companies, collections of published papers, and reviews of published data. This information is likely to cover the company's product range and requires expertise to interpret.
- Published literature includes papers reporting original findings (primary papers), review papers, books, monographs, and reports of proceedings, meetings, and conferences. It covers many more chemicals than does the product range of any company. It requires expertise in identifying and interpreting information.
- Databases and databanks may include relevant information depending on the objectives of the hosts or providers (which may change). Databases generally direct searchers to original sources, while databanks generally contain limited information from original sources, and give little insight into the quality of test information. Databases and databanks are only routes to the original sources, rather than

sources themselves. They cover many more chemicals than does the product range of any company. They require expertise in searching numerous systems and interpreting information.

- QSAR and SAR models are sometimes freely and sometimes commercially available. In theory, they may be applied to any untested chemical, but domain applicability is a potential problem. Specialised expertise is needed to run models and interpret results.
- The internet has search engines that identify electronic versions of a diverse range of data sources. In addition, the websites of various expert organisations and regulatory bodies contain useful information. Much 'grey' (that is, not formally published) literature is available via this route.

Individual chapters in this user guide contain links to electronic data sources. These links were current at the time of publication.

1.3.7. Acceptable test methodologies

Acceptable test methods for assessing each hazardous property are identified in the relevant hazardous property chapters.

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2. Substances with Explosive Properties – Class 1

2.1. Introduction

The term 'explosive' is defined in section 2 of the Hazardous Substances and New Organisms Act 1996 (HSNO Act) as meaning:

capable of sudden expansion owing to a release of internal energy, and includes the capability to generate:

- (a) *Deflagration*
- (b) *Pyrotechnic effects.*

It is useful to also recall that the HSNO Act provides a broad definition of 'substance', which, in the case of explosive substances, includes 'any manufactured article containing, incorporating, or including any hazardous substance with explosive properties'.

The criteria for deeming a substance explosive are derived from the recommendations of the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods (UNSCETDG). They cover how easily a substance explodes, and the type of explosive force generated when it is set off, for example, a blast, projectile movement, or pyrotechnic (fireworks-like) effect.

The details of the threshold tests and classification levels can be found in the United Nations Recommendations on the Transport of Dangerous Goods Model Regulations (United Nations, 1999b) (UN Model Regulations) and its companion volume Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria (United Nations, 1999a) (UN Manual of Tests and Criteria). These versions of the documents are referred to in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and Hazardous Substances (Classification) Regulations 2001. Equivalent material can be found in the more recent versions of these documents.

The threshold limits adopted under the HSNO Act will maintain the present levels of intervention for explosive hazards used by the Chief Inspector of Explosives under the Explosives Act 1957. This ensures that all fireworks, emergency flares, and other pyrotechnic devices as well as explosive powders, explosive articles (for example, detonators), and blasting explosives are captured. The new limits, however, adopt the internationally accepted performance criteria for explosive effects used by the UN, formalising the shift from the current mix of general chemical types, specific product formulations, and broad use descriptions under the Explosives Act. Substances that have minor explosive characteristics may fall outside the explosive threshold, but will usually be checked for their flammability or capacity to oxidise.

It is important to note that otherwise inert materials with a fine particle size distribution, that possess solely a dust explosibility hazard when dispersed at above a minimum concentration in air, are excluded from the HSNO Act definition of an explosive substance. These substances will not pass the threshold tests for explosiveness described below.

2.2. Definitions

The following terms are used in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and the Hazardous Substances (Classification) Regulations 2001 (the classification regulations) in respect of explosive substances.

| Term | Definition |
|------------------------------|---|
| deflagrate | In relation to a substance that is initiated or ignited, means the production in that substance of a chemical reaction that proceeds through, or along the surface of, the substance at subsonic velocity, where that chemical reaction: <ol style="list-style-type: none"> results in the steady production of hot gases at high pressures; and if the substance is sufficiently confined, results in an increase in pressure, rate of reaction, and temperature that may produce a detonation of the substance. |
| detonate | In relation to a substance that is initiated, means the production in that substance of a chemical reaction that proceeds through that substance at supersonic velocity, resulting in the production of heat and a supersonic shock wave through the surrounding medium. |
| effective protective feature | A device incorporated into an explosive article that will prevent accidental functioning during normal conditions of transport, storage, or handling. |
| g | gram(s) |
| gas | A substance that: <ol style="list-style-type: none"> is completely gaseous at 20°C and at 101.3 kPa absolute pressure; or has a vapour pressure of more than 300 kPa absolute pressure at 50°C. |
| J | joule(s) |
| kg | kilogram(s) |
| kPa | kilopascal(s) |
| LD ₅₀ | The median lethal dose, being a statistically derived single dose of a substance that can be expected to cause death in 50% of animals. |
| liquid | A substance that is <ol style="list-style-type: none"> a substance that has a melting point of less than or equal to 20°C at 101.3 kPa absolute pressure; or a viscous substance without a defined melting point, if: <ol style="list-style-type: none"> more than the quantity of the substance specified in ASTM D4359-90, called 'Test method for determining whether a material is a liquid or a solid', collects on a watch glass when tested in the manner specified in that test; or a penetrometer penetrates into the substance the distance defined in the test for determining fluidity prescribed in Appendix A.3 of the European Agreement Concerning the International Carriage of Dangerous Goods by Road (United Nations, 1994), when the method specified in that test is followed. |
| m | metre(s) |
| mg | milligram(s) |

| | |
|--|---|
| mm | millimetre(s) |
| primary explosive substance | A substance that: <ul style="list-style-type: none"> • has the necessary sensitivity to heat, friction, or shock to make it suitable for initiating secondary detonating explosive substances and articles; and • when incorporated into an explosive article, is known as a primer or detonator. |
| propellant explosive substance | A substance that deflagrates (that is, is capable of a steady high rate of production of gas sufficient to generate a force capable of producing movement or physical change, the rate of gas production under confinement is able to result in a detonation). |
| pyrotechnic effect | In relation to a substance that is initiated, means the production in that substance of a self-sustaining exothermic chemical reaction resulting in heat, sound, light, smoke, gas, or motion, or a combination of these. |
| pyrotechnic substance | A substance that produces pyrotechnic effects. |
| secondary detonating explosive substance | A substance designed to detonate that requires stimulation equivalent to the detonation of a primary explosive substance to initiate it. |
| solid | A substance that is neither a liquid nor a gas. |
| Test Series | When followed by a letter or number, means one or more tests as prescribed in the UN Manual of Tests and Criteria. |
| UN Manual of Tests and Criteria | Third revised edition of <i>Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria</i> (United Nations, 1999a). Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. |
| UN Model Regulations | Eleventh revised edition of <i>Recommendations on the Transport of Dangerous Goods Model Regulations</i> (United Nations, 1999b). Note: Equivalent material can be found in more recent versions of this document, for example, the 15th revised edition. |

2.3. Threshold for substances with an explosive property

2.3.1. Two elements to the threshold

The two elements to the threshold for substances with an explosive property are:

- an ability to cause an explosive effect (explosiveness), coupled with a sufficient likelihood of detonation or deflagration, when stimulated (sensitiveness); and
- whether substances are designed to detonate, deflagrate, or produce a pyrotechnic effect.

The first criterion requires results of the quantitative tests set out in the UN Manual of Tests and Criteria. As well as following the UN, the second criterion carries over the current scope of the Explosives Act 1957. It provides for any article designed to have an explosive effect to be assessed under the HSNO Act, without the need for the first two test types covered in the first criterion. These criteria are expanded, in more technical detail, in section 2.3.2.

2.3.2. Explosive threshold technical description

The threshold criteria for substances with explosive properties, including manufactured articles containing, incorporating, or including hazardous substances with explosive properties, are defined in Schedule 1 of the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. These criteria are described below.

If a substance meets any one of the following threshold criteria, it is considered explosive within the meaning of the HSNO Act.

UN Model Regulations – ‘Orange Book’ listing

Any substance or manufactured article listed in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations (United Nations, 1999b), as being class 1 (denoting it as an explosive substance).

Sensitiveness and explosiveness threshold

Sensitiveness measures the response of an explosive substance to some accidental stimuli. A substance is above the sensitiveness threshold if it gives a positive result to any of the three types of test in Test Series 2 of UN Manual of Tests and Criteria (pp 471–466).

- i. In a type 2(a) or UN gap test (section 12.4, UN Manual of Tests and Criteria), when confined in the prescribed steel tube and subjected to detonative shock by initiating the prescribed booster charge, which is separated from the test substance by the prescribed spacer, the substance is able to propagate a detonation as shown by fragmenting the tube completely or punching a hole through the prescribed witness plate (section 12.4.1.4, UN Manual of Tests and Criteria).
 - ii. In a type 2(b) or Koenen test (section 12.5, UN Manual of Tests and Criteria), when confined in the prescribed steel tube with a closing plate orifice of 2.0 mm or more and subjected to intense heat as prescribed, the substance is able to propagate a detonation as shown by the tube being: fragmented into three or more large pieces (which can still be connected by a narrow strip); or fragmented into many mainly small pieces; or fragmented into many very small pieces and the closing device bulged out or fragmented (section 12.5.1.4, UN Manual of Tests and Criteria).
 - iii. In a type 2(c) time/pressure test of the effect of ignition (section 12.6, UN Manual of Tests and Criteria), when confined in the prescribed steel pressure vessel and ignited by the prescribed electric fusehead, the substance is able to produce a pressure increase from 690–2,070 kPa absolute pressure or more, within 30 ms or less (section 12.6.1.4, UN Manual of Tests and Criteria).
- a. A substance designed to detonate, deflagrate, or produce a pyrotechnic effect
- Any substance expressly designed to detonate, deflagrate, or produce a pyrotechnic effect is above the HSNO Act threshold for the explosive property.
- A substance designed to detonate will, when initiated, produce a violent chemical reaction that proceeds through the reacted material at supersonic velocity producing heat and high pressure. The result of the reaction is the exertion of extremely high pressures on the surrounding medium, forming a propagating shock wave of supersonic velocity; that is, the substance explodes with a sudden loud noise.
- A substance designed to deflagrate will, when initiated or ignited, produce a chemical reaction that proceeds at subsonic velocity along the surface of and/or through the reacted material, producing hot gases at high pressures; that is, the substance bursts into flames and burns away rapidly. A

deflagration under confinement results in an increase in pressure, rate of reaction, and temperature, which may cause detonation.

A substance designed to produce a pyrotechnic effect will, when initiated, produce a non-detonative, self-sustaining, exothermic chemical reaction, producing an effect of heat, light, sound, smoke, gas, or motion, or a combination of these. Pyrotechnic effect refers to a display of fireworks or to the ignition of a substance for technical or military purposes.

b. External bonfire test for manufactured articles

This is a test performed on explosive articles or packages of explosive articles to determine whether there is a mass explosion or a hazard from dangerous projections, radiant heat, and/or violent burning, or any other dangerous effect when the articles are involved in a fire. An article is above this threshold if it produces some effect of projection of fragments, fire, smoke, heat, or loud noise external to the article when tested as a stack of articles in accordance with test type 6(c) in section 16.6 of the UN Manual of Tests and Criteria (test criteria in para 16.6.1.4.7, pp 155–156).

2.4. Classification criteria for explosive substances and articles

The explosive property classification scheme groups explosive substances in terms of three effects. These effects are the:

- degree of sensitiveness to stimuli;
- type of explosive effect; and
- different levels at which those explosive effects might be displayed.

The HSNO Act classification scheme uses the system in the UN Model Regulations. Thus, the classification for substances with an explosive property is based on:

- subclasses (UN divisions) for types and levels of explosiveness and for the sensitiveness of the substance to stimuli; and
- categories (UN compatibility groupings) for explosive type.

Classification requires allocation to both a subclass and a category. A substance or article is classified as being in a particular subclass or category if it meets the criteria set out in Schedule 1 of the classification regulations for that subclass or category. These criteria are taken from the UN Model Regulations and UN Manual of Tests and Criteria, and are described in the sections below. Substances and articles may be classified only into the combinations of subclasses and categories (divisions and groupings) permitted by the UN Model Regulations, as shown in Table 2.1. The assignment of substances to cells where there is no entry is prohibited.

Table 2.1: Scheme of classification of explosive substances and articles

| Category for explosive type and properties | Subclass for type and level of explosive hazard | | | | | |
|--|---|----------------|--|------------------------------|-------------------------------------|---------------------------|
| | Mass explosion 1.1 | Projection 1.2 | Fire and minor blast or projection 1.3 | Minor fire or projection 1.4 | Very insensitive mass explosion 1.5 | Extremely insensitive 1.6 |
| A | 1.1A | | | | | |
| B | 1.1B | 1.2B | | 1.4B | | |
| C | 1.1C | 1.2C | 1.3C | 1.4C | | |
| D | 1.1D | 1.2D | | 1.4D | 1.5D | |
| E | 1.1E | 1.2E | | 1.4E | | |
| F | 1.1F | 1.2F | 1.3F | 1.4F | | |
| G | 1.1G | 1.2G | 1.3G | 1.4G | | |
| H | | 1.2H | 1.3H | | | |
| J | 1.1J | 1.2J | 1.3J | | | |
| K | | 1.2K | 1.3K | | | |
| L | 1.1L | 1.2L | 1.3L | | | |
| N | | | | | | 1.6N |
| S | | | | 1.4S | | |

Note: Categories for explosive substances do not indicate the degree of hazard.

2.4.1. Subclasses for explosive substances and articles

Explosive substances are divided into the subclasses (UN divisions) 1.1–1.6 described in (a)–(f) below. A substance or article is classified as being in a particular subclass if it meets the criteria for that subclass. The criteria for the subclasses for the nature and level of explosive effect, and for the sensitiveness of the substance to stimuli are given in the table in Part 1 of Schedule 1 of the classification regulations, as follows.

a. Subclass 1.1 – substances and articles that have a mass explosion hazard

Substances and articles that have a ‘mass explosion hazard’, as evidenced by:

- i. a crater at the test site, or damage to the witness plate, or producing a measurable blast, or disrupting and scattering the confining material, when an individual article or package is tested as prescribed in test type (a) of Test Series 6, section 16.4, pp 149–150, of the UN Manual of Tests and Criteria (test criteria are in para 16.4.1.4); or
- ii. more than one package or article exploding practically instantaneously; as shown by: a crater at the test site appreciably larger than that given by a single package or article; or damage to the witness plate appreciably bigger than that from a single package or article; or the measured blast

significantly exceeds that from a single package or article; or violent disruption and the scattering of the confining material, when a stack of explosive articles or packages of an explosive substance is tested as prescribed in test type (b) of Test Series 6, section 16.5, pp 151–152, of the UN Manual of Tests and Criteria (test criteria are in para 16.5.1.8); or

- iii. a substantial proportion of the articles or packages explode when a stack of explosive articles or packages are subjected to fire as prescribed in test type (c) of Test Series 6, section 16.6, pp 153–156, of the UN Manual of Tests and Criteria (test criteria are in para 16.6.1.4.2).

b. Subclass 1.2 – substances and articles that have a projection hazard

Substances and articles that have a ‘projection hazard’ but not a mass explosion hazard, as evidenced by the failure to show the criteria for mass explosion (para (a) above), but when a stack of explosives articles or packages is subjected to fire as prescribed in test type (c) of Test Series 6, section 16.6, pp 153–156, of the UN Manual of Tests and Criteria, demonstrate either of:

- i. perforation of any of the three witness screens; or
- ii. metallic projection with a kinetic energy exceeding 20 J, as assessed by the distance–mass relation given in Figure 16.6.1.1 in the UN Manual of Tests and Criteria.

(These criteria are in section 16.6.1.4.3 of the UN Manual of Tests and Criteria.)

c. Subclass 1.3 – substances and articles that have a fire hazard and either a minor blast hazard or a minor projection hazard or both, but not a mass explosion hazard

Substances and articles which have a ‘fire hazard’ and either a minor blast hazard or a minor projection hazard or both, but not a mass explosion hazard, as evidenced by a failure to show the criteria for mass explosion or projection hazards (paras (a) and (b) above), but, when a stack of explosive articles or packages is subjected to fire as prescribed in test type (c) of Test Series 6, section 16.6, pp 153–156, of the UN Manual of Tests and Criteria, demonstrate any one of the following criteria.

- i. A fireball or jet of flame extending beyond any of the three witness screens.
- ii. A fiery projection emanating from the product is thrown more than 15 m from the edges of the packages or unpackaged articles.
- iii. A burning time of the product measured to be less than 35 seconds for 100 kg of net explosive mass. (Alternatively, in the case of articles and low energy substances, the irradiance of the burning product exceeds that of the test fire by more than 4 kW/m² at a distance of 15 m from the edge of the packages or unpackaged articles. The irradiance is measured over 5 seconds, during the period of maximum output.)

(These criteria are in section 16.6.1.4.4 of the UN Manual of Tests and Criteria.)

d. Subclass 1.4 – substances and articles that present no significant explosive hazard

Substances and articles that present ‘no significant hazard’, as evidenced by a failure to show the criteria for mass explosion, projection hazard, or fire hazard (paras (a)–(c) above), but, when a stack of explosive articles or packages are subjected to fire as prescribed in test type (c) of Test Series 6,

section 16.6, pp 153–156, of the UN Manual of Tests and Criteria, demonstrate any of the following criteria are assigned to subclass 1.4 and to a category other than category S.

- i. A fireball or jet of flame that extends more than 1 m from the flames of the test fire.
- ii. A fiery projection emanating from the product is thrown more than 5 m from the edges of the packages or unpackaged articles.
- iii. An indentation of any of the three witness screens of more than 4 mm.
- iv. A metallic projection with a kinetic energy exceeding 8 J, as assessed by the distance–mass relation given in Figure 16.6.1.1 in the UN Manual of Tests and Criteria.
- v. A burning time of the product measured to be less than 330 seconds for 100 kg of net explosive mass.

(These criteria are in section 16.6.1.4.5 of the UN Manual of Tests and Criteria.)

However, if the substance produces a thermal, blast, or projection effect that is less than any of the criteria above (that is, it is not sufficient to hinder fire-fighting or other emergency response efforts in the immediate vicinity, and would not be capable of causing bodily harm within 5 m of the articles), the substance (or article) is allocated to subclass 1.4 and category S.

(These criteria are in section 16.6.1.4.6 of the UN Manual of Tests and Criteria.)

If the substance produces no hazardous effects, as described above, but there is some effect (that is, projection, fire, smoke, heat, or a loud noise) external to the device itself, and the product is an article manufactured specifically to produce a practical explosive or pyrotechnic effect, then the product is assigned to subclass 1.4 and category S.

(These criteria are in section 16.6.1.4.7(a)(i) of the UN Manual of Tests and Criteria. Note that it is usually necessary to make this assessment on the basis of a test involving the functioning of the article without packaging or confinement.)

e. Subclass 1.5 – very insensitive substances that have a mass explosion hazard

Substances that have a mass explosion hazard but are so insensitive there is very little probability of initiation or transition from burning to detonation under normal conditions of transport. This is evidenced by the following.

- i. A lack of a positive response to shock from intense mechanical stimulus in the prescribed cap sensitivity test type (a), Test Series 5, section 15.4, p 132, of the UN Manual of Tests and Criteria.

(The criteria for a positive response are described in para 15.4.1.4 of the UN Manual of Tests and Criteria as:

- the witness plate is torn or otherwise penetrated – bulges, cracks, or folds in the witness plate do not indicate cap sensitivity; or
- the centre of the lead cylinder is compressed from its initial length by an amount of 3.2 mm or greater.)

- ii. (A failure to undergo a transition from deflagration to detonation when tested in any one of the three type 5(b) tests, prescribed in section 15.5, pp 136–144, of the UN Manual of Tests and Criteria.
 - iii. A transition from deflagration to detonation is considered to have occurred in a type 5(b)(i) (French DDT test) if the criteria in para 15.5.1.4 of the UN Manual of Tests and Criteria are met. These criteria are:
 - the lead witness plate is compressed in a manner characteristic of detonation; and
 - the measured propagation velocity is greater than the speed of sound in the substance and constant in the part of the test tube furthest from the initiator.
 - iv. A transition from deflagration to detonation is considered to have occurred in a type 5(b)(ii) (USA DDT test) if the criteria in para 15.5.2.4 of the UN Manual of Tests and Criteria are met. In this case, the test result is considered positive if a hole is punched through the witness plate.
 - v. A transition from deflagration to detonation is considered to have occurred in a type 5(b)(iii) (deflagration to detonation transition test) if the criteria in para 15.5.3.4 of the UN Manual of Tests and Criteria are met. In this case, test results are assessed by the tube rupture character or explosion of the detonating cord. The result is considered positive if the tube fragments.)
 - (vi) A lack of an intense crack or projection of fragments from the fire area when a pile of packages of the explosive substance is tested in the prescribed external fire test type 5(c), as prescribed in section 15.6, pp 145–146, UN Manual of Tests and Criteria. (The test criteria are in paras 15.6.1.3.5 and 15.6.1.4 of the UN Manual of Tests and Criteria.)

Note that if a substance gives a positive result in any of the Test Series 5 tests discussed above, then the substance should not be classified in subclass 1.5, but should be tested and classified according to Test Series 6.
- f. Subclass 1.6 – extremely insensitive articles that do not have a mass explosion hazard
- Articles that contain only extremely insensitive detonating substances and that demonstrate a negligible probability of accidental initiation or propagation. This is evidenced by the following.
- i. In the case of explosive substances, a failure to give a positive response to any of the first six tests of Test Series 7 (test types 7(a)–(f)) for sensitivity to shock, impact, external fire, and elevated temperature, as prescribed in section 17, pp 159–175, of the UN Manual of Tests and Criteria. (The test criteria are in paras 17.4.1.4, 17.5.1.4, 17.6.1.4, 17.6.2.4, 17.7.1.4, 17.7.2.4, 17.8.1.4, and 17.9.1.4 of the UN Manual of Tests and Criteria.)
 - ii. In the case of explosive articles containing extremely insensitive detonating substances, a failure to give a positive response to any of the last four tests of Test Series 7 (test types 7(g), 7(h), 7(j), and 7(k)) for sensitiveness to external fire, elevated temperature, impact, and detonation of an adjacent article, as prescribed in section 17, pp 176–179, of the UN Manual of Tests and Criteria. (The test criteria are in paras 17.10.1.4, 17.11.1.4, 17.12.1.4, and 17.13.1.4 respectively of the UN Manual of Tests and Criteria.)

Note that substances that are accepted for classification into subclasses 1.1–1.6 are those that pass the threshold tests as given in the technical specifications above for defining the threshold for substances with an explosive property, but not including substances that show insufficient thermal stability by giving a positive result in test type 3(c), Test Series 3, in section 13.6, pp 117–119, of the UN Manual of Tests and Criteria, and articles, packaged articles, or packaged substances that show insufficient thermal stability and/or impact resistance by giving a positive result in any of the tests described in Test Series 4, section 14, pp 123–130, of the UN Manual of Tests and Criteria (these are items considered too dangerous to transport).

2.4.2. Categories for explosive substances and articles

Explosive substances are also divided into the categories (UN compatibility groups) A–H, J, K, L, N, and S described below. A substance or an article is classified as being in a particular category if it meets the criteria for that category. The criteria for the categories for types of explosives and their properties are based on the premise that substances within groups are unlikely to result in unintended detonation or deflagration when in proximity to each other, and are given in the Table 1 in Part 2 of Schedule 1 of the classification regulations as follows.

Category A

Primary explosive substances that are very sensitive to heat, impact, or friction, or are able to transmit detonation or deflagration to secondary explosive substances close to them, as measured by the impact, friction, and small-scale burn tests in test types 3(a), (b), and (d), Test Series 3, section 13, pp 67–122, of the UN Manual of Tests and Criteria. (The test criteria for these test types are in paras 13.4.1.4, 13.4.2.4, 13.4.3.4, 13.4.4.4, 13.4.5.4, 13.4.6.4, 13.5.1.4, 13.5.2.4, 13.5.3.4, and 13.7.1.3 of the UN Manual of Tests and Criteria.)

Category B

Articles containing a primary explosive substance but not containing two or more effective protective features, or articles designed to be primers, detonators, or detonator assemblies for blasting.

Category C

Propellant explosive substances (deflagrating explosive used for propulsion) or other deflagrating explosive substances, and articles containing such explosive substances.

Category D

Secondary detonating explosive substances that are less sensitive than primary explosive substances and more sensitive than substances falling into category N, or black powder, or articles containing such secondary detonating explosive substances; in each case without means of initiation and without a propelling charge; or articles containing a primary explosive substance and two or more effective protective features.

Category E

Articles containing a secondary detonating explosive substance, without means of initiation, but with a propelling charge (other than one containing a flammable liquid or gel or hypergolic liquids that ignite spontaneously on contact with an oxidant).

Category F

Articles containing a secondary detonating explosive substance with its own means of initiation (being an article containing a primary explosive substance designed to initiate the secondary explosive substance), without a propelling charge or with a propelling charge other than one containing a flammable liquid or gel or hypergolic liquids.

Category G

Pyrotechnic substances, or articles containing a pyrotechnic substance, or articles containing both an explosive substance and an illuminating, incendiary, tear- or smoke-producing substance (other than a water-activated article or an article containing white phosphorus, phosphides, a pyrophoric substance, a flammable liquid or gel, or hypergolic liquids).

Category H

Articles containing both an explosive substance and white phosphorus (for smoke generation but represents a fire hazard from spontaneous ignition on contact with air).

Category J

Articles containing both an explosive substance and a flammable liquid or gel.

Category K

Articles containing both an explosive substance and an acutely toxic substance with a HSNO Act hazard classification of 6.1A, 6.1B, or 6.1C (oral LD50 of less than 300 mg/kg bodyweight).

Category L

A mixture or an article that contains both an explosive substance and a substance that spontaneously combusts, detonates, or deflagrates when exposed to air, water, oxidising substances, or flammable substances, or generates a substance that spontaneously combusts, detonates, or deflagrates when exposed to air or water. These substances can present special risks and need isolation of each type within category L.

Category N

Articles containing only extremely insensitive detonating substances, where 'extremely insensitive' is as defined in the criteria for subclass 1.6.

Category S

Substances or articles so packed that any hazardous effects arising from their accidental functioning are confined within the package, unless the package has been degraded by fire, in which case any blast or projection effects are so limited they would not be capable of causing bodily harm within 5 m of the articles. This category also includes articles that produce only non-hazardous effects of projection, fire, smoke, heat, or loud noise, if these effects are external to the article. These criteria are in paras 16.6.1.4.6 and 16.6.1.4.7(a)(i) of the UN Manual of Tests and Criteria and relate to test type 6(c) of Test Series 6, para 16.6, of the UN Manual of Tests and Criteria.

2.5. Notes on explosive thresholds and classifications

2.5.1. Acceptable test results

Apart from the criterion of being designed to detonate, deflagrate, or produce a pyrotechnic effect, the HSNO Act explosive threshold specifies the UN tests as the measures for the threshold of explosiveness. No other tests appear to be in common international use. Accordingly, the first element of the threshold requires test results from the test methods as set out in the UN Manual of Tests and Criteria. As these methods require relatively sophisticated testing facilities, it is expected that overseas test data will be the basis for assessing applications.

Similarly, the classification regulations specify the UN tests as the measures for the classification of explosiveness. As explosive substances and articles are required to be classified and identified in accordance with the UN Model Regulations for the purposes of transport, they will be essentially classified for the purposes of the HSNO Act (in relation to explosiveness) at the time they arrive in the country or following manufacture, as the two classification systems are identical.

2.5.2. Screening procedures for substances that may have explosive properties

Screening procedures, involving theoretical appraisal and/or small-scale tests, can be used to identify the hazard potential of new substances that are suspected of having explosive properties without the need for the larger scale tests mentioned above. If the screening procedures indicate that there is a hazard, then the full explosive classification procedure should be applied. The screening procedures should not be used for substances expressly manufactured with the intention of producing a practical explosive or pyrotechnic effect. Similarly, when the substance is a mixture containing any known explosives then the full explosive classification procedure should be applied.

Explosive properties are associated with the presence of certain chemical groups in a molecule that can react to produce very rapid increases in temperature or pressure. A substance is unlikely to have explosive properties in one of the following cases.

- No chemical groups are typically associated with explosive properties present in the molecule. Examples of such groups are:
 - C-C unsaturation such as acetylenes, acetylides, and 1,2-dienes;

- C-metal such as Grignard reagents or organo-lithium compounds;
 - N-metal;
 - Contiguous nitrogen atoms such as azides, aliphatic azo compounds, diazonium salts, hydrazines, and sulphonylhydrazides;
 - Contiguous oxygen atoms such as peroxides and ozonides;
 - N-O such as hydroxylamines, nitrates, nitro compounds, and nitroso compounds;
 - N-oxides and 1,2-oxazoles;
 - N-halogen such as chloramines and fluoroamines; and
 - O-halogen such as chlorates, perchlorates, and iodosyl compounds.
- The substance contains chemical groups associated with explosive properties that include oxygen but the calculated oxygen balance is less than -200, where the oxygen balance is calculated for the chemical reaction:

$$C_xH_yO_z + [x + (y/4) - (z/2)]. O_2 \leftrightarrow x. CO_2 + (y/2). H_2O$$
 using the formula:

$$\text{oxygen balance} = -1,600[2x + (y/2) - z]/\text{molecular weight}$$
 - The organic substance or a homogeneous mixture of organic substances contains chemical groups associated with explosive properties but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500°C.
 - For mixtures of inorganic oxidising substances (subclass 5.1.1) with organic materials, the concentration of the inorganic oxidising substance is less than 15%, by mass, of the mixture, if the oxidising substance is classified as 5.1.1A or 5.1.1B (UN 5.1, Packing Group I or Packing Group II); or is less than 30%, by mass, of the mixture, if the oxidising substance is classified as 5.1.1C (UN 5.1, Packing Group III).

2.5.3. Mixture rule for explosive substances

In general, no mixture rules apply to explosive hazards. The direct testing of mixtures for explosive hazards is usually required since the hazards of a mixture are not always reliably predicted from component data.

2.5.4. Criterion of being designed to detonate, deflagrate, or produce a pyrotechnic effect

The criterion of being designed to detonate, deflagrate, or produce a pyrotechnic effect carries over the current scope of the Explosives Act 1957. For example, the criterion covers airbag igniters and model rockets, which are designed to deflagrate, and caps (amorces), which are designed to produce sound by a pyrotechnic effect.

The classification of fireworks, signal flares, and model rockets under the previous explosives legislation was covered by the Schedule to the Explosives Act 1957, the explosives regulations, and the Explosives Authorisation Order. The Schedule to the Act described the three divisions within class 7 Fireworks, with the use of a quantity (40 g), of firework composition, to differentiate fireworks available to the public and those available only to permit holders. Signal flares and model rockets were also limited in their availability to certain persons by the explosives regulations.

The restrictions explosive regulations placed on fireworks that could be approved for sale to the public included:

- the composition was not to include chlorate mixed with sulphur, phosphorus, or any sulphide (unless it was an amorce);
- the composition was not to include poisonous substances;
- the construction was not to allow any firework composition to escape;
- a firework must not contain its own means of ignition (unless it is an amorce, throw-down, snap, or bon-bon cracker);
- rockets were not to be projected erratically or unpredictably;
- rockets were not to have a sharp, pointed, rigid cone;
- a firework was not to be shaped as a hand-held firework (port fire or squib) if on ignition it commenced with a discharge of fire and concluded with an explosion that burst the case;
- a firework was not to discharge hot or burning material onto the ground; and
- the size and construction of cannons, bangers, and bungers were limited.

The Explosives (Fireworks) Order 1990 removed cannons, bangers, and bungers or fireworks whose principal effect was percussive. The Explosives (Skyrockets Restriction) Amendment Act 1994 removed rockets, tourbillions, and fireworks whose principal effect was vertical or horizontal flight.

The provisions above are carried over as specific regulations under section 140(1)(R) of the HSNO Act in the Hazardous Substances (Fireworks) Regulations 2001.

2.5.5. British Home Office classification

While New Zealand has previously used the British Home Office classification together with the UN classification system, it is considered that, increasingly, data on explosive substances would relate to UN tests and criteria for classification, and that using the British Home Office system with the UN classification system would create confusion. Accordingly, the HSNO classification system for explosives uses only the system described in the UN Model Regulations.

References

United Nations 1994. *European Agreement Concerning the International Carriage of Dangerous Goods by Road* United Nations, Geneva.

United Nations 1999a. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria* 3rd revised edition. United Nations, United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 1999b. *Recommendations on the Transport of Dangerous Goods Model Regulations* 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more

recent versions of this document, for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

3. Introduction to Substances with Flammable Properties – Classes 2–4

3.1. Introduction

Under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification system for flammability, there are separate thresholds and classifications for substances in gas, liquid, and solid forms, with solid substances being further subdivided into different types of flammable property.

The nine subclasses to the classification system for flammable substances, with corresponding threshold levels, are:

- a. ignitibility for flammable gases (see chapter 4 [below](#));
- b. flammable components for flammable aerosols (see chapter 4 [below](#));
- c. ignitibility for flammable liquids (see chapter 5 [below](#));
- d. liquid desensitised explosives, and (see in chapter 5 [below](#));
- e. flammable solids (see chapter 6 [below](#)), which are divided into:
 - i. flammable solids (readily combustible solids and solids which may cause fire through friction [below](#));
 - ii. self-reactive substances (see [below](#));
 - iii. desensitised explosives (see [below](#));
 - iv. substances liable to spontaneous combustion and pyrophoric and self-heating substances (see [below](#)); and
 - v. substances that in contact with water emit flammable gases (see [below](#)).

The criteria and test procedures to classify substances with flammable properties are closely aligned with the United Nations Recommendations on the Transport of Dangerous Goods Model Regulations (United Nations, 1999b) (UN Model Regulations) and its companion volume Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria (United Nations, 1999a) (UN Manual of Tests and Criteria). These versions of the documents are referred to in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and Hazardous Substances (Classification) Regulations 2001. (Equivalent material can be found in more recent versions of these documents.)

The HSNO Act, however, covers all aspects of the lifecycle of substances at which the substances present a hazard (for example, manufacture, storage, transport, use, and disposal); whereas the UN Model Regulations are generally concerned with only the transport sector. Accordingly, the HSNO Act classification systems depart from the UN Model Regulations to enable the control of hazards associated with elements of the lifecycle other than transport.

The HSNO Act thresholds also broadly correspond with those previously used under the Dangerous Goods Act 1974, although they have been amended to align with the criteria agreed in the international harmonisation process. For example, the HSNO Act includes some substances not already subject to the

Dangerous Goods Act (for example, flammable liquids with flashpoints between 61°C and 93°C that are not fuel oils such as some high flashpoint solvents and cutback bitumen). On the other hand, some substances subject to the Dangerous Goods Act are not captured by the HSNO Act threshold for flammability (for example, fuel oils with a flashpoint higher than 93°C). The HSNO Act definition of a flammable gas reflects the definitions in the Globally Harmonised System for Classification and Labelling of Chemicals (United Nations, 2007) and is wider than the previous Dangerous Goods Act definition.

3.2. Definitions

The following terms include those used in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and the Hazardous Substances (Classification) Regulations 2001 with respect to flammable substances. They are particularly relevant to chapters 3–6.

| Term | Definition |
|----------------------------|---|
| ASTM | When followed by letters and numbers, means the document identified by those letters and numbers that is published by the American Society for Testing and Materials. |
| closed cup flashpoint test | An internationally recognised test method in which a set volume of a liquid is heated in a closed vessel of prescribed dimensions, and to which an ignition source is periodically introduced, until a temperature is reached at which the vapour above the liquid ignites. This temperature is known as the flashpoint of the liquid. Several standard methods can be used for this test (see section 5.1.3 in chapter 5). |
| Data | Includes values that are directly measured, calculated, or estimated for any of the measures given. |
| desensitising agent | A substance or material that, when mixed with a class 1, class 4.1.2, or class 5.2 substance produces a mixture that has reduced hazardous properties (in terms of those classifications) compared with the original class 1, class 4.1.2, or class 5.2 substance. |
| flammability, flammable | The ability of a substance to be ignited and to support combustion in air at 20°C and 101.3 kPa absolute pressure. |
| flammable ingredient | Any substance that meets one or more of the threshold criteria for a 'flammable gas', 'flammable liquid', 'flammable solid', or any combination thereof. |
| flammable range | The range between two ratios of flammable gas or vapour to air, the lower of which contains too much air and the upper of which contains too little air, to be able to support combustion. It includes a minimal range effectively equivalent to a single value. |
| flammable vapour | The gaseous form of a normally liquid or solid substance that is flammable. |
| flashpoint | The lowest temperature at which a flammable liquid gives off sufficient vapour to form a flammable mixture with air that ignites momentarily, when tested in any closed cup flashpoint test. |
| g | gram(s) |

| Term | Definition |
|--|--|
| gas | A substance that: <ol style="list-style-type: none"> is completely gaseous at 20°C and at 101.3 kPa absolute pressure; or has a vapour pressure of more than 300 kPa absolute pressure at 50°C. |
| ignitable | Able to be set on fire. |
| initial boiling point (IBP) | The temperature at which a flammable substance begins to boil at a pressure of 101.3 kPa absolute. |
| ISO | International Organization for Standardization |
| ISO 10156:1996 | ISO standard <i>Gases and Gas Mixtures: Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets</i> (ISO, 1996). |
| kg | kilogram(s) |
| kPa | kilopascal(s) |
| kV | kilovolt(s) |
| L | litre(s) |
| liquid | A substance that is: <ol style="list-style-type: none"> a substance with a melting point of less than or equal to 20°C at 101.3 kPa absolute pressure; or a viscous substance, without a defined melting point, if: <ol style="list-style-type: none"> more than the quantity of the substance specified in ASTM D4359-90 (ASTM, 2006), collects on a watch glass when tested in the manner specified in that test; or a penetrometer penetrates into the substance the distance defined in the test for determining fluidity prescribed in Appendix A.3 of the <i>European Agreement Concerning the International Carriage of Dangerous Goods by Road</i> (United Nations, 1994), when the method specified in that test is followed |
| m | metre(s) |
| mL | millilitre(s) |
| mm | millimetre(s) |
| SADT | See self-accelerating decomposition temperature (SADT). |
| self-accelerating decomposition temperature (SADT) | The lowest temperature at which self-accelerating decomposition of the substance occurs in the packaging in which it is tested as prescribed in Test Series H in section 28 of the UN Manual of Tests and Criteria. |
| solid | A substance that is neither a liquid nor a gas. |
| Test Series | When followed by a letter or number, means one or more tests as prescribed in the UN Manual of Tests and Criteria. |
| UN Manual of Tests and Criteria | Third revised edition of the <i>Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria</i> (United Nations, 1999a). |

| Term | Definition |
|----------------------|---|
| UN Model Regulations | Eleventh revised edition of the <i>Recommendations on the Transport of Dangerous Goods Model Regulations</i> (United Nations, 1999b). |
| UN PG I, II, and III | United Nations Packing Groups I, II, and III. Following the UN Model Regulations, they relate to packaging with a high level of ability to contain the contents through to packaging with a lesser ability to contain the contents. They are used to refer to some classification categories as having a high degree of hazard through to a lesser degree of hazard respectively. |

3.3. Threshold tests for substances with flammable properties

Tests for flammability include finding the temperature at which a substance ignites, and testing the rate at which a substance burns. For example, the threshold test for flammable liquids is the determination of its flashpoint.

The approach taken to defining tests and criteria for thresholds for flammable substances is as follows.

- a. In some cases, because of the sensitivity and degree of hazard of the substance and for the sake of consistency in results, the criteria depend on precise testing procedures being followed. In these cases, the Hazardous Substances (Classification) Regulations 2001 specify the specific testing procedures in one of two ways:
 - i. A narrative description of the test method (this allows equivalent tests to be performed); or
 - ii. The reference to a particular test is specified in the regulations, in which case only that test will be accepted.
- b. When the criteria have a well-defined and universally understood meaning (for example, the closed cup flashpoint test), the regulations are limited to specifying the criteria, enabling any appropriate test to be used. This approach also permits calculation or estimation methods to be considered (for example, for mixtures).

This document gives some guidance about the test protocols or methods recognised as acceptable tests for the specified threshold criteria. In general, the test protocols or methods that are acceptable are specified in the:

- UN Model Regulations (United Nations, 1999b (11th revised edition)); or
- UN Manual of Tests and Criteria (United Nations, 1999a (3rd revised edition)).

3.4. Technical description of the elements of the flammable property thresholds and classification system

If a substance meets any one of the threshold criteria described in the following sections, it is considered a flammable substance within the meaning of the HSNO Act. These criteria are contained in Schedule 2 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.

A flammable substance is classified as having a particular hazard classification if it meets the criteria set out in the table in Schedule 2 to the Hazardous Substances (Classification) Regulations 2001 for that hazard classification.

The classification schemes for the various subclasses of flammable substances are summarised in Table 3.1.

Note that in the case of subclass 4.1.2 (self-reactive flammable solids), if a substance does not meet the criteria for a 4.1.2A, 4.1.2B, or 4.1.2C hazard classification, then a 4.1.2D classification applies, unless sufficient data are provided that show that the substance meets the criteria for hazard classification 4.1.2E, 4.1.2F, or 4.1.2G. With respect to the criteria in the Hazardous Substances (Classification) Regulations 2001 for subclass 4.1.2, Test Series A–G refer to the tests for self-reactive substances and organic peroxides in sections 21–27, respectively, of the UN Manual of Tests and Criteria.

Table 3.1: Flammable property classification

| Category of hazard | Subclass of flammable hazard | | | | | | | | |
|--------------------|--|---|---|---------------------------------------|-------------------------------------|--|------------------------------------|---|---------------------------------------|
| | Gases* 2.1.1 | Aerosols 2.1.2 | Liquids 3.1 | Liquid desensitised explosives 3.2 | Flammable solids 4.1.1 | Self-reactive flammable solids† 4.1.2 | Desensitised explosives‡ 4.1.3 | Spontaneously combustible substances§ 4.2 | Substances dangerous when wet# 4.3 |
| A | Ignitable at ≤ 13% volume in air or have a flammable range with air of ≥12%, regardless of LFL | Pressurised mixture containing a gas, compressed, liquefied, or dissolved under pressure; comprising ≥ 5%, by mass, flammable ingredients; under a pressure > 100 kPa | 3.1A Flashpoint (closed cup) < 23°C and initial boiling point ≤ 35°C (equivalent to UN PG I) | 3.2A (equivalent to UN PG I) | 4.1.1A (equivalent to UN PG II) | 4.1.2A | 4.1.3A (equivalent to UN PG I) | 4.2A Pyrophoric substances (equivalent to UN PG I) | 4.3A (equivalent to UN PG I) |
| B | Have a flammable range in mixture in air, other than those in category A | | 3.1B Flashpoint (closed cup) < 23°C but initial boiling point > 35°C (equivalent to UN PG II) | 3.2B (equivalent to UN PG II) | 4.1.1B (equivalent to UN PG III) | 4.1.2B | 4.1.3B (equivalent to UN PG II) | 4.2B Self-heating substances (equivalent to UN PG II) | 4.3B (equivalent to UN PG II) |

| User Guide for Thresholds and Classifications | | | | | | | | | |
|---|--|--|--|-----------------------------------|--|--------|-------------------------------------|--|-----------------------------------|
| C | | | 3.1C Flashpoint (closed cup) $\geq 23^{\circ}\text{C}$, but $\leq 60^{\circ}\text{C}$ (equivalent to UN PG III) | 3.2C (equivalent to UN PG III) | | 4.1.2C | 4.1.3C (equivalent to UN PG III) | 4.2C Self-heating substances (equivalent to UN PG III) | 4.3C (equivalent to UN PG III) |
| D | | | 3.1D Flashpoint (closed cup) $> 60^{\circ}\text{C}$ but $\leq 93^{\circ}\text{C}$ | | | 4.1.2D | | | |
| E | | | | | | 4.1.2E | | | |
| F | | | | | | 4.1.2F | | | |
| G | | | | | | 4.1.2G | | | |

Notes:

LFL lower flammable limit

UN PG United Nations Packing Group.

* Gases or gas mixtures at 20°C and at a standard pressure of 101.3 kPa.

§ This classification is equivalent to UN Division 4.2, UN Model Regulations.

† This classification is equivalent to UN Division 4.1(b), UN Model Regulations.

This classification is equivalent to UN Division 4.3, UN Model Regulations.

‡ This classification is equivalent to UN Division 4.1(c), with the classification criteria as per the UN Model Regulations.

References

ASTM 2006. *Test Method for Determining Whether a Material is a Liquid or a Solid*, ASTM D4359-90. American Society for Testing and Materials, Philadelphia, PA.

ISO 1996. *Gases and Gas Mixtures: Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets*, ISO 10156:1996. International Organization for Standardization, Geneva.

United Nations 1994. *European Agreement Concerning the International Carriage of Dangerous Goods by Road* United Nations, Geneva.

United Nations 1999a. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria*, 3rd revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 1999b. *Recommendations on the Transport of Dangerous Goods Model Regulations*, 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 2007. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

4. Flammable Gases and Aerosols – Subclasses 2.1.1 and 2.1.2

4.1. Flammable gases – subclass 2.1.1

Subclass 2.1.1 is equivalent to division 2.1 of the United Nations Model Regulations (United Nations, 1999).

Key terms are defined in section 3.2 in chapter 3.

4.1.1. Threshold criteria for flammable gases

A flammable gas is any gas or gas mixture that is sufficiently flammable that it can be ignited when mixed with air in a proportion within a flammable range for that substance at 20°C and at a pressure of 101.3 kPa absolute pressure.

4.1.2. Classification of flammable gases

There are two categories for flammable gases that exceed the defined threshold.

- Category A (high hazard) – classification 2.1.1A

Any gas or gas mixture that at 20°C at a pressure of 101.3 kPa absolute:

- a. is ignitable when in a mixture of 13% or less by volume with air; or
- b. has a flammable range with air of at least 12%, regardless of the lower flammability limit.

(Flammability should be determined by tests or by calculation in accordance with methods adopted in section 5 of ISO 10156: 1996 (ISO, 1996).

- Category B (medium hazard) – classification 2.1.1B

Any gas or gas mixture, other than those of high hazard (classification 2.1.1A), that at 20°C and a pressure of 101.3 kPa absolute is sufficiently flammable to be capable of ignition when mixed with air in a proportion within any flammable range.

Examples of flammable gases

Liquefied petroleum gas has, at 20°C and a standard pressure of 101.3 kPa, a lower flammable level in air of 2% and an upper flammable level in air of 9%. Therefore, it is a class 2.1.1A flammable gas according to the criteria above.

Ammonia has a lower flammable level in air of 16% and an upper flammable level in air of 25%. Therefore, it is a class 2.1.1B flammable gas according to the criteria above.

See also the examples in Table 4.1.

Table 4.1: Examples of gases that would meet the criteria for the Hazardous Substances and New Organisms Act 1996 flammable classification categories

| Classification category | Description | Example gases |
|---|--|---|
| Category A (high hazard): criterion (a) | Gases that are ignitable when in a mixture of 13% or less by volume with air | Butane, ethane, methane, propane, carbon monoxide, ethylene, hydrogen sulphide, formaldehyde, |

| | | |
|---|--|-------------------------|
| | | hydrogen, acetylene |
| Category A (high hazard): criterion (b) | Gases that have a flammable range with air of at least 12% regardless of the lower flammable limit | Chlorotrifluoroethylene |
| Category B (medium hazard) | Gases or gas mixtures, other than those of high hazard, that at 20°C and a pressure of 101.3 kPa absolute have a flammable range in mixture in air | Methyl bromide, ammonia |

4.1.3. Discussion

Classification criteria

Flammable gas classification 2.1.1A is consistent with division 2.1 of the UN Model Regulations.

Flammable gas classification 2.1.1B originates from a December 1996 recommendation of the United Nations Committee of Experts on the Transport of Dangerous Goods subcommittee, advising the Inter-Organisation Programme on the Sound Management of Chemicals on flammability.

The criterion for category B extends the threshold of gases that are considered flammable gases to such substances as methyl bromide and ammonia. However, these substances are already subject to New Zealand controls under the Dangerous Goods Act 1974.

Gas mixtures

If a substance is a gas mixture made up of one or more chemical elements or compounds, and any one of those elements or compounds meets one or more of the classification criteria specified above for flammable gases, then the mixture may be assumed to have the same classification as its flammable components, unless it can be shown that the mixture has a different classification according to the above criteria.

The means of determining the flammability of gas mixtures (including vapours of substances from other subclasses), and therefore their classification, is by applying the tests or calculation in accordance with methods adopted by the International Organization for Standardization (see ISO 10156:1996 (ISO, 1996)). Where insufficient data are available to use these methods, then tests by a comparable method recognised by the EPA may be used.

Further details of test methods are given below.

Measuring the flammability of gases

The ISO 10156:1996 test involves introducing a known concentration of a gas and air mixture, in a reaction tube fitted with an ignition spark plug with 5 mm gap, connected to a 15 kV spark generator, and observing whether a spark results in a flame rising up the tube.

The procedure involves beginning with a low concentration of gas, and repeating the test several times, each time gradually increasing the concentration of gas until a spark results in a flame rising up the tube.

The calculation methods in the ISO standard appear to apply only to certain applications such as special gas mixtures produced to order (in small quantities).

Alternative test methods

For alternative test methods see:

- 'Limits of flammability of gases and vapours' (Coward and Jones, 1952);
- 'Flammability characteristics of combustible gases and vapours' (Zabetakis, 1965);
- Flammability of Mixed Gases (Burgess et al, 1982);
- Code of Practice for Selection, Installation and Maintenance of Electrical Apparatus for Use in Potentially Explosive Atmospheres (Other Than in Mining Applications or Explosives Processing and Manufacture), BS 5345 Part 1: 1989 (British Standards, 1989); and
- Determining Limits of Flammability of Chemicals at Elevated Temperature and Pressure, ASTM Standard E918-83: 1988 (ASTM, 1988).

Comparison with previous Dangerous Goods Act 1974 criteria

The Dangerous Goods Act 1974 (which was repealed by the Hazardous Substances and New Organisms Act 1996 (HSNO Act)) scheduled flammable gases into the four categories.

- Class 2(b): ethane, ethylene, hydrogen, methane, and any other flammable gas (other than that covered by the following bullets) included under any succeeding paragraph of this class).
- Class 2(c): acetylene, compressed or dissolved, and contained within a porous substance.
- Class 2(d): liquefied petroleum gas and any other liquefied flammable gas.
- Class 2(f): anhydrous ammonia.

The Dangerous Goods (Class 2 – Gases) Regulations 1980 classified gases in many cases by their intended use, for example, ammonia. In other cases, the regulations subdivided the gases into groups such as permanent flammable gases and liquefied flammable gases. By comparison, the HSNO Act has only two classes, which relate to the level of the flammable effect.

Relationship to the Gas Act 1992

The regulations made under the HSNO Act do not apply to any gas distribution and transmission system, which comes under the provisions of the Gas Act 1992. While substances such as natural gas are clearly flammable, the Gas Act generally provides up-to-date and effective control in these circumstances. Consequently, specific provisions are included in the Hazardous Substances (Classes 1 to 5 Controls)

Regulations 2001 to avoid any overlap with the Gas Act controls. This follows the previous practice as defined in the Dangerous Goods (Class 2 – Gases) Regulations 1980, which stated in regulation 4:

Nothing in these regulations shall apply to—

(a) coal gas or natural gas except when packed, stored, conveyed or handled in cylinders or transportable tanks.

4.2. Flammable aerosols – subclass 2.1.2

4.2.1. Threshold criteria for flammable aerosols

An aerosol is a flammable aerosol if it is a pressurised mixture containing a gas, compressed, liquefied, or dissolved under pressure, with or without a liquid, paste, or powder; comprising at least 45% by mass of flammable ingredients. The substance also must be packed under pressure in a way that is designed to be released as solid or liquid particles in suspension in a gas; or as a foam, paste, or powder; or in a liquid state; or in a gaseous state.

In this context, ‘flammable ingredient’ means any substance that meets the threshold for a ‘flammable gas’, a ‘flammable liquid’, a ‘flammable solid’, or any combination of these.

4.2.2. Classification of flammable aerosols

The one classification category for flammable aerosols (2.1.2A) has the same threshold criteria as above.

4.2.3. Discussion

The UN Model Regulations definition of ‘aerosols’ (chapter 3.3: notes 63 and 190) combines a description of the substance and a description of the package, as follows.

A flammable aerosol is a substance that includes more than 45% by mass or more than 250 g of flammable components, which are defined as ‘gases that are flammable in air at normal pressure, or are substances or preparations that are in liquid form that have a flashpoint $\leq 100^{\circ}\text{C}$ ’ (note 63).

Aerosols, meaning the aerosol dispensers, are any non-refillable receptacles made of metal, glass, or plastic that contain a gas compressed, liquefied, or dissolved under pressure, with or without a liquid, paste, or powder, and fitted with a release device allowing the contents to be ejected as solid or liquid particles in suspension in a gas, as a foam, paste, or powder, in a liquid state, or in a gaseous state (note 190).

Aerosols are classified under the UN Model Regulations as division 2.1 when the criteria of note 63 are met.

The definitions of aerosol in European Commission Directive 75/324/EEC (EC, 1975) and the International Civil Aviation Organization’s Technical Instructions for Safe Transport of Dangerous Goods by Air (ICAO, 2006) are virtually identical to the definition in the UN Model Regulations. The EC directive sets a maximum capacity of metal aerosol dispensers of 1,000 ml. A recent amendment to the directive requires all aerosols with any flammable contents to be considered flammable unless tests indicate that they are not.

The approach used to specify the threshold was required because the regulation-making powers in the HSNO Act differentiate between the substance and the controls applied to it. When the above specification of aerosol is combined with the controls on flammable aerosols and the requirements for packages, the result is equivalent to the approach taken by the UN, European Community, and International Civil Aviation Organisation.

References

ASTM 1988. *Determining Limits of Flammability of Chemicals at Elevated Temperature and Pressure*, Standard E918-83: 1988. American Society for Testing and Materials, Philadelphia, PA.

British Standards 1989. *Code of Practice for Selection, Installation and Maintenance of Electrical Apparatus for Use in Potentially Explosive Atmospheres (Other than in Mining Applications or Explosives Processing and Manufacture)*, BS 5345 Part 1: 1989. British Standards, London.

Burgess, DS, Furno, AL, Kutch, JM, Mura, KE 1982. *Flammability of Mixed Gases*, report of investigation 8709. Bureau of Mines, United States Department of the Interior, Washington DC.

Coward, HF, Jones, GW 1952. *Limits of flammability of gases and vapours*. US Bureau of Mines Bulletin 503.

EC 1975. *Aerosols Dispensers Directive*, Directive 75/324/EEC. European Commission, Brussels.

ICAO 2006. *Technical Instructions for Safe Transport of Dangerous Goods by Air*, 2007–08 edition. International Civil Aviation Organization.

ISO 1996. *Gases and Gas Mixtures: Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets*, ISO 10156:1996. International Organization for Standardization, Geneva.

United Nations 1999. *Recommendations on the Transport of Dangerous Goods Model Regulations*, 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

Zabetakis, MG 1965. Flammability characteristics of combustible gases and vapours. *US Bureau of Mines Bulletin 627*.

5. Flammable Liquids and Liquid Desensitised Explosives – Subclasses 3.1 and 3.2

5.1. Flammable liquids – subclass 3.1

Subclass 3.1 is equivalent to class 3 of the UN Model Regulations (United Nations, 1999b).

Key terms are defined in section 3.2 in chapter 3.

5.1.1. Threshold criteria for flammable liquids

Any liquid that gives off a vapour that ignites at a temperature of less than or equal to 93°C in a closed cup flashpoint test is considered to be a flammable substance within the meaning of the Hazardous Substances and New Organisms Act 1996 (HSNO Act).

Examples

Xylene has a flashpoint of 28°C, so is a flammable liquid.

Ethylene glycol has a flashpoint of 111°C, so is not classified as a flammable liquid.

5.1.2. Classification of flammable liquids

The classification category for flammable liquids is determined in accordance with the following criteria.

- Category A (very high hazard) – classification 3.1A (equivalent to United Nations Packing Group (UN PG) I)
Any liquid that gives off a flammable vapour that ignites in a closed cup flashpoint test at a temperature less than 23°C, and has an initial boiling point (IBP) of less than or equal to 35°C.
- Category B (high hazard) – classification 3.1B (equivalent to UN PG II)
Any liquid that gives off a flammable vapour that ignites in a closed cup flashpoint test at a temperature less than 23°C, but has an initial boiling point (IBP) greater than 35°C.
- Category C (medium hazard) – classification 3.1C (equivalent to UN PG III)
Any liquid that gives off a flammable vapour that ignites in a closed cup flashpoint test at a temperature greater than or equal to 23°C, but less than or equal to 60°C.
- Category D (low hazard) – classification 3.1D
Any liquid that gives off a flammable vapour that ignites in a closed cup flashpoint test at a temperature greater than 60°C but less than or equal to 93°C.

5.1.3. Discussion

These classification categories are equivalent to the recommendations from the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG), noting that category D is as proposed by the UNCETDG subcommittee advising the Inter-Organisation Programme on the Sound Management of Chemicals (IOMC) on flammability.

Measuring the flammability of liquids

The classification criteria require the flashpoint to be determined using a closed cup method. There are a number of internationally recognised closed cup test methods, of which several are specified in section 2.3.3 of the UN Model Regulations.

These test methods are acceptable means of determining the classification criteria, because the regulations made under the HSNO Act do not specify a particular means. However, the EPA generally expects flashpoints to be determined by one of the following methods:

- Pensky Martens Closed Cup test method (ASTM D93, British Standard (BS) EN 22719, BS 2000 Part 404, IP 404, International Organization for Standardization (ISO) 2719, Australia Standard/New Zealand Standard (AS/NZS) 2106);
- Abel Closed Cup test method (BS 2000 Part 170, IP 170, AS/NZS 2106);
- Abel-Pensky test method (DIN 51755);
- Tag Closed Cup test method (ASTM D56); or
- Setaflash Closed Cup test method (ASTM D3278).

Closed cup flashpoints may be able to be estimated from open cup measurements. The UN Model Regulations (para 2.3.1.2) give the UN class 3 Packing Group III limit of 60.5°C, closed cup, as being equivalent to an open cup value of 65.6°C.

The difference between open cup and closed cup values for a substance increases as the flashpoint increases, due to the nature of the two test methods. Therefore, it can be assumed that open cup flashpoint values of greater than 103°C are correlated with closed cup values in excess of the threshold level of 93°C.

Flashpoint limit

The threshold criterion of a flashpoint $\leq 93^{\circ}\text{C}$ originates from an August 1996 proposal of the UNCETDG subcommittee advising the IOMC co-ordinating group on flammability to modify the UN Model Regulations criteria to make it applicable to other aspects of the lifecycle. This was subsequently adopted under the Globally Harmonized System for Classification and Labelling of Chemicals (United Nations, 2007)

Viscous substances

The UN Model Regulations exempt some specific types of viscous flammable substances from land transport controls, but the exemption does not apply to sea and air transport. (See section 2.3.2.5, UN Model Regulations.)

Accordingly, no exemptions are provided in the HSNO Act classification system by reason of viscosity, although, as with other hazards, a substance above the threshold is captured for assessment rather than automatically having controls imposed.

Screening procedures for mixtures that may be flammable liquids

Screening procedures, involving a theoretical appraisal, can be used to identify the hazard potential of mixtures that are suspected of having flammable properties instead of experimental determination.

A suitable method for calculating the flashpoint of mixtures containing both volatile, flammable components and non-volatile components (for example, polymers or additives) is that described by Gmehling and Rasmussen (1982). The basis of this approach is that the non-volatile components only slightly decrease the partial pressure of the solvents and thus, the flashpoint of the mixture can be calculated from the measured flashpoints of the flammable volatile components. The criteria used are as follows.

The flashpoint of mixtures need not be determined experimentally if the calculated flashpoint of the mixture is at least 5°C greater than the threshold value (93°C) and provided the:

- composition of the mixture is accurately known;
- flashpoint (closed cup) of each flammable component is known;
- activity coefficient is known for each component as present in the mixture including the temperature dependence; and
- liquid phase is homogeneous.

Correlation with Dangerous Goods Act 1974 classes

The HSNO Act categories 3A and 3B combined (corresponding to UN class 3 PGs I and II, respectively) match Dangerous Goods Act 1974 class 3(a).

The HSNO Act category 3C (UN class 3 PG III) equates to Dangerous Goods Act 1974 class 3(b).

The HSNO Act category 3D is a new category into which Dangerous Goods Act 1974 class 3(c), fuel oils, is largely included.

Example substances

Category A substances include:

- carbon disulphide (UN 1131);
- diethyl ether (UN 1155); and
- acetaldehyde (UN 1089).

Category B substances include:

- acetone (UN 1090);
- ethyl acetate (UN 1173);
- petrol (UN 1203); and
- ethanol (UN 1170).

Category C substances include:

- amyl acetate (UN 1104);
- ethylene glycol monoethyl ether (UN 1171); and
- kerosene (UN 1223).

Category D substances include:

- n-methyl pyrrolidinone;
- dipropylene glycol monomethyl ether;

- octanol; and
- diesel.

5.2. Liquid desensitised explosives – subclass 3.2

This subclass was recently introduced to the UN classification system in the 11th edition of class 3 of the UN Model Regulations).

5.2.1. Threshold criteria for liquid desensitised explosives

Liquid desensitised explosives are explosive (class 1) substances that are dissolved or suspended in water or other liquid substances to form an homogeneous liquid mixture to suppress their explosive properties, where the concentration of the explosive substance is at or above the minimum level deemed subject to the UN Model Regulations. This criterion is in para 2.3.1.4 of the UN Model Regulations.

Current entries in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations are UN 1204, 2059, 3064, 3343, and 3357.

5.2.2. Classification of liquid desensitised explosives

There are three classification categories to subclass 3.2, liquid desensitised explosives. The criteria for inclusion in these categories are as follows.

- Category A (high hazard) – classification 3.2A (equivalent to UN PG I)
 - a. Any substance that is listed in para 2.3.1.4 of the UN Model Regulations as a liquid desensitised explosive and is assigned UN PG I in the UN Dangerous Goods List in chapter 3.2 of the UN Model Regulations (the only such substance currently listed is UN 2059, nitrocellulose solution, PG I).
 - b. Any liquid desensitised explosive that is formed from an explosive by adding a desensitising agent to form a liquid substance that no longer meets a threshold for class 1, is not already listed in the UN Model Regulations, and has not been assigned a packing group in the UN Model Regulations.
- Category B (medium hazard) – classification 3.2B (equivalent to UN PG II)

Any substance that is listed in para 2.3.1.4 of the UN Model Regulations as a liquid desensitised explosive and is assigned UN PG II in the UN Dangerous Goods List in chapter 3.2 of the UN Model Regulations (such substances currently listed are UN 1204, 2059, 3064, and 3357).
- Category C (low hazard) – classification 3.2C (equivalent to UN PG III)

Any substance that is listed in para 2.3.1.4 of the UN Model Regulations as a liquid desensitised explosive and is assigned UN PG III in the UN Dangerous Goods List in chapter 3.2 of the UN Model Regulations (the only such substance currently listed is UN 2059, nitrocellulose solution, PG III).

5.2.3. Discussion

Desensitised explosives are substances that have been assigned to class 1 (explosives) but which have been diluted to suppress their explosive properties to the extent that they are excluded from class 1 by UN Test Series 6, as listed in section 16 of the UN Manual of Tests and Criteria (United Nations, 1999a). They

are generally listed in the UN Dangerous Goods List with an indication of the highest concentration that still excludes them from class 1. In some cases, the concentration below which it is no longer considered to be even a desensitised explosive and so subject to the UN Model Regulations is also listed.

The only entries for liquid desensitised explosives in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations are listed in Table 5.1.

Table 5.1: Entries for liquid desensitised explosives in the Dangerous Goods List in the United Nations (UN) Model Regulations

| UN Number | Name and description | UN Packing Group |
|-----------|---|------------------|
| 1204 | Nitroglycerin solution in alcohol (with not more than 1% nitroglycerin) | II |
| 2059 | Nitrocellulose solution, flammable (with not more than 12.6% nitrogen, by dry mass, and not more than 55% nitrocellulose) | I, II, III |
| 3064 | Nitroglycerin solution in alcohol (with more than 1% but not more than 5% nitroglycerin) | II |
| 3343 | Nitroglycerin mixture, desensitised, liquid, flammable, not otherwise specified (with not more than 30% nitroglycerin, by mass) | |
| 3357 | Nitroglycerin mixture, desensitised, liquid, not otherwise specified (with not more than 30% nitroglycerin, by mass) | II |

Source: United Nations (1999b, chapter 3.2).

References

Gmehling J, Rasmussen, P 1982. Flash points of flammable liquid mixtures using UNIFAC. *Industrial and Engineering Chemistry Fundamentals* 21, 186–188.

United Nations 1999a. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria*, 3rd revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 1999b. *Recommendations on the Transport of Dangerous Goods Model Regulations*, 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 2007. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

6. Flammable Solids, Self-Reactive Flammable Solids, Solid Desensitised Explosives, Spontaneously Combustible Flammable Solids, and Substances Dangerous When Wet – Subclasses 4.1.1, 4.1.2, 4.1.3, 4.2, and 4.3

6.1. Flammable solids – subclass 4.1.1

The Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification system for solids with flammable properties provides for five subclasses, reflecting different manifestations of the flammable property. This generally mirrors the system under the UN Model Regulations (United Nations, 1999b) for class 4 flammable solids, which provides for three divisions within the class, with one of these subdivided into three types of substance.

The HSNO Act subclasses for flammable solids are:

- subclass 4.1.1 – flammable solids, which includes solids that may cause fire through friction (see section 6.1 [above](#));
- subclass 4.1.2 – self-reactive substances (see section 6.2 [below](#));
- subclass 4.1.3 – solid desensitised explosives (see section 6.3 [below](#));
- subclass 4.2 – substances liable to spontaneous combustion and pyrophoric and self-heating substances (see section 6.4 [below](#)); and
- subclass 4.3 – substances that in contact with water emit flammable gases (see section 6.5 [below](#)).

Key terms are defined in section 3.2 in chapter 3.

6.1.1. Threshold criteria for flammable solids subclass 4.1.1 (equivalent to UN division 4.1(a))

This subclass covers substances that are readily combustible or may cause or contribute to fire through friction.

a. The threshold criterion for substances considered easily ignitable and readily combustible is as follows.

Any solid that meets the criteria of para 33.2.1.4.4 (test criteria and method of assessing results) of the UN Manual of Tests and Criteria (United Nations, 1999a), when tested in accordance with the burning rate test method for readily combustible solids set out in Test Series N.1 (para 33.2.1.4, UN Manual of Tests and Criteria).

b. The threshold criterion for substances that may cause or contribute to fire through friction is as follows.

Any solid listed in the Dangerous Goods List, chapter 3.2, of the UN Model Regulations, with the serial number: UN 1331, 1343, 1944, 1945, or 2254.

For any substance subjected to the threshold test method in (a) above, the result must be determined using:

- the finest particle form in which that substance is reasonably capable of being used or rendered; or
- where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then that finer form.

6.1.2. Classification criteria for flammable solids subclass 4.1.1

There are two classification categories to subclass 4.1.1, easily ignitable, readily combustible flammable solids and solids that may cause fire through friction. The criteria for inclusion in these categories are as follows.

- Category A (medium hazard) – classification 4.1.1A (equivalent to UN PG II)
 - a. Any readily combustible solid (other than a metal powder) that, when tested in accordance with section 33.2.1.4 (burning rate test of Test Series N.1) of the UN Manual of Tests and Criteria, has a burning time of less than 45 seconds and the flame passes the wetted zone.
 - b. Any metal powder or metal alloy powder for which the zone of reaction spreads over the whole length of the sample in five minutes or less, when tested in accordance with section 33.2.1.4 (burning rate test of Test Series N.1) of the UN Manual of Tests and Criteria.
 - c. A substance listed in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations numbered UN 1343 (phosphorus trisulphide).
 - d. Any other substance that may cause fire through friction, and has a similar degree of flammability to the foregoing UN numbered substances in (c) above (that is, where the amount of friction required to cause ignition, when tested in accordance with Test Series 3(b) (para 13.5, UN Manual of Tests and Criteria), is less than 120% of that for any of the substances in the preceding paragraph).
- Category B (low hazard) – classification 4.1.1B (equivalent to UN PG III)
 - a. Any readily combustible solid (other than a metal powder) which, when tested in accordance with para 33.2.1.4 (burning rate test of Test Series N.1) of the UN Manual of Tests and Criteria, has a burning time of less than 45 seconds and the wetted zone stops the flame propagation for at least 4 minutes.
 - b. Any metal powder or metal alloy powder for which the reaction spreads over the whole length of the sample in more than 5 minutes but not more than 10 minutes, when tested in accordance with section 33.2.1.4 (burning rate test of Test Series N.1) of the UN Manual of Tests and Criteria.
 - c. Any substance listed in the Dangerous Goods List, chapter 3.2, UN Model Regulations and numbered UN 1331, 1944, 1945, or 2254.
 - d. Any other solid that may cause fire through friction, and has a similar degree of flammability to the foregoing UN numbered substances in (c) above (that is, where the amount of friction required to cause ignition, when tested in accordance with Test Series 3(b) (para 13.5, UN Manual of Tests and Criteria), is less than 120% of that for any of the substances in the preceding paragraph).

6.1.3. Discussion

Threshold for flammable solids

The HSNO Act threshold criteria are equivalent to those for UN division 4.1(a) as set out in the UN Model Regulations and UN Manual of Tests and Criteria.

Correlation with United Nations Packing Groups



The classification criteria for HSNO Act categories A and B correspond with UN PGs II and III respectively, as described in section 2.4.2.2 of the UN Model Regulations.

Particle size of flammable solids for testing

The UN/International Labor Organization working group on the harmonisation of the classification criteria for physical hazards considered the question of particle size for the testing of flammable solids. It concluded in a United Nations Committee of Experts on the Transport of Dangerous Goods report (UNCETDG, 1998) that:

- tests for solids should be carried out on substances in the form as presented, for example, for transport;
- if the substance was to be presented for use in a different form that might alter its behaviour on testing, it should be re-tested in its different form; and
- if it was reasonably foreseeable that a substance would considerably change its material form during its lifetime, the potential hazards of its changed form should also be taken into consideration.

This corresponds to the requirement above that testing should be done on the finest particle form in which the substance is reasonably capable of being used or rendered, or, where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then testing should be done on that finer form.

Test methods

The apparatus and procedure for the burning rate test are set out in section 33.2.1.4 of the UN Manual of Tests and Criteria. If the substance fails the preliminary screening test (para 33.2.1.4.3.1, UN Manual of Tests and Criteria), the substance may be considered not to be a flammable solid, and no further testing need be carried out. If the substance passes the preliminary screening test, the burning rate test should be carried out. The EPA is not aware of any alternative comparable method recognised by any overseas national competent authority.

This particular test has been specified in regulation, and information used in the evaluation must have been obtained using this test. This is because the rate of propagation of the flame depends on how the test is conducted, for example, the cross-sectional area of the powder trail, and on how compacted the powder is in the trail.

Solids that may cause fire through friction

Only a small number of individual 'substances' that may cause fire through friction are listed in the UN Model Regulations. Accordingly, this part of the threshold has been included simply by listing these substances, the UN serial number of which is:

- UN 1331: matches, 'strike anywhere';
- UN 1343: phosphorus trisulphide (free from yellow and white phosphorus);
- UN 1944: matches, safety (book, card and strike-on-a-box);
- UN 1945: matches, wax 'vesta'; and
- UN 2254: matches, fusee.

Current New Zealand criteria

The Dangerous Goods Act 1974 groups class 4.1 flammable solids into categories A, B, and C, and lists specific substances. The entries in these categories in the Schedule to the Dangerous Goods Act do not readily correlate with UN division 4.1(a) or the HSNO Act classification subclasses 4.1.1, 4.1.2, and 4.1.3.

Example substances

The HSNO Act category A (equivalent to UN PG II) includes:

- hafnium powder, wetted;
- titanium powder, wetted;
- zirconium powder, wetted;
- aluminium powder, coated; and
- and 'solids that cause fire through friction', listed above.

The HSNO Act category B (equivalent to UN PG III) includes:

- sulphur;
- silicon powder (amorphous); and
- 'solids that cause fire through friction', listed above.

6.2. Self-reactive flammable solids – subclass 4.1.2

6.2.1. Threshold criteria for self-reactive flammable solids subclass 4.1.2 (equivalent to UN division 4.1(b))

A substance is considered a subclass 4.1.2 self-reactive flammable solid within the meaning of the HSNO Act when:

- a. in a quantity of 50 kg, it has a self-acceleration decomposition temperature (SADT) of less than or equal to 75°C when tested in accordance with any of the test methods set out in Test Series H (section 28, pp 279–300, UN Manual of Tests and Criteria), and it has a heat of decomposition greater than 300 J/g as required in para 2.4.2.3 of the UN Model Regulations; or
- b. it is a substance listed in para 2.4.2.3.2.3 of the UN Model Regulations as having a class and division of a self-reactive substance (UN division 4.1(b)).

6.2.2. Discussion

Notes on thresholds for self-reactive flammable solids

The HSNO Act threshold criteria for subclass 4.1.2 are equivalent to those for UN division 4.1(b) (including Type G) as set out in the UN Model Regulations (para 2.4.2.3).

Test methods

For any substance subjected to the threshold test method in (a) above, the result must be determined using:

- a. the finest particle form in which that substance is reasonably capable of being used or rendered; or

- b. where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then that finer form.

The degree to which these self-reactive substances will heat up internally depends on the:

- surface to volume ratio of the quantity of substance presented; and
- nature (for example, the thermal conductivity) of the container or package it is in.

Accordingly, the test procedures described in the UN Manual of Tests and Criteria are directly referred to for this threshold.

The heat of decomposition can be determined using any internationally recognised method, such as differential scanning calorimetry or adiabatic calorimetry. In using such techniques, special care should be taken in interpreting the results when:

- sampling and testing mixtures;
- the material of the sample vessel may influence the result;
- endotherms immediately precede exotherms;
- evaporation of constituents will lower the exothermicity;
- the presence of air may critically affect the measured decomposition energy;
- there is a large difference between the specific heats of the reactants and products; and
- using rapid heating rates.

Screening procedures for substances that may be self-reactive substances

A substance does not need to be evaluated as a self-reactive substance if no chemical groups present in the molecule are associated with explosive or self-heating properties. Examples of the former are groups such as C-C unsaturation, C-metal, N-metal, N-N unsaturation, peroxides, N-O, N-halogen, O-halogen. Examples of the latter are groups such as mutually reactive groups (for example, aminonitriles, haloanilines, and organic salts of oxidising acids), S=O, P-O, strained rings, and unsaturation.

6.2.3. Classification criteria for self-reactive flammable solids subclass 4.1.2 (equivalent to UN division 4.1(b))

There are seven classification categories to subclass 4.1.2, self-reactive flammable solids, which relate to the degree of hazard that the substances present. The criteria for inclusion in these categories are as follows. (See also the tables in Appendix 6A.)

- Subclass 4.1.2A – self-reactive flammable solid category A (equivalent to UN Type A)
 - a. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a detonation as defined in UN Test Series A (see ‘Definitions’ in section 6.2.4 for an explanation of ‘as defined in UN Test Series’); and
 - iii. propagates a detonation in confined conditions as specified in UN Test Series B.
 - b. The substance:
 - i. is a self-reactive flammable solid;

- ii. propagates a detonation as defined in UN Test Series A;
 - iii. does not propagate a detonation in confined conditions as defined in UN Test Series B;
 - iv. propagates a rapid deflagration as defined in UN Test Series C; and
 - v. propagates a rapid deflagration in confined conditions as defined in UN Test Series D.
 - c. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C; and
 - iv. propagates a rapid deflagration under confined conditions as defined in UN Test Series D.
 - d. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C; and
 - iv. propagates a rapid deflagration when ignited under confinement as defined in UN Test Series D.
- Subclass 4.1.2B – self-reactive flammable solid category B (equivalent to UN Type B)
 - a. The substance is listed in the UN Model Regulations as having a classification of a self-reactive flammable solid division 4.1 of Type B.
 - b. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a detonation as defined in UN Test Series A;
 - iii. does not propagate a detonation under confined conditions as defined in UN Test Series B;
 - iv. propagates a rapid deflagration as defined in UN Test Series C;
 - v. does not propagate a rapid deflagration under confined conditions as defined in UN Test Series D;
 - vi. exhibits violent effect when heated under confinement as defined in UN Test Series E; and
 - vii. undergoes a thermal explosion under confined conditions as defined in UN Test Series G.
 - c. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a detonation as defined in UN Test Series A;
 - iii. does not propagate a detonation under confinement as defined in UN Test Series B;
 - iv. propagates a slow deflagration or does not propagate a deflagration as defined in UN Test Series C;
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - v. undergoes a thermal explosion under confinement as defined in UN Test Series G.
 - d. The substance:
 - i. is a self-reactive flammable solid;

- ii. propagates a partial detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C; and
 - iv. does not propagate a rapid deflagration under confinement as defined in UN Test Series D and
 - v. displays a violent effect when heated under confinement as defined in UN Test Series E; and
 - vi. undergoes a thermal explosion under confinement as defined in UN Test Series G.
- e. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as defined in UN Test Series A
 - iii. propagates slow or no deflagration as defined in UN Test Series C
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - v. undergoes a thermal explosion under confinement as defined in UN Test Series G.
- f. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C;
 - iv. does not propagate a rapid deflagration under confinement as defined in UN Test Series D;
 - v. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - vi. undergoes a thermal explosion under confinement as defined in UN Test Series G.
- g. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a slow deflagration as defined in UN Test Series C;
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - iii. undergoes a thermal explosion under confinement as described in UN Test Series G.
- h. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as described in UN Test Series A;
 - iii. does not propagate a deflagration as described in UN Test Series C;
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - v. undergoes a thermal explosion under confinement as described in UN Test Series G.
- Subclass 4.1.2C – self-reactive flammable solid category C (equivalent to UN Type C)
 - a. The substance is listed in the UN Model Regulations as having a classification of a self-reactive flammable solid division 4.1 of Type C.
 - b. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a detonation as described in UN Test Series A;
 - iii. does not propagate a detonation under confinement as described in UN Test Series B;

- iv. propagates a rapid deflagration as described in UN Test Series C;
 - v. does not propagate a rapid deflagration under confinement as described in UN Test Series D;
 - vi. displays violent effect when heated under confinement as described in UN Test Series E; and
 - vii. does not undergo a thermal explosion when heated under confinement as prescribed in Test Series G.
- c. The substance:
- i. is a self-reactive flammable solid;
 - ii. propagates a detonation as described in UN Test Series A;
 - iii. does not propagate a detonation under confinement as described in UN Test Series B;
 - iv. propagates a slow deflagration or propagates no deflagration as described in UN Test Series C;
 - v. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - vi. does not undergo a thermal explosion when heated under confinement as prescribed in Test Series G.
- d. The substance:
- i. is a self-reactive flammable solid;
 - ii. propagates a detonation as described in UN Test Series A;
 - iii. does not propagate a detonation under confinement as described in UN Test Series B;
 - iv. propagates a rapid deflagration as described in UN Test Series C;
 - v. does not propagate a rapid deflagration under confinement as described in UN Test Series D; and
 - vi. displays medium effect, low effect or no effect when heated under confinement as described in UN Test Series E.
- e. The substance:
- i. is a self-reactive flammable solid;
 - ii. propagates a detonation as defined in UN Test Series A;
 - iii. does not propagate a detonation under confinement as described in UN Test Series B;
 - iv. propagates a slow deflagration or no deflagration as defined in UN Test Series C; and
 - v. displays medium effect, low effect, or no effect when heated under confinement as described in UN Test Series E.
- f. The substance:
- i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C;
 - iv. does not propagate a rapid deflagration under confinement as described in UN Test Series D;
 - v. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - vi. does not undergo a thermal explosion under confinement as described in UN Test Series G.
- g. The substance:

- i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C;
 - iv. does not propagate a rapid deflagration under confinement as defined in UN Test Series D; and
 - v. displays medium effect, low effect, or no effect when heated under confinement as defined in UN Test Series E.
- h. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as defined in UN Test Series A;
 - iii. propagates a slow or no deflagration as defined in UN Test Series C;
 - iv. displays violent effect when heated under confinement as described in UN Test Series E; and
 - v. does not undergo a thermal explosion under confinement as described in UN Test Series G.
- i. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined under UN Test Series A;
 - iii. propagates a rapid deflagration as defined under UN Test Series C;
 - iv. does not propagate a rapid deflagration under confinement as defined in UN Test Series D;
 - v. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - vi. does not undergo a thermal explosion under confinement as defined in UN Test Series G.
- j. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a rapid deflagration as defined in UN Test Series C;
 - iv. does not propagate a rapid deflagration under confinement as defined in UN Test Series D; and
 - v. displays medium effect, low effect, or no effect when heated under confinement as described in UN Test Series E.
- k. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a slow deflagration as defined in UN Test Series C;
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and
 - v. does not undergo a thermal explosion under confinement as defined in UN Test Series G.
- l. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C;
 - iv. displays violent effect when heated under confinement as defined in UN Test Series E; and

- v. does not undergo a thermal explosion under confinement as defined in UN Test Series G.
- Subclass 4.1.2D – self-reactive flammable solid category D (equivalent to UN Type D)
 - a. The substance is listed in the UN Model Regulations as having a classification of a self-reactive flammable solid division 4.1 of Type D.
 - b. The substance:
 - i. is a self-reactive flammable solid;
 - ii. propagates a partial detonation as described in UN Test Series A;
 - iii. propagates a slow deflagration or no deflagration as defined in UN Test Series C;
 - iv. displays medium effect, low effect, or no effect when heated under confinement as defined UN Test Series E.
 - c. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. propagates a slow deflagration as defined in UN Test Series C; and
 - iv. displays medium effect, low effect, or no effect when heated under confinement as defined in UN Series E.
 - d. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C; and
 - iv. displays medium effect when heated under confinement as defined in UN Test Series E.
- Subclass 4.1.2E – self-reactive flammable solid category E equivalent to UN Type E)
 - a. The substance is listed in the UN Model Regulations as having a classification of a self-reactive flammable solid division 4.1 of Type E.
 - b. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C;
 - iv. displays low effect or no effect when heated under confinement as defined in UN Test Series E;
 - and
 - v. is not intended to be stored or transported in bulk or no data are available for Test Series F.
 - c. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C;
 - v. displays low effect or no effect when heated under confinement as defined in UN Test Series E;
 - vi. is intended to be stored or transported in bulk;

- vii. displays an explosive power at a level of 'not low', as defined in UN Test Series F, or no data are available for UN Test Series F.
- Subclass 4.1.2F – self-reactive flammable solid category F equivalent to UN Type F)
 - a. The substance is listed in the UN Model Regulations as having a classification of a self-reactive flammable solid division 4.1 of Type F.
 - b. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C;
 - iv. displays low effect or no effect when heated under confinement as defined in UN Test Series E;
 - v. displays no explosive power as defined in UN Test Series F when tested for bulk containers; and
 - vi. displays a low effect when heated under confinement as defined in UN Test Series E.
 - c. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. not propagate a deflagration as defined in UN Test Series C;
 - iv. displays low effect or no effect when heated under confinement as defined in UN Test Series E;
 - v. is intended to be stored or transported in bulk; and
 - vi. displays 'low' explosive power as defined in UN Test Series F.
 - d. The substance:
 - i. is a self-reactive flammable solid;
 - ii. does not propagate a detonation as defined in UN Test Series A;
 - iii. does not propagate a deflagration as defined in UN Test Series C;
 - iv. displays no effect when heated under confinement as defined in UN Test Series E, including when it is assessed for bulk containers;
 - v. has no explosive power as defined in UN Test Series F; and
 - vi. has either an SADT less than 60°C (for a 50 kg quantity) or, if the substance is a mixture that contains a solvent or desensitising agent, that solvent or desensitising agent has a boiling point less than 150°C.
- Subclass 4.1.2G – self-reactive flammable solid category G (equivalent to UN Type G)
The substance:
 - a. is a self-reactive flammable solid;
 - b. does not propagate a detonation as defined in UN Test Series A;
 - c. does not propagate a deflagration as defined in UN Test Series C;

- d. displays no effect when heated under confinement as defined in UN Test Series E, including when it is assessed for bulk containers;
- e. has no explosive power as defined in UN Test Series F; and
- f. has an SADT greater than or equal to 60°C (for a 50 kg quantity), and, if the substance is a mixture that contains a solvent or desensitising agent, that solvent or desensitising agent is a liquid that has a boiling point greater than or equal to 150°C.

6.2.4. Discussion

Classification of category E, F, or G is provided only for substances that in response to:

- Test Series A do not detonate;
- Test Series C do not deflagrate; and
- Test Series E show either a low effect or no effect of heating under confinement.

These classifications determine the degree to which the explosive power or heating under confinement may be related to quantities in excess of 50 kg. Where these data are not sought, a classification of category D is sufficient.

That is, if a substance does not meet the criteria for a 4.1.2A, 4.1.2B, or 4.1.2C hazard classification, a 4.1.2D classification applies, unless sufficient data are provided that shows the effects meet the criteria for hazard classification 4.1.2E, 4.1.2F, or 4.1.2G.

The classification scheme and criteria above are summarised in tables in Appendix 6A. The full UN classification scheme is set out in section 2.4.2.3 of the UN Model Regulations.

The HSNO Act classification system for self-reactive substances is consistent with the UN Model Regulations. The UN Model Regulations classify self-reactive substances into seven types according to the degree of danger associated with explosive and flammable effects. Because the magnitude of such effects is dependent on quantity, the classification of Types B to F is directly related to a maximum quantity. Each classification 'type' has different controls. For presentation purposes, aspects of control (for example, labelling, temperature control, and requirements in relation to desensitisation (the addition of diluents)) have not been included here because under the HSNO Act framework, such controls are more appropriately included in the Hazardous Substances (Classes 1 to 5 Controls) Regulations 2001 and in the controls under the Hazardous Substances (Packaging) Regulations 2001 and Hazardous Substances (Identification) Regulations 2001.

The UN Model Regulations classify the substances as packaged for transport. Type G substances are not considered to be a significant hazard (for transport). However, decomposition can be initiated by contact with catalytic impurities. This is more likely to occur when the substance is stored in bulk, not packed, or taken out of the package. Since the HSNO Act requires consideration of other parts of the lifecycle besides transport (for example, manufacturing, bulk storage, use, occupational health and safety), Type G self-reactive substances are classified under the HSNO Act to enable controls to be imposed when required.

Under the UN Model Regulations, substances are not classified as 4.1(b), self-reactive substances, if they fall within the criteria for class 1, explosives, subclass 5.1, oxidising substances, or subclass 5.2, organic peroxides. Any substance that exceeds the threshold tests for both subclasses 4.1.2, self-reactive substance, and 4.2, substance liable to spontaneous combustion, is classified as subclass 4.1.2, self-reactive substance.

Definitions

The following definitions relate to the classification criteria above.

| Term | Definition |
|--------------------------------|--|
| as defined in UN Test Series A | Tested using the procedures specified in section 21, Test Series A, pp 197–212, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series B | Tested using the procedures specified in section 22, Test Series B, pp 213–216, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series C | Tested using the procedures specified in section 23, Test Series C, pp 217–228, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series D | Tested using the procedures specified in section 24, Test Series D, pp 229–231, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series E | Tested using the procedures specified in section 25, Test Series E, pp 233–247, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series F | Tested using the procedures specified in section 26, Test Series F, pp 249–271, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series G | Tested using the procedures specified in section 27, Test Series G, pp 273–278, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series H | tested using the procedures specified in section 28, Test Series H, pp 279–300, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |

Description and properties of self-reactive substances

Self-reactive substances are thermally unstable substances liable to undergo a strongly exothermic decomposition even without the participation of oxygen (air).

The decomposition of self-reactive substances can be initiated by heat, contact with catalytic impurities (for example, acids, heavy metal compounds, and bases), friction, or impact. The rate of decomposition

increases with temperature and varies with the substance. Decomposition may result in the evolution of toxic gases and vapours, particularly if no ignition occurs.

Some self-reactive substances may decompose explosively, particularly if confined. This characteristic may be modified by the addition of diluents or by the use of appropriate packaging.

Some self-reactive substances burn vigorously. For certain self-reactive substances, the temperature at which they are held must be controlled.

Examples of self-reactive substances are compounds such as:

- aliphatic azo compounds (-C-N=N-C-);
- organic azides (-C-N₃);
- diazonium salts (-CN₂+Z-);
- N-nitroso compounds (-N-N=O); and
- aromatic sulphohydrazides (-SO₂-NH-NH₂).

This list is not exhaustive and substances with other reactive groups and some mixtures of substances may have similar properties.

Current New Zealand criteria

The Dangerous Goods Act 1974 groups class 4.1 flammable solids into categories A, B, and C, and lists specific substances. The entries in these categories in the Schedule to the Dangerous Goods Act do not readily correlate with UN division 4.1(b) or the HSNO Act classification subclasses 4.1.1, 4.1.2, and 4.1.3.

Example substances

Table 6.1: Example substances classified as self-reactive flammable solids in the UN Model Regulations

| Type | Examples of substances meeting the criteria for that type |
|------|--|
| A | None currently assigned* |
| B | 2-diazo-1-naphthol-4-sulfochloride, 2-diazo-1-naphthol-5-sulfochloride Azodicarbonamide formulation Type B, temperature controlled |
| C | Azodicarbonamide formulation Type C 2,2' -azodi (isobutyronitrile) Tetramine palladium(II) nitrate |
| D | Azodicarbonamide formulation Type D Benzene sulfohydrazide Diphenyloxide-4,4'-disulfohydrazide 4-nitrosophenol |
| E | Diethyleneglycol bis(allyl carbonate) + di-isopropylperoxydicarbonate |
| F | None currently assigned |

| | |
|---|-------------------------|
| G | None currently assigned |
|---|-------------------------|

Note

- * Extremely sensitive substances – too sensitive for transport: The classification is dependent on the type of packaging. A change of packaging could change the classification from Type A. Such substances will exist in workplaces, for example. Accordingly, a classification category is needed for specifying controls for these substances, including the performance requirements associated with their safe handling before their being made into an article that can meet the requirements for use and packaged for transport and storage.

6.3. Solid desensitised explosives – subclass 4.1.3

6.3.1. Threshold criteria for flammable solids subclass 4.1.3 desensitised explosives (equivalent to UN division 4.1(c))

A substance is considered to be a subclass 4.1.3 solid desensitised explosive, within the meaning of the HSNO Act, if:

- a. before being desensitised, it would meet one or more of the threshold criteria for substances with explosive properties (class 1); and it has been desensitised to the extent that it would, when tested under Test Series 6 type (c) (para 16.6, UN Manual of Tests and Criteria), show no projection, fire, smoke, heat, or noise effect external to the substance itself; and
- b. it does not meet any of the threshold criteria for substances with oxidising properties (subclasses 5.1 and 5.2) or for self-reactive substances of subclass 4.1.2; or
- c. it is an explosive substance that has been wetted with water or alcohols or diluted with other substances, to form an homogeneous mixture in order to suppress its explosive properties, where the concentration of the explosive substance is at or above the minimum level deemed subject to the UN Model Regulations; or
- d. it is listed in para 2.4.2.4.1 of the UN Model Regulations; or
- e. it is listed in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations as having a class and division of a solid desensitised explosive (UN division 4.1(c)).

Substances meeting the criteria in (a) above are listed in para 2.4.2.4.2 of the UN Model Regulations as:

- UN 2956: 5-tert-Butyl-2,4,6-trinitro-m-xylene (musk xylene);
- UN 3241: 2-Bromo-2-nitropropane-1,2-diol;
- UN 3242: Azodicarbonamide; and
- UN 3251: Iso-Sorbide-5-mononitrate.

6.3.2. Classification criteria for subclass 4.1.3 desensitised explosives and related substances (equivalent to UN division 4.1(c))

There are three classification categories to subclass 4.1.3, solid desensitised explosives and related substances. The criteria for inclusion in these categories are as follows.

- Category A (high hazard) – classification 4.1.3A (equivalent to UN PG I)

A substance that either:

- has one of the following UN numbers in the Dangerous Goods List, chapter 3.2 of the UN Model Regulations:
 - UN 1310: Ammonium picrate (wetted with not less than 10% water, by mass);
 - UN 1320: Dinitrophenol (wetted with not less than 15% water, by mass);
 - UN 1321: Dinitrophenolates (wetted with not less than 15% water, by mass);
 - UN 1322: Dinitroresorcinol (wetted with not less than 15% water, by mass);
 - UN 1336: Nitroguanidine (wetted with not less than 20% water, by mass);
 - UN 1337: Nitrostarch (wetted with not less than 20% water, by mass);
 - UN 1344: Trinitrophenol (wetted with not less than 30% water, by mass);
 - UN 1347: Silver picrate (wetted with not less than 30% water, by mass);
 - UN 1348: Sodium dinitro-*o*-cresolate (wetted with not less than 15% water, by mass);
 - UN 1349: Sodium picramate (wetted with not less than 20% water, by mass);
 - UN 1354: Trinitrobenzene (wetted with not less than 30% water, by mass);
 - UN 1355: Trinitrobenzoic acid (wetted with not less than 30% water, by mass);
 - UN 1356: Trinitrotoluene (wetted with not less than 30% water, by mass);
 - UN 1357: Urea nitrate (wetted with not less than 20% water, by mass);
 - UN 1517: Zirconium picramate (wetted with not less than 20% water, by mass);
 - UN 1571: Barium azide (wetted with not less than 50% water, by mass);
 - UN 2852: Dipicryl sulphide (wetted with not less than 10% water, by mass);
 - UN 3317: 2-amino-4,6-dinitrophenol (wetted with not less than 20% water, by mass); or
- any solid desensitised explosive that is formed from an explosive of class 1 by adding a desensitising agent to form a solid substance that no longer meets the threshold criteria for class 1.

- Category B (medium hazard) – classification 4.1.2B (equivalent to UN PG II)

A substance that has one of the following UN numbers in the Dangerous Goods List, chapter 3.2 of the UN Model Regulations:

- UN 2555: Nitrocellulose with water (not less than 25% water, by mass);
- UN 2556: Nitrocellulose with alcohol (not less than 25% alcohol, by mass and not more than 12.6% nitrogen, by dry mass);
- UN 2557: Nitrocellulose, with not more than 12.6% nitrogen, by dry mass, mixture with or without plasticiser, with or without pigment;
- UN 2907: Isosorbide dinitrate mixture, with not less than 60% lactose, mannose, starch, or calcium hydrogen phosphate;
- UN 3270: Nitrocellulose membrane filters, with not more than 12.6% nitrogen, by dry mass;
- UN 3319: Nitroglycerin mixture, desensitised, solid, with more than 2% but not more than 10% nitroglycerin, by mass;
- UN 3344: Pentaerythrite tetranitrate mixture, desensitised, solid, not otherwise specified, with more than 10% but not more than 20% PETN, by mass; or

- UN 3242: Azodicarbonamide.
- Category C (low hazard) – classification 4.1.3C (equivalent to UN PG III)
A substance that has one of the following UN numbers in the Dangerous Goods List, chapter 3.2 of the UN Model Regulations:
 - UN 2956: 5-tert-Butyl-2,4,6-trinitro-m-xylene (musk xylene);
 - UN 3241: 2-Bromo-2-nitropropane-1,2-diol; or
 - UN 3251: Iso-Sorbide-5-mononitrate.

6.3.3. Discussion

Threshold for desensitised explosives

The HSNO Act threshold criteria for subclass 4.1.3 are equivalent to those for division 4.1(c) as set out in the UN Model Regulations, including substances previously known as substances related to self-reactive substances.

Classification of desensitised explosives

The HSNO Act classification categories A, B, and C for subclass 4.1.3, desensitised explosives and related substances, are equivalent to UN PGs I, II, and III, respectively, of division 4.1(c), solid desensitised explosives, as described in the UN Model Regulations.

The UN Model Regulations assign substances to this classification by analogy with existing substances. Subclass 4.1.3 also includes substances formerly classified by the UN as 'substances related to self-reactive substances'. These substances are similar to division 4.1.2 'self-reactive substances' but have an SADT greater than 75°C. They are liable to undergo a strongly exothermic decomposition and are liable, in certain packaging, to meet the criteria for explosive substances in class 1.

New products that are thermally stable and have, or are suspected of having, explosive properties should first be considered for class 1 using the class 1 acceptance procedure and, if necessary, the assignment procedure.

When a substance is assigned to class 1 but is diluted to be exempted from class 1 by Test Series 6 (see above), this diluted substance, when meeting the classification criteria or definition for another class or subclass, should be classified in that class or subclass at the highest concentration at which it is exempt from class 1. When sufficiently diluted, such substances may not meet the criteria for any class or subclass, and may be deemed non-hazardous in terms of the flammability criteria.

Nature of desensitised explosives

Desensitised explosives are substances that are generally wetted with water or alcohols or are diluted with other substances to suppress their explosive properties. In their unwetted or undiluted form, they are substances that would meet the criteria for class 1.

Explosive substances can be desensitised to different extents. For example, an explosive substance that is too sensitive to transport can be desensitised to a degree that enables it to be transported; but in this

desensitised state, it still meets the criteria for a substance with explosive properties. Such substances should still be classified as explosive substances.

When substances are diluted so as to exempt them from the explosives classification by failing Test Series 6, but they have flammable properties, then they are classified as subclass 4.1.3, solid desensitised explosives.

Current New Zealand criteria

The Dangerous Goods Act 1974 grouped class 4.1, flammable solids, into categories A, B, and C, and listed specific substances. Although overall these Dangerous Goods Act categories did not readily correlate with UN class 4.1 or the HSNO Act classification subclasses 4.1.1, 4.1.2, and 4.1.3, DG class 4.1 category B did list substances now classified as HSNO Act subclass (UN division) 4.1.3, desensitised explosives.

6.4. Spontaneously combustible flammable solids – subclass 4.2

6.4.1. Threshold criteria for subclass 4.2 substances liable to spontaneous combustion and pyrophoric and self-heating substances (equivalent to UN division 4.2)

A substance is considered to be a subclass 4.2 substance (substances liable to spontaneous combustion and pyrophoric and self-heating substances), within the meaning of the HSNO Act, if, when tested as described in para 33.3.1 of the UN Manual of Tests and Criteria, it meets one or more of the following criteria.

- a. It is a substance that is a solid in powder form and, when tested in accordance with Test N.2 (para 33.3.1.4, UN Manual of Tests and Criteria), ignites in one of the tests (pyrophoric solids).
- b. It is a substance that is a liquid and, when tested in accordance with Test N.3 (para 33.3.1.5, UN Manual of Tests and Criteria), ignites in the first part of the test (para 33.3.1.5.3.1) or ignites or chars the filter paper in the second part of the test (para 33.3.1.5.3.2) (pyrophoric liquids).
- c. It is a substance that is a solid and, when tested in accordance with Test N.4 (para 33.3.1.6, UN Manual of Tests and Criteria), gives a positive result in a test using a 100 mm sample cube at 140°C (self-heating solids).

6.4.2. Notes on the threshold for subclass 4.2

The threshold criteria for HSNO Act subclass 4.2 are equivalent to those for division 4.2 as set out in the UN Manual of Tests and Criteria.

The EPA is not aware of any alternative comparable test methods recognised by any overseas national competent authority, so the methods described above from the UN Manual of Tests and Criteria have been used to define the threshold.

For any substance subjected to the threshold test methods described in (a) and (c) above (that is, test methods N3 and N4), the result must be determined using either:

- the finest particle form in which that substance is reasonably capable of being used or rendered; or
- where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then that finer form.

Details of the test criteria for the test methods mentioned above follow.

Test N2 (pyrophoric solids)

A substance meets criterion (a) above if it is a solid in powder form and in its commercial form ignites while falling, or within five minutes of settling, when poured from about a 1 m height onto a non-combustible surface, in one or more times out of six.

Test N3 (pyrophoric liquids)

A substance meets criterion (b) above if it is a liquid, and if 5 mL of the liquid, when:

- poured into an inert container containing an inert solid powder, ignites when exposed to air for five minutes, in one or more times out of six; or
- added to a dry filter paper at 25°C, ignition or charring occurs on the filter paper within five minutes of addition of the liquid, in one or more times out of three.

Test N4 (self-heating substance)

A substance meets criterion (c) above if a solid cube of the substance with sides 100 mm long, when heated to 140°C, either spontaneously ignites or experiences a 60°C rise in temperature during a 24-hour period.

6.4.3. Classification criteria for subclass 4.2 substances liable to spontaneous combustion, pyrophoric and self heating substances (equivalent to UN division 4.2)

There are three classification categories to subclass 4.2, substances liable to spontaneous combustion and pyrophoric and self-heating substances. The criteria for inclusion in these categories are as follows.

- Category A (pyrophoric substances: high hazard) – classification 4.2A (equivalent to UN PG I) ‘Pyrophoric substances’, which do not meet the criteria for subclass 4.1.2, that ignite within five minutes on contact with air under the following test conditions.
 - a. For pyrophoric solids, if, when tested in accordance with the procedure set out in Test N.2 (section 33.3.1.4, UN Manual of Tests and Criteria), the substance ignites in one of the tests, the substance is classified category A.
 - b. For pyrophoric liquids, if, when tested in accordance with the procedure set out in Test N.3 (section 33.3.1.5, UN Manual of Tests and Criteria), the liquid ignites in the first part of the test (para 33.3.1.5.3.1) or ignites or chars the filter paper in the second part of the test (para 33.3.1.5.3.2), the substance is classified category A.
- Category B (self-heating substances: medium hazard) – classification 4.2B (equivalent to UN PG II) ‘Self-heating substances’, which do not meet the criteria for subclass 4.1.2, that fail to qualify as category A, but when tested in accordance with Test N.4 (section 33.3.1.6, UN Manual of Tests and Criteria), give

a positive result with a 25 mm cube of the substance at 140°C (the criteria are in paras 33.3.1.6.4.1 and 33.3.1.6.4.3).

- Category C (self-heating substances: low hazard) – classification 4.2C (equivalent to UN PG III) ‘Self-heating substances’, which do not meet the criteria for subclass 4.1.2, that fail to qualify for category A or B, but when tested in accordance with Test N.4 (section 33.3.1.6, UN Manual of Tests and Criteria), obtain a positive result in a test using:
 - a. a 100 mm sample cube at 140°C and the substance is in a volume of more than 3 m³; or
 - b. a 100 mm sample cube at 140°C and a positive result is obtained in a test using a 100 mm cube at 120°C and the substance is in a volume of more than 450 L; or
 - c. a 100 mm sample cube at 140°C and a positive result is obtained in a test using a 100 mm cube at 100°C.

6.4.4. Discussion

Substances giving a positive result with tests for both subclass 4.1.2, self-reactive substances, and subclass 4.2, substances liable to spontaneous combustion, should be classified as subclass 4.1.2, self-reactive substances.

The classification criteria and UN test methods referred to above are as follows.

- Category A (equivalent to UN PG I)

‘Pyrophoric substances’ are substances that ignite within five minutes on contact with air under the following test conditions.

 - a. For pyrophoric solids, if when 1–2 mL of the powdery substance is poured from a 1 m height onto a non-combustible surface, it ignites during dropping or within five minutes of settling. This procedure should be performed six times unless a positive result is obtained earlier. If ignition occurs in one of the tests, the substance is in category A. The procedure set out in section 33.3.1.4, p 328, of the UN Manual of Tests and Criteria, should be followed in determining this result.
 - b. For pyrophoric liquids:
 - i. If, when 5 mL of the liquid is poured into a porcelain cup of about 100 mm diameter (filled with diatomaceous earth or silica gel at room temperature to a height of about 5 mm), the liquid ignites when exposed to air for five minutes. This procedure should be performed six times unless a positive result is obtained earlier. If no ignition occurs then the second part of the test below is performed to determine if it chars or ignites a filter paper.
 - ii. A 0.5 mL test sample should be delivered from a syringe to an indented dry filter paper. The test should be conducted at 25 ± 2°C and a relative humidity of 50 ± 5%. Observations are made to see if ignition or charring occurs on the filter paper within five minutes of addition of the liquid. This procedure should be performed three times using fresh filter paper each time unless a positive result is obtained earlier.
 - iii. If the liquid ignites in the first part of the test, or it ignites or chars the filter paper, it is above the criteria for category A.

The procedures in section 33.3.1.5, p 329, of the UN Manual of Tests and Criteria, should be followed in determining these results.

Substances in the lower categories are self-heating substances requiring longer periods and larger quantities to ignite.

- Category B (equivalent to UN PG II)

'Self-heating substances' are substances that fail to qualify as category A, but ignite when a 2.5 cm cube of the substance is heated to 140°C, in contact with air.

The procedures in section 33.3.1.6, pp 330–331, of the UN Manual of Tests and Criteria, should be followed in determining these results.

- Category C (equivalent to UN PG III)

'Self-heating substances' are substances that fail to qualify for category A or B, but:

- a. a positive result (that is, spontaneous ignition or a 60°C or greater rise in temperature during the 24-hour testing time) is obtained in a test using a 100 mm sample cube at 140°C, but a negative result (that is, no spontaneous ignition or a less than 60°C temperature rise) is obtained in a test using a 25 mm cube sample at 140°C, and the substance is in a volume of more than 3m³; or
- b. a positive result is obtained in a test using a 100 mm sample cube at 140°C, a negative result is obtained in a test using a 25 mm cube sample at 140°C, a positive result is obtained in a test using a 100 mm cube at 120°C, and the substance is in a volume of more than 450 L; or
- c. a positive result is obtained in a test using a 100 mm sample cube at 140°C, a negative result is obtained in a test using a 25 mm cube sample at 140°C, and a positive result is obtained in a test using a 100 mm cube at 100°C.

The procedures in section 33.3.1.6, pp 330–331, of the UN Manual of Tests and Criteria, should be followed in determining these results.

The HSNO Act classification categories A, B, and C are consistent with the UN Model Regulations PGs I, II, and III, except that for category C, the words 'and the substance is to be transported in packages with a volume of' have been changed to 'and the substance is in volumes of'. This is because, under the HSNO Act, we are interested in the whole lifecycle of the substance, not just transportation, and because the substance may be stored in bulk, unpackaged.

The main reason for the HSNO Act threshold being different from that in UN PG III, is because it was considered that the threshold criteria should not contain references to transport or packaging of a certain size; these being aspects that should be taken into account when setting controls.

Screening procedures for substances that may be liable to spontaneous combustion

A substance does not need to be evaluated as a pyrophoric substance when experience, in production or handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures (that is, the substance is known to be stable at room temperature for prolonged periods).

Previous New Zealand criteria

The Dangerous Goods Act 1974 listed division 4.2, substances liable to spontaneous combustion, being solids or liquids possessing the common property of being liable spontaneously to heat and to ignite, in groups A and B. There is no clear correlation between the HSNO Act categories (UN PGs) and the Dangerous Goods Act groups A and B. These Dangerous Goods Act groups are no longer applicable.

Example substances

Category A (equivalent to UN PG I) substances include UN 1366: diethylzinc.

Category B (equivalent to UN PG II) substances include UN 1369: p-nitrosodimethylaniline.

Category C (equivalent to UN PG III) substances include:

- UN 1376: iron oxide spent; and
- UN 2002: celluloid scrap.

6.5. Substances dangerous when wet – subclass 4.3

6.5.1. Threshold criteria for subclass 4.3 substances that in contact with water emit flammable gases (equivalent to UN division 4.3)

Certain substances in contact with water may emit flammable gases that can form explosive mixtures with air. This subclass classifies substances where the reaction with water may lead to the development of dangerous amounts of gases that may be flammable.

A substance is considered a subclass 4.3 substance, substances that in contact with water emit flammable gases, within the meaning of the HSNO Act, if it meets one or both of the following criteria.

- a. Any substance that, when in contact with water, may emit a flammable gas, and when tested in accordance with Test N.5 (section 33.4.1.4, UN Manual of Tests and Criteria), reacts with water at ambient temperatures to produce a gas which ignites spontaneously.
- b. Any substance that, when in contact with water, may emit a flammable gas, and when tested in accordance with Test N.5 (section 33.4.1.4, UN Manual of Tests and Criteria), reacts with water to produce a flammable gas at a rate of 1 L or greater per kilogram of substance per hour.

6.5.2. Notes to threshold criteria

The HSNO Act threshold criteria for subclass 4.3 are equivalent to those for UN division 4.3 as set out in para 33.4.1.4.4 of the UN Manual of Tests and Criteria.

Details of the test method for substances that in contact with water emit flammable gases, are in section 33.4.1.4 (Test N.5, UN Manual of Tests and Criteria). No alternative comparable methods have been recognised by any overseas national competent authority, so the UN tests have been used to define the HSNO Act threshold.

For any substance subjected to the above threshold test method, the result must be determined using either:

- the finest particle form in which that substance is reasonably capable of being used or rendered; or
- where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then that finer form.

6.5.3. Classification criteria for subclass 4.3 substances which in contact with water emit flammable gases (equivalent to UN division 4.3)

There are three classification categories to subclass 4.3, substances that in contact with water emit flammable gases. The criteria for inclusion in these categories are as follows.

- Category A (high hazard) – classification 4.3A (equivalent to UN PG I)
Any substance that:
 - a. emits a gas that ignites when a small quantity of the substance is brought into contact with water when tested in accordance with the procedure set out in Test N.5 (para 33.4.1.4, UN Manual of Tests and Criteria); or
 - b. reacts readily with water at ambient temperatures such that the rate of evolution of flammable gas is greater than or equal to 10 L of gas per kilogram of substance over any one minute, when the rate of emission of flammable gas is determined in accordance with Test N.5 (para 33.4.1.4, UN Manual of Tests and Criteria).
- Category B (medium hazard) – classification 4.3B (equivalent to UN PG II)
Any substance that reacts readily with water at ambient temperatures such that the maximum rate of evolution of flammable gas, determined in accordance with the procedures of Test N.5 (para 33.4.1.4, UN Manual of Tests and Criteria), is greater than or equal to 20 L of gas per kilogram of substance per hour, but less than 10 L per kilogram per minute.
- Category C (low hazard) – classification 4.3C (equivalent to UN PG III)
Any substance that reacts slowly with water at ambient temperatures such that the maximum rate of evolution of flammable gas, determined in accordance with the procedures of Test N.5 (para 33.4.1.4, UN Manual of Tests and Criteria), is equal to or greater than 1 L of gas per kilogram of substance per hour, but less than 20 L per kilogram per hour.

6.5.4. Discussion

The above classification is in accordance with section 2.4.4.3 of the UN Model Regulations for division 4.3, PGs I, II, and III.

Screening procedures for substances that in contact with water may react to emit flammable gases

A substance does not need to be evaluated as a substance that may react with water to emit flammable gases if:

- the chemical structure of the substance does not contain metals or metalloids; or
- experience in production or handling shows that the substance does not react with water (for example, the substance is manufactured in water or washed with water); or
- the substance is known to be soluble in water to form a stable mixture.

Current New Zealand criteria

The Dangerous Goods Act 1974 defines division 4.3 as 'substances which, in contact with water, emit flammable gases, being substances which, by interaction with water, are liable to become spontaneously flammable or to emit flammable gases in dangerous quantities'. It also provides for three categories: A, B, and C. There is some general correlation between the Dangerous Goods Act categories A and B and UN PGs I and II (and HSNO Act categories A and B). This is not complete, however, and there are inconsistencies.

The one substance listed in Dangerous Goods Act category C, lithium aluminium hydride ethereal, is actually a UN PG I (HSNO Act category A) substance.

Example substances

Category A (equivalent to UN PG I) substances include:

- sodium;
- caesium; and
- lithium.

Category B: (equivalent to UN PG II) substances include:

- calcium;
- barium; and
- aluminium carbide

Category C: (equivalent to UN PG III) substances include:

- calcium cyanamide;
- ferrosilicon; and
- zinc ashes.

References

UNCETDG 1998. *Report of the Committee of Experts to the Fourteenth Session, Addendum 3*, ST/SG/AC.10/C.3/28/Add.3. United Nations Committee of Experts on the Transport of Dangerous Goods, Geneva.

United Nations 1999a. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria*, 3rd revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 1999b. *Recommendations on the Transport of Dangerous Goods Model Regulations*, 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document., for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

Appendix 6A: Scheme of classification of self-reactive flammable solids

A substance is assigned to a category if it meets all of the criteria in any of the rows comprising the table relevant to that category.

Table 6A.1: Criteria for allocation to self-reactive flammable solid category A

| | | | | |
|-----|--|--|--|--|
| i. | Propagates a detonation UN Test Series A | Propagates a detonation as confined UN Test Series B | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D |
| ii. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | |
| iv. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | |

Table 6A.2: Criteria for allocation to self-reactive flammable solid category B

| | | | | | |
|------|---|--|--|---|---|
| i. | Listed in UN Model Regulations as 4.1(b) category C | | | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E |
| iii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| iv. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E |
| v. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN | |

| | | Series B | | Test Series E | |
|-------|--|---|---|---|---|
| vi. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| vii. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| viii. | Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| ix. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| x. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| xi. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| xii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |

Table 6A.3: Criteria for allocation to self-reactive flammable solid category C

| | | | | | |
|-------|---|--|---|---|---|
| i. | Listed in UN Model Regulations as 4.1(b) category C | | | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E |
| iii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| iv. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E |
| v. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| vi. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| vii. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| viii. | Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| ix. | Does not propagate a | Propagates a rapid deflagration | Does not propagate a rapid | Violent effect when heated | Does not undergo a thermal |

| | | | | | |
|------|--|--|---|---|--|
| | detonation UN Test Series A | UN Test Series C | deflagration as confined UN Test Series D | under defined confinement UN Test Series E | explosion as confined UN Test Series G |
| x. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| xi. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| xii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |

Table 6A.4: Criteria for allocation to self-reactive flammable solid category D

| | | | |
|------|---|--|---|
| i. | Listed in UN Model Regulations as 4.1(b) category D | | |
| ii. | Propagates a partial detonation UN Test Series A | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E |
| iii. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E |
| iv. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Medium effect when heated under defined confinement UN Test Series E |

Note: Substances classified as 4.1.2 category D may be eligible for classification as category E, F, or G if quantities in excess of 50 kg meet the requirements in Tables 6A.5, 6A.6, and 6A.7.

Table 6A.5: Criteria for allocation to self-reactive flammable solid category E

| | | | | | | |
|------|---|--|---|--|--|--|
| i. | Listed in UN Model Regulations as 4.1(b) category E | | | | | |
| ii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Not intended to be stored or transported in bulk | | |
| iii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power not low UN Test Series F | |

Table 6A.6: Criteria for allocation to self-reactive flammable solid category F

| | | | | | | |
|------|---|--|---|--|--------------------------------------|---|
| i. | Listed in UN Model Regulations as 4.1(b) category F | | | | | |
| ii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | Low effect when heated under defined confinement UN Test Series E |
| iii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power low UN Test Series F | |
| iv. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* |

Note

* And the self-accelerating thermal decomposition temperature from Test Series H is less than 60°C for 50 kg of

the substance, or, if the substance is a mixture containing a solvent or desensitising agent, that solvent or desensitising agent has a boiling point less than 150°C .

Table 6A.7: Criteria for allocation to self-reactive flammable solid category G

| | | | | | | |
|----|---|---|--|--|-------------------------------------|---|
| i. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* |
|----|---|---|--|--|-------------------------------------|---|

Note

* And the self-accelerating thermal decomposition temperature from Test Series H is greater than or equal to 60°C for 50 kg of the substance, and, if the substance is a mixture containing a solvent or desensitising agent, that solvent or desensitising agent is a liquid with a boiling point greater than or equal to 150°C.

7. Substances with Oxidising Properties – Class 5

7.1. Introduction

Substances that have the capacity to oxidise are assessed for their ability to promote fire, usually by providing oxygen and releasing chemical energy. The Hazardous Substances and New Organisms Act 1996 (HSNO Act) thresholds distinguish between two categories: those that are organic peroxides and those that are not.

For both types of oxidising substances, the Recommendations on the Transport of Dangerous Goods Model Regulations (United Nations, 1999b) (UN Model Regulations) test criteria have been adopted for the establishment of the HSNO Act thresholds and classification levels. The thresholds are equivalent to the limits already used in practice by the Chief Inspector of Dangerous Goods under the Dangerous Goods Act 1974.

Swimming pool cleaner (calcium hypochlorite) is an example of a substance that would be included, while organic peroxide mixtures with less than 1% available oxygen would be excluded, for example, some anti-acne skin treatments where benzoyl peroxide is the active ingredient.

7.2. Definitions

The following terms are used in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and Hazardous Substances (Classification) Regulations 2001 in respect of substances with oxidising properties, or relate to the classification criteria.

| Term | Definition |
|--------------------------------|---|
| as defined in UN Test Series A | Tested using the procedures specified in section 21, pp 197–212, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series B | Tested using the procedures specified in section 22, pp 213–216, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series C | Tested using the procedures specified in section 23, pp 217–228, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series D | Tested using the procedures specified in section 24, UN, pp 229–231, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series E | Tested using the procedures specified in section 25, pp 233–247, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |

| Term | Definition |
|--------------------------------|---|
| as defined in UN Test Series F | Tested using the procedures specified in section 26, pp 249–271, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series G | Tested using the procedures specified in section 27, pp 273–278, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| as defined in UN Test Series H | Tested using the procedures specified in section 28, pp 279–300, of the UN Manual of Tests and Criteria, and includes both the testing methods and descriptions of the results contained in that section. |
| data | Values that are directly measured, calculated, or estimated for any of the measures given. |
| desensitising agent | A substance or material that, when mixed with a class 1, class 4.1.2, or class 5.2 substance, produces a mixture that has reduced properties (in terms of those classifications) compared with the original class 1, class 4.1.2, or class 5.2 substance. ‘Desensitised’ has the corresponding meaning. |
| gas | A substance that: <ul style="list-style-type: none"> a. is completely gaseous at 20°C and at 101.3 kPa absolute pressure; or b. has a vapour pressure of more than 300 kPa absolute pressure at 50°C. |
| ISO | International Organization for Standardization |
| ISO 10156:1996 | ISO standard <i>Gases and Gas Mixtures: Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets</i> (ISO, 1996). |
| kPa | kilopascal(s) |
| liquid | A substance that is: <ul style="list-style-type: none"> a. a substance with a melting point of less than or equal to 20°C at 101.3 kPa absolute pressure; or b. a viscous substance, without a defined melting point, if: <ul style="list-style-type: none"> i. more than the quantity of the substance specified in ASTM D4359-90, <i>Test Method for Determining Whether a Material is a Liquid or a Solid</i> (ASTM, 2006) collects on a watch glass when tested in the manner specified in that test; or ii. a penetrometer penetrates into the substance the distance defined in the test for determining fluidity prescribed in Appendix A.3 of the <i>European Agreement Concerning the International Carriage of Dangerous Goods by Road</i> (United Nations, 1994), when the method specified in that test is followed. |
| organic peroxide | A substance containing one or more chemical compounds that: <ul style="list-style-type: none"> a. contains the bivalent oxygen [-O-O-] structure; and b. may be considered as a derivative of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical; and c. may cause or contribute to combustion by the release of chemical energy or compounds that may cause or contribute to fire, explosion, or chemical |

| Term | Definition |
|--|--|
| | decomposition. |
| oxidising substance | A substance that, while not necessarily combustible in itself, may cause or contribute to the combustion of other substances or materials. |
| SADT | See self-accelerating decomposition temperature. |
| self-accelerating decomposition temperature (SADT) | The lowest temperature at which the self-accelerating decomposition of the substance occurs in the packaging in which it is tested as prescribed in Test Series H in section 28 of the UN Manual of Tests and Criteria (United Nations, 1999b). |
| solid | A substance that is neither a liquid nor a gas. |
| Test Series | When followed by a letter or number, means one or more tests as prescribed in the UN Manual of Tests and Criteria (United Nations, 1999b). |
| tested | Means tested according to the methods set out as follows. a. For testing oxidising solids or liquids: In the UN Model Regulations and in the UN Manual of Tests and Criteria; except that for a solid, where the substance is known to be likely to crumble into a finer particle form, or to be used in a finer particle form than the form as transported, then the test should be conducted using that finer form. b. For testing a gas: Those parts of ISO 10156:1996 that relate to determining the oxidising potential of a gas. |
| UN Manual of Tests and Criteria | Third revised edition of <i>Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria</i> (United Nations, 1999a). |
| UN Model Regulations | Eleventh revised edition of <i>Recommendations on the Transport of Dangerous Goods Model Regulations</i> (United Nations, 1999b). |

7.3. Threshold for substances with oxidising properties

The threshold for substances with an oxidising property has two elements.

- Oxidising substances not organic peroxides
These are substances that, while in themselves not necessarily combustible, may cause or contribute to the combustion of other substances or materials.
- Organic peroxides
These are substances that contain the bivalent oxygen [-O-O-] structure and may be considered as derivatives of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical.

The criteria used to define the thresholds follow exactly the internationally harmonised criteria developed by the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG). To meet the threshold, oxidising substances that are not organic peroxides are tested for the rate at which they promote burning. Organic peroxides are tested for the amount of oxygen that they can provide for combustion.

7.4. Oxidising property classification

For substances with an oxidising property, classification generally follows the degree to which these effects are observed to occur when the substance is tested. The classification systems for oxidising substances and organic peroxides are generally consistent with those given in the UN Model Regulations, with the test criteria being those contained in the UN Manual of Tests and Criteria (United Nations, 1999a).

Oxidising substances are divided into:

- subclass 5.1.1 for solids and liquids (see section 7.5);
- subclass 5.1.2 for gases (see section 7.5); and
- subclass 5.2 for organic peroxides (see section 7.6).

Subclasses 5.1.1 and 5.2 are divided into several categories representing different degrees of hazard.

Subclass 5.1.2 has only one category.

An oxidising substance or organic peroxide is classified as having a particular hazard classification if it meets the criteria set out in the table in Schedule 3 to the Hazardous Substances (Classification) Regulations 2001 for that hazard classification.

The classification systems for oxidising substances and organic peroxides are summarised in Table 7.1.

Table 7.1: Classification systems for oxidising substances and organic peroxides

| Degree of hazard | Nature of oxidising hazard | | |
|------------------|-------------------------------------|---|---------------------------|
| | Oxidisers (liquids/solids) 5.1.1 | Oxidising gases* 5.1.2 | Organic peroxides† 5.2 |
| A | 5.1.1A (equivalent to UN PG I) | 5.1.2A Promotes combustion at a greater rate than air does | 5.2A |
| B | 5.1.1B (equivalent to UN PG II) | | 5.2B |
| C | 5.1.1C (equivalent to UN PG III) | | 5.2C |
| D | | | 5.2D |
| E | | | 5.2E |
| F | | | 5.2F |

G

5.2G

Notes:

UN PG United Nations Packing Group.

* Gas means a substance that: (a) has a vapour pressure > 300 kPa at 50°C, or (b) is completely gaseous at 20°C and a standard pressure of 101.3 kPa.

† Generally equivalent to the classification system for UN class 5.2, as contained in the UN Model Regulations.

Note that in the case of subclass 5.2 (organic peroxides), if a substance does not meet the criteria for a 5.2A, 5.2B, or 5.2C hazard classification, then a 5.2D classification applies, unless sufficient data are provided that show the substance meets the criteria for hazard classifications 5.2E, 5.2F, or 5.2G.

With respect to the criteria in the Hazardous Substances (Classification) Regulations 2001 for subclass 5.2, Test Series A–G refer to the tests for self-reactive substances and organic peroxides in sections 21–27, respectively, of the UN Manual of Tests and Criteria.

7.5. Oxidising substances – subclasses 5.1.1 and 5.1.2

If a substance meets any one of the threshold criteria described in the following sections, it is considered an oxidising substance within the meaning of the HSNO Act.

7.5.1. Threshold criteria for oxidising substances not organic peroxides – subclasses 5.1.1 and 5.1.2 (equivalent to UN division 5.1)

A substance is considered an oxidising substance (not an organic peroxide), within the meaning of the HSNO Act if one of the following is true.

- a. It is a substance that is not an organic peroxide and is listed in the Dangerous Goods List, chapter 3.2 of the UN Model Regulations as having a class, division, or subsidiary risk of 5.1 (denoting it as an oxidising substance).
- b. It is a solid that is not an organic peroxide, which, when tested in the form in which it is generally available, it is found that the test mixture of the substance with dried cellulose either spontaneously ignites or shows a mean burn time equal to or faster than that of the 3:7 reference mixture by mass of potassium bromate and cellulose when tested under the same conditions as described in the standard test. The standard test is that prescribed for oxidising solids in Test Series O.1 (para 34.4.1, UN Manual of Tests and Criteria).
- c. It is a liquid that is not an organic peroxide, and that when mixed with dried cellulose either spontaneously ignites or shows a mean pressure rise time that is equal to or faster than the mean pressure rise time of the 1:1 reference mixture of 65% aqueous nitric acid solution and cellulose under the same conditions when tested in accordance with the test method for oxidising liquids set out in Test O.2 (para 34.4.2, UN Manual of Tests and Criteria).

- d. It is a gas that is not an organic peroxide, and that will cause or contribute to combustion at a faster rate than air when tested in accordance with the test procedure for determining the oxidising power of gases and gas mixtures set out in ISO 10156:1996 (ISO, 1996).

In the case of criterion (b) above for oxidising solids, the physical form of the substance presented for testing should also be considered. The substance should be tested in the finest particle form in which it is reasonably capable of being used or rendered. Where it is likely or known that more than 10% of the mass of the substance will crumble into a finer particle form, then the substance should be prepared and tested using that finer form.

Where the substance is a mixture and is made up of one or more chemical elements or compounds, any one of which meets one or more of the threshold criteria for oxidising solids or liquids given in (b) and (c) above, then the mixture will have a capacity to oxidise unless it can be shown that the exact mixture itself does not meet any of the threshold criteria described above.

7.5.2. Classification criteria for subclass 5.1.1 and 5.1.2 oxidising substances (excluding organic peroxides)

The following classification schemes apply to oxidising substances that meet any of the criteria as set out in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 for oxidising substances other than organic peroxides. That is, substances that are organic peroxides are excluded from this classification and are classified separately.

The principal oxidising hazards arise from the ability of the substance to ignite or cause fire or combustion, usually after coming into contact with some other substance or other material. For substances with an oxidising property, classification generally follows the degree to which these effects are observed to occur when the substance is tested. Thus, the classification of substances with an intrinsic capacity to oxidise (that are not organic peroxides) is based on the:

- ability of the substance to cause or contribute to combustion when compared with one or more reference materials or reference mixtures; and
- physical form of the substance.

An oxidising substance that is in solid or liquid form is assigned one of three classification categories to denote the relative degree to which it may cause or contribute to combustion. These three categories are generally equivalent to the UN Packing Groups (PGs) I, II, and III. An oxidising substance that is a gas is assigned to a separate category of 'oxidising gas'.

Technical description of the classification criteria

The properties of a substance that will cause it to fall within a classification category for oxidising substances are as follows.

- Category A (oxidising substances that are solids or liquids: high hazard) – classification 5.1.1A (equivalent to UN PG I)

- a. The substance is listed in the UN Model Regulations as having a classification, division, or subsidiary risk of an oxidising substance, division 5.1, and is assigned PG I.
 - b. The substance is a solid and when mixed with dried cellulose it forms a mixture that either spontaneously ignites or shows a mean burning time faster (that is, a shorter time) than the mean burning time of a 3:2 reference mixture by mass of potassium bromate and cellulose, under the same conditions when tested as prescribed for solids in Test O.1 (para 34.4.1, UN Manual of Tests and Criteria).
 - c. The substance is a liquid and when mixed with dry cellulose it forms a mixture that either spontaneously ignites or shows a mean pressure rise time faster than the mean pressure rise time of a 1:1 reference mixture by mass of 50% perchloric acid and cellulose, under the same conditions when tested as prescribed for liquids in Test O.2 (para 34.4.2, UN Manual of Tests and Criteria).
- Category B (oxidising substances that are solids or liquids: medium hazard) – classification 5.1.1B (equivalent to UN PG II)
 - a. The substance is listed in the UN Model Regulations as having a classification, division, or subsidiary risk of an oxidising substance, division 5.1, and is assigned PG II.
 - b. The substance is a solid and when mixed with dry cellulose it forms a mixture that shows a mean burning time equal to or faster (that is, a shorter time) than the mean burning time of a 2:3 reference mixture by mass of potassium bromate and cellulose, under the same conditions when tested as prescribed for solids in Test O.1 (para 34.4.1, UN Manual of Tests and Criteria), and the criteria for a classification of category A are not met.
 - c. The substance is a liquid and when mixed with dry cellulose it forms a mixture that shows a mean pressure rise time equal to or faster than the mean pressure rise time of a 1:1 reference mixture by mass of 40% aqueous sodium chlorate solution and cellulose, under the same conditions when tested as prescribed for liquids in Test O.2 (para 34.4.2, UN Manual of Tests and Criteria), and the criteria for a classification of category A are not met.
- Category C (oxidising substances that are solids or liquids: low hazard) – classification 5.1.1C (equivalent to UN PG III)
 - a. The substance is listed in the UN Model Regulations as having a classification, division, or subsidiary risk of an oxidising substance, division 5.1, and is assigned UN PG III.
 - b. The substance is a solid and when mixed with dry cellulose it forms a mixture which shows a mean burning time equal to or faster (that is, a shorter time) than the mean burning time of a 3:7 reference mixture by mass of potassium bromate and cellulose, under the same conditions when tested as prescribed for solids in Test O.1 (para 34.4.1, UN Manual of Tests and Criteria), and the criteria for a classification of category A or category B are not met.
 - c. The substance is a liquid and when mixed with dry cellulose it forms a mixture which shows a mean pressure rise time equal to or faster than the mean pressure rise time of a 1:1 reference mixture by

mass of 65% aqueous nitric acid and cellulose, under the same conditions when tested as prescribed for liquids in Test O.2 (para 34.4.2, UN Manual of Tests and Criteria), and the criteria for a classification category A or category B are not met.

- Classification 5.1.2A (oxidising substances that are gases) (equivalent to UN division 2.2 (in part))
A substance is classified as an oxidising gas if:
 - a. the substance is listed in the UN Model Regulations as having a classification of UN division 2.2 and a classification, division, or subsidiary risk of an oxidising substance, division 5.1; or
 - b. the substance is a gas and when tested or evaluated as prescribed in section 5 of ISO 10156:1996 for determining the oxidising power of gases and gas mixtures it is found to cause or contribute to combustion of other material at a faster rate than air does.

7.5.3. Discussion

Multiple hazards classification

A substance may have different hazard classifications where this is necessary to indicate different hazard levels according to:

- different physical forms of the substance, if it is a solid; and
- different concentrations of the substance, if it is a mixture.

Threshold tests for substances with oxidising properties

The approach taken to defining tests and criteria for thresholds for oxidising substances is to specify the specific testing procedures based on those in the UN Manual of Tests and Criteria, and to provide that substances listed in the UN Model Regulations be included. The latter is to minimise the need for re-testing substances already accepted as having an oxidising property. This approach has been taken because there appears to be no other recognised procedures in common use. Similarly, for gases, the criterion used is a single test procedure laid out in ISO 10156:1996.

Test procedures

The test methods set out in the UN Manual of Tests and Criteria are designed to observe the effects of the test substance and cellulose mixture, relative to a mixture made up of a reference substance and cellulose, under set conditions. Consequently, the description of the test refers to the observed effects of the substance and cellulose mixture, not the 'substance' itself. For solids, two different mixing ratios of 1:1 and 4:1 of substance to cellulose are tested, because a test substance may react differently with each ratio.

The description for the assessment of mixtures follows current best practice as described in the UN Manual of Tests and Criteria. Generally, a substance with an oxidising property should be mixed with only 'compatible substances', that is, substances that it will not react with to cause a fire or combustion. For a mixture, the classification is based on the component substance if only one component has an oxidising property sufficient to meet the test for classification, or the mixture is tested and classified accordingly.

Solids

The particle form of solids is referred to in the definition of the test for solid substances. This recognises that during a substance's lifecycle, some substances occur in a finer particle form than as originally presented for testing. However, such an occurrence is of interest only if it will affect the overall hazard classification and is known to be likely to occur. This avoids unnecessary extra testing.

Liquids

The risk of liquids that are themselves not oxidising but contain anhydrous combustible salts, leading to combustible residues on exposure to air through evaporation, is a matter for the EPA to consider when it assesses the hazardous properties of the mixture. There is no special classification category to deal with these liquids.

Gases

ISO 10156:1996 has been adopted as the test method for determining the oxidising potential of a gas.

The test details are described in ISO 10156:1996. Substances in gas form that contribute to combustion at a greater rate than does air are considered as having oxidising properties above the threshold. This means the gas in question is more oxidising than air.

It should be noted that while the UNCETDG has not settled on a specific test, it has agreed to the definition of an oxidising gas. The UN Model Regulations suggest using the test method set out in ISO 10156:1996 or an equivalent approved by a competent authority.

ISO 10156:1996 also provides calculation methods for determining the flammability of gases.

If the oxidising properties of a gas mixture have not been determined by test, then they may be estimated by the following method.

The principle of the method is to compare the oxidising potential of gases in a mixture with the oxidising potential of oxygen in air. The concentration of gases in the mixture is expressed as '% volume'.

The gas mixture is as oxidising as, or more oxidising than, air, if the following condition is verified:

$$\sum x_i c_i \geq 21$$

Where:

x_i is the concentration of gas i in % volume

c_i is the coefficient of oxygen equivalency for gas i (specific to each gas)

The coefficients used in the above calculation to determine the oxidising capacity of certain gases in a mixture with respect to the oxidising capacity of oxygen in air are listed in section 5.2 of ISO 10156:1996. This gives, for example, for oxygen $c = 1$, and for nitrous oxide, $c = 0.6$. When no value for c is given for a gas in this standard, a value of 40 is attributed to this coefficient.

Mixture rules

Where the substance is a mixture of one or more chemical elements or compounds, any one of which meets any of the threshold criteria for an oxidising solid or a liquid, then the mixture (that is, as it is imported or

manufactured) is deemed to have a capacity to oxidise equivalent to the most hazardous element or compound unless:

- a. it can be shown that the exact mixture itself has a different classification; or
- b. the EPA has previously determined that the mixture has, or falls within a range that has, an alternate classification, in which case that classification applies.

Where a substance has a defined range of compositions or mixtures it may fall into more than one classification step, according to the effect of different concentrations of the ingredients in the mixture.

Screening procedures for substances that may be oxidising substances

Organic compounds do not need to be considered against the criteria for oxidising substances if they do not contain oxygen, fluorine, or chlorine, or if these elements are present in the compound but are chemically bonded only to carbon or hydrogen.

Inorganic substances do not need to be considered against the criteria for oxidising substances if they do not contain any oxygen or halogen atoms.

Classification for gases

The UN Model Regulations do not classify gases further, having established the gas has an oxidising property. The focus is principally on the carriage of the gas, typically under pressure, and the use of pan lifecycle controls such as labelling to warn and otherwise inform people about safe handling.

In line with the UN Model Regulations, the classification of oxidising gases translates into certain labelling and other hazard identification controls. For example, the UN Model Regulations require gases that are neither flammable (class 2.1) nor toxic (class 2.3) to be classified as class 2.2, and they may have a secondary hazard identification according to a test that can determine the gases ability to contribute to combustion. For example, compressed oxygen is an oxidising gas and it has a United Nations classification of class 2.2 (non-flammable, non-toxic gas) with subsidiary hazard class 5.1 (which indicates it is an oxidising substance).

7.6. Organic peroxides – subclass 5.2

If a substance meets any one of the threshold criteria described in the following sections, it is considered an organic peroxide within the meaning of the HSNO Act.

7.6.1. Threshold criteria for organic peroxides – subclass 5.2 (equivalent to UN division 5.2)

A substance is considered an organic peroxide, within the meaning of the HSNO Act, if any of the following criteria applies.

- a. The substance is listed in para 2.5.3.2.4 of the UN Model Regulations as an organic peroxide or is listed in the Dangerous Goods List in chapter 3.2 of the UN Model Regulations as having a class or division of an organic peroxide (division 5.2).
- b. Any substance that is an organic peroxide or contains organic peroxides and has more than 1.0% available oxygen from the organic peroxides when containing not more than 1.0% hydrogen peroxide by mass.
- c. Any substance that is an organic peroxide or contains organic peroxides and has more than 0.5% available oxygen from the organic peroxides when containing not less than 1.0% but not more than 7.0% hydrogen peroxide by mass.

Definition of 'available oxygen' content

Where any substance or mixture is considered for the purposes of threshold criteria (b) and (c) above, the available oxygen content as a percentage by mass is determined by the formula:

$$O\% = 16\sum(n_i(c_i/m_i))$$

Where:

O% = the percentage of available oxygen content to be determined

n_i = number of peroxygen groups per molecule of organic peroxide i

c_i = concentration (mass %) of organic peroxide i

m_i = molecular mass of organic peroxide i

This formula calculates the availability of oxygen as a percent by mass. The 'i' is a mathematical expression to allow for adding up the available oxygen for each component in a mixture. Mixtures of different organic peroxides with or without hydrogen peroxide are common.

Notes on the threshold criteria

Where the substance is a mixture and is made up of one or more compounds, any one of which meets one or both of the threshold criteria (b) and (c) above, then the mixture will have a capacity to oxidise, and will be considered hazardous for the purposes of the HSNO Act unless:

- a. it can be shown that the exact mixture itself does not meet any of the threshold criteria described above; or
- b. the EPA has previously determined that the mixture is within a range that is considered not to be hazardous.

Effects of some organic peroxides

Some organic peroxide formulations:

- may, under increased temperature, evolve oxygen and thus depress the temperature at which other flammable materials may ignite; and
- can form peroxides that are unstable when left to 'stand', and may be explosive on exposure to light or air.

Consideration of the stability of a substance should take account of information on such hazards.

7.6.2. Classification criteria for organic peroxides – subclass 5.2

Introduction

The following classification system applies to a substance with oxidising properties that meets any of the criteria for an organic peroxide set out in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001, as described above. Where a substance satisfies the criteria for being above the threshold, the EPA may require additional information to help it to identify and assess the degree and nature of its oxidising property or capacity to oxidise.

The principal oxidising hazards arise from the ability of organic peroxides to ignite or cause fire or combustion, sometimes with explosive force, and sometimes only on exposure to air or water or ambient temperatures. Organic peroxides may be thermally unstable or self-ignite, and this may release sufficient energy and products to sustain or promote a chemical decomposition that is hazardous.

The classification of an organic peroxide generally follows the degree to which these effects are observed to occur. The process is set out in Figure 7.1 and the criteria are summarised in Table 7.2. From Figure 7.1, it can be seen that each classification category may be achieved by various combinations of test results.

Mixtures containing organic peroxides, including those using desensitising agents, are also subject to classification according to these criteria.

Figure 7.1: Procedure for classification of organic peroxides

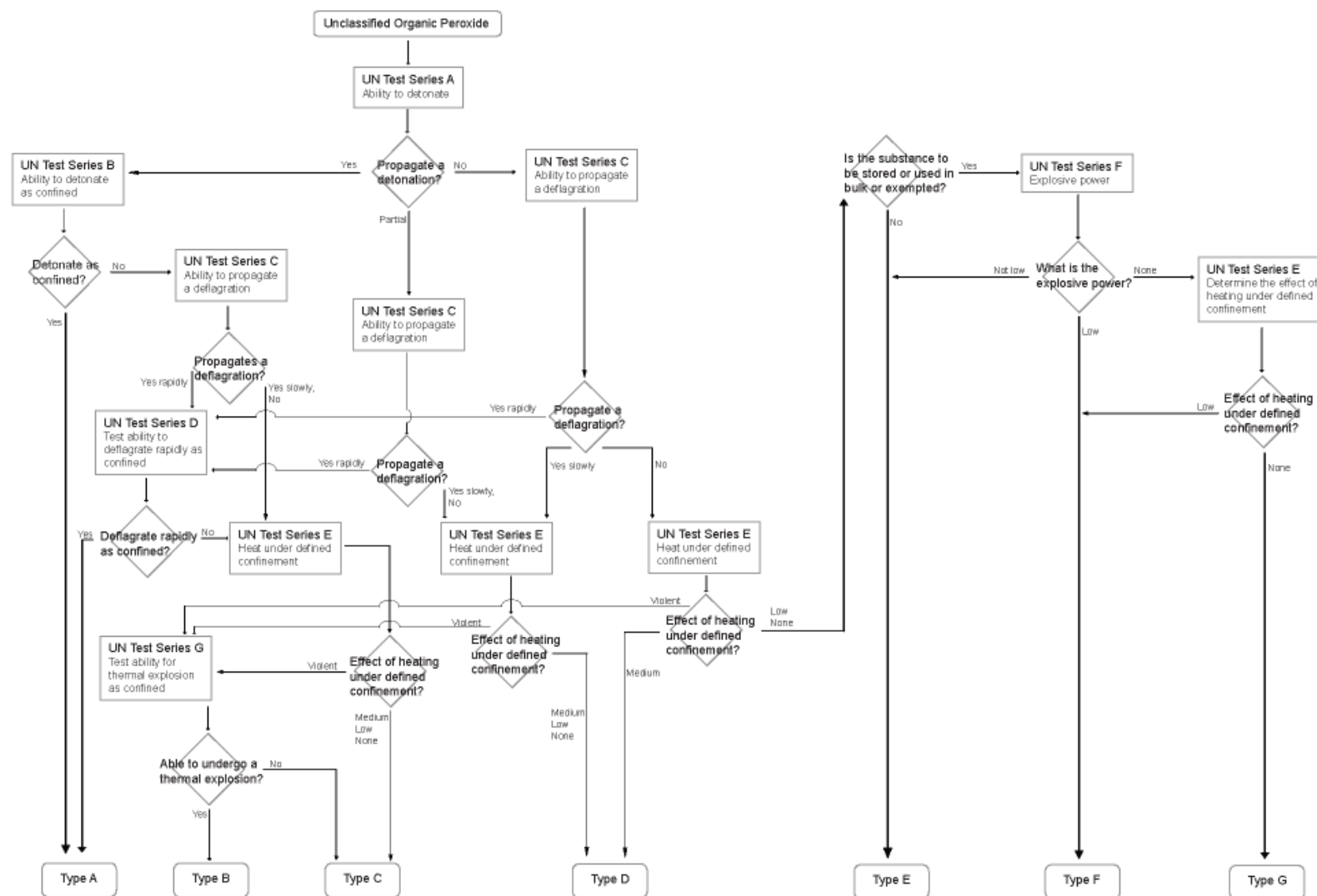


Table 7.2: HSNO Act classification criteria and classification categories for organic peroxides (subclass 5.2)

| Summary of classification criteria * | Overall HSNO Act classification | Secondary classification |
|--|---------------------------------|---------------------------|
| Shows detonation and/or rapid deflagration, including ability to propagate rapid deflagration under confinement | Category A | A1-A4 See Table 7A.1† |
| Propagates a partial or no detonation Propagates a slow or no deflagration Reacts violently and is thermally explosive under confinement | Category B | B1-B7 See Table 7A.2† |
| Substances that may detonate or deflagrate but in response to Test Series B or D or G do not detonate, or do not deflagrate rapidly, and show no thermal explosive effects | Category C | C1-C11 See Table 7A.3† |
| Propagates a partial or no detonation Propagates a slow or no deflagration Medium, low, or no effect when heated under confinement | Category D | D1-D3 See Table 7A.4† |
| Does not propagate a detonation Does not propagate a deflagration Low or no effect when heated under defined confinement Explosive power is not low | Category E | E1-E2 See Table 7A.5† |
| Does not propagate a detonation Does not propagate a deflagration Low or no effect when heated under defined confinement Explosive power is low | Category F | F1-F2 See Table 7A.6† |
| Does not propagate a detonation Does not propagate a deflagration No effect when heated under defined confinement No explosive power Is thermally stable, with a self-accelerating decomposition temperature greater than or equal to 60°C And, if liquid dilutant is used to form a mixture, it is an organic liquid with a boiling point of not less than 150°C | Category G | G1-G2 See Table 7A.7† |

Notes

* In response to test procedures in UN Manual of Tests and Criteria.

† The secondary classification is expected to inform the innate hazards of the organic peroxide for its management outside a container. It is not expected to affect generic packaging or labelling requirements, or the property performance controls for exposure to these hazards.

Technical description of the classification criteria for subclass 5.2 organic peroxides

A substance that meets the qualifying threshold criteria for a capacity to oxidise and is an organic peroxide is assigned a general classification of subclass 5.2.

The classification of an organic peroxide is based on its capacity to cause or contribute to combustion by the release of chemical energy or compounds that may cause or contribute to fire, explosion, or chemical decomposition. The classification also specifies the degree to which these effects occur by assigning a category from A to G, as determined and evaluated by the methods described in Part II of the UN Manual of Tests and Criteria. The sequence of hazard, category A to G, is from high hazard to low hazard.

Where a substance or mixture is not listed in the UN Model Regulations, the organic peroxide is assigned a classification category using the following procedure (and set out in Figure 7.1).

- Preliminary tests to ascertain the potential for fire, explosive effects, or decomposition to occur are based on the:
 - effects of ignition sources, using any test method that will identify those materials that will react violently under little or no confinement;
 - sensitivity of the substance to impact and to friction, using the tests described in Test Series 3 for explosive potential in the UN Manual of Tests and Criteria (pp 67–122); and
 - thermal stability and sensitivity to exothermic decomposition of the substance, using an appropriate calorimetric test method such as differential scanning calorimetry or adiabatic calorimetry.
- Based on the results of the preliminary tests, the organic peroxide's capacity to oxidise can be determined by its response to one or more of the following tests.
 - The self-accelerating decomposition temperature (SADT), determined as described in Test Series H.
 - The degree of heating under confinement, determined as described in Test Series E. If the test result is a 'violent reaction', the degree of thermal explosive power of the substance is determined as described in Test Series G.
 - The degree of mass hazard explosive power, determined as prescribed in Test Series F. Test Series F may also be used as a preliminary test to determine sensitivity to propagate detonations.
 - The degree of propagation of deflagration determined as prescribed in Test Series C. If the test result is 'positive', the degree of deflagration when confined is determined as prescribed in Test Series D.
 - The degree of propagation of detonation determined as prescribed in Test Series A. If the test result is a 'positive', the degree of detonation when confined is determined as prescribed in Test Series B. However, Test Series A is not required if the result of Test Series E is 'no' and the Test Series F result is 'low' or 'no'.

Tests Series B, D, or G is used to establish the degree to which the effects of the organic peroxide may be related to mass or confinement in a container or package.

Test Series A–H refer to the tests for organic peroxides and self-reactive substances in sections 21–28, respectively, of the UN Manual of Tests and Criteria.

Where a substance is a defined range or mixture, it may have more than one classification to indicate different degrees of hazard according to the different concentrations of the ingredients in the mixture. Note this may occur by desensitising the substance.

Classification criteria for the categories of organic peroxides

The properties of a substance that will cause it to fall within a particular classification category for organic peroxides are as follows.

a. Organic peroxide subclass 5.2 category A (equivalent to UN Type A)

A substance is classified as organic peroxide subclass 5.2 category A if:

- i. the substance is an organic peroxide and propagates a detonation as defined in UN Test Series A and propagates a detonation in confined conditions as defined in UN Test Series B (see section 7.2 for an explanation of 'as defined in UN Test Series'); or
- ii. the substance is an organic peroxide and propagates a detonation as defined in UN Test Series A and does not propagate a detonation in confined conditions as defined in UN Test Series B and propagates a rapid deflagration as defined in UN Test Series C and propagates a rapid deflagration in confined conditions as defined in UN Test Series D; or
- iii. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and propagates a rapid deflagration under confined conditions as defined in UN Test Series D; or
- iv. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and propagates a rapid deflagration when confined as defined in UN Test Series D.

These classification criteria are summarised in Table 7.3. A substance is assigned to this category if it meets all of the criteria in any of the rows in the table.

Table 7.3: Criteria for allocation to organic peroxide category A

| | | | | |
|------|--|--|--|--|
| i. | Propagates a detonation UN Test Series A | Propagates a detonation as confined UN Test Series B | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D |
| iii. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | |
| iv. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | |

b. Organic peroxide subclass 5.2 category B (equivalent to UN Type B)

A substance is classified as organic peroxide subclass 5.2 category B if:

- i. the substance is listed in the UN Model Regulations as having a classification or division of an organic peroxide (classification division 5.2) and is designated as Type B; or
- ii. the substance is an organic peroxide and propagates a detonation as defined in UN Test Series A and does not propagate a detonation under confined conditions as defined in UN Test Series B and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confined conditions as defined in UN Test Series D and exhibits violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confined conditions as defined in UN Test Series G; or
- iii. the substance is an organic peroxide and propagates a detonation as defined in UN Test Series A and does not propagate a detonation under confinement as defined in UN Test Series B and either propagates a slow deflagration or does not propagate deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as defined in UN Test Series G; or
- iv. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confinement as defined in UN Test Series D and displays a violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as defined in UN Test Series G; or
- v. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test Series A and propagates a slow or no deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as defined in UN Test Series G; or
- vi. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confinement as defined in UN Test Series D and displays violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as defined in UN Test Series G; or
- vii. The substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a slow deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as described in UN Test Series G; or
- viii. The substance is an organic peroxide and does not propagate a detonation as described in UN Test Series A and does not propagate a deflagration as described in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and undergoes a thermal explosion under confinement as described in UN Test Series G.

These classification criteria are summarised in Table 7.4. A substance is assigned to this category if it meets all of the criteria in any of the rows comprising the table.

Table 7.4: Criteria for allocation to organic peroxide category B

| | | | | | | |
|-------|--|--|---|---|---|--|
| i. | Listed in UN Recommendations as 5.2 Type B | | | | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G |
| iii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | |
| iv. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | |
| v. | Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | |
| vi. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | |
| vii. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | |
| viii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | |

c. Organic peroxide subclass 5.2 category C (equivalent to UN Type C)

A substance is classified as organic peroxide subclass 5.2 category C if:

- i. the substance is listed in the UN Model Regulations as having a classification or division of an organic peroxide (classification division 5.2) and is designated as Type C; or
- ii. the substance is an organic peroxide and propagates a detonation as described in UN Test Series A and does not propagate a detonation under confinement as described in UN Test Series B and propagates a rapid deflagration as described in UN Test Series C and does not propagate a rapid deflagration under confinement as described in UN Test Series D and displays violent effect when heated under confinement as described in UN Test Series E and does not undergo a thermal explosion when heated under confinement as prescribed in Test Series G; or
- iii. the substance is an organic peroxide and propagates a detonation as described in UN Test Series A and does not propagate a detonation under confinement as described in UN Test Series B and propagates a slow deflagration or propagates no deflagration as described in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and does not undergo a thermal explosion when heated under confinement as prescribed in Test Series G; or
- iv. the substance is an organic peroxide and propagates a detonation as described in UN Test Series A and does not propagate a detonation under confinement as described in UN Test Series B and propagates a rapid deflagration as described in UN Test Series C and does not propagate a rapid deflagration under confinement as described in UN Test Series D and displays medium effect, low effect or no effect when heated under confinement as described in UN Test Series E; or
- v. the substance is an organic peroxide and propagates a detonation as defined in UN Test Series A and does not propagate a detonation under confinement as described in UN Test Series B and propagates a slow deflagration or no deflagration as defined in UN Test Series C and displays medium effect, low effect or no effect when heated under confinement as described in UN Test Series E; or
- vi. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test series A and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confinement as described in UN Test Series D and displays violent effect when heated under confinement as defined in UN Test Series E and does not undergo a thermal explosion under confinement as described in UN Test Series G; or
- vii. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confinement as defined in UN Test Series D and displays medium effect, low effect or no effect when heated under confinement as defined in UN Test Series E; or
- viii. the substance is an organic peroxide and propagates a partial detonation as defined in UN Test Series A and propagates a slow or no deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as described in UN Test Series E and does not undergo a thermal explosion under confinement as described in UN Test Series G; or

- ix. the substance is an organic peroxide and does not propagate a detonation as defined under UN Test Series A and propagates a rapid deflagration as defined under UN Test Series C and does not propagate a rapid deflagration under confinement as defined in UN Test Series D and displays violent effect when heated under confinement as defined in UN Test Series E and does not undergo a thermal explosion under confinement as defined in UN Test Series G; or
- x. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a rapid deflagration as defined in UN Test Series C and does not propagate a rapid deflagration under confinement as defined in UN Test Series D and displays medium effect, low effect or no effect when heated under confinement as described in UN Test Series E; or
- xi. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a slow deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and does not undergo a thermal explosion under confinement as defined in UN Test Series G; or
- xii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays violent effect when heated under confinement as defined in UN Test Series E and does not undergo a thermal explosion under confinement as defined in UN Test Series G.

These classification criteria are summarised in Table 7.5. A substance is assigned to this category if it meets all of the criteria in any of the rows comprising the table.

Table 7.5: Criteria for allocation to organic peroxide category C

| | | | | | |
|------|--|--|--|---|---|
| i. | Listed in UN Recommendations as 5.2 Type B | | | | |
| ii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E |
| iii. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| iv. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E |

| | | | | | |
|-------|--|--|---|---|---|
| v. | Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| vi. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| vii. | Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| viii. | Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| ix. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G |
| x. | Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | |
| xi. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |
| xii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | |

d. Organic peroxide subclass 5.2 category D (equivalent to UN Type D)

A substance is classified as organic peroxide subclass 5.2 category D if:

- i. the substance is listed in the UN Model Regulations as having a classification or division of an organic peroxide (classification division 5.2) and is designated as Type D; or
- ii. the substance is an organic peroxide and propagates a partial detonation as described in UN Test Series A and propagates a slow deflagration or no deflagration as defined in UN Test Series C and displays medium effect, low effect or no effect when heated under confinement as defined UN Test Series E; or
- iii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and propagates a slow deflagration as defined in UN Test Series C and displays medium effect, low effect or no effect when heated under confinement as defined in UN Test Series E; or
- iv. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays medium effect when heated under confinement as defined in UN Test Series E.

These classification criteria are summarised in Table 7.6. A substance is assigned to this category if it meets all of the criteria in any of the rows comprising the table.

Table 7.6: Criteria for allocation to organic peroxide category D

| | | | |
|------|--|--|---|
| i. | Listed in UN Recommendations as 5.2 Type D | | |
| ii. | Propagates a partial detonation UN Test Series A | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E |
| iii. | Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E |
| (iv. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Medium effect when heated under defined confinement UN Test Series E |

e. Organic peroxide subclass 5.2 category e (equivalent to UN Type E)

A substance is classified as organic peroxide subclass 5.2 category E if:

- i. the substance is listed in the UN Model Regulations as having a classification or division of an organic peroxide (classification division 5.2) and is designated as Type E; or
- ii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays low effect or no effect when heated under confinement as defined in UN Test Series E and is not intended to be stored or transported in bulk; or

- iii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays low effect or no effect when heated under confinement as defined in UN Test Series E and is intended to be stored or transported in bulk and displays an explosive power of 'not low' as defined in UN Test Series F or no data is available for Test Series F.

These classification criteria are summarised in Table 7.7. A substance is assigned to this category if it meets all of the criteria in any of the rows comprising the table.

Table 7.7: Criteria for allocation to organic peroxide category E

| | | | | | |
|------|--|--|---|--|--|
| i. | Listed in UN Recommendation as 5.2 Type E | | | | |
| ii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Not intended to be stored or transported in bulk | |
| iii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power not low UN Test Series F |

- f. Organic peroxide subclass 5.2 category F (equivalent to UN Type F)

A substance is classified as organic peroxide subclass 5.2 category F if:

- i. the substance is listed in the UN Model Regulations as having a classification or division of an organic peroxide (classification division 5.2) and is designated as Type F; or
- ii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays low or no effect when heated under confinement as defined in UN Test Series E and, when tested for bulk containers, displays no explosive power as defined in UN Test Series F and displays a low effect when heated under confinement as defined in UN Test Series E; or
- iii. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays low or no effect when heated under confinement as defined in UN Test Series E and is intended to be stored or transported in bulk and displays low explosive power as defined UN Test Series F; or
- iv. the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays no effect when heated under confinement as defined in UN Test Series E including when it is assessed for bulk containers and it has no explosive power as defined in UN Test Series F and has either an SADT less than 60°C or a 50 kg quantity of the substance or, if the substance is a mixture that

contains a solvent or desensitising agent, the solvent or desensitising agent is not an organic liquid with a boiling point greater than or equal to 150°C.

These classification criteria are summarised in Table 7.8. A substance is assigned to this category if it meets all of the criteria in any of the rows comprising the table.

Table 7.8: Criteria for allocation to organic peroxide category F

| | | | | | | |
|------|--|--|---|--|--------------------------------------|---|
| i. | Listed in UN Recommendations as 5.2 Type F | | | | | |
| ii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | Low effect when heated under defined confinement UN Test Series E |
| iii. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power low UN Test Series F | |
| iv. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* |

Note

* The self-accelerating thermal decomposition temperature for a 50 kg quantity of the substance in Test Series H is less than 60°C; or, if the substance is a mixture containing a solvent or desensitising agent, that solvent or desensitising agent is not an organic liquid with a boiling point greater than or equal to 150°C.

g. Organic peroxide subclass 5.2 category G (equivalent to UN Type G)

A substance is classified as organic peroxide subclass 5.2 category G if the substance is an organic peroxide and does not propagate a detonation as defined in UN Test Series A and does not propagate a deflagration as defined in UN Test Series C and displays no effect when heated under confinement as defined in UN Test Series E, including when it is assessed for bulk containers, and it has no explosive power as defined in UN Test Series F and has an SADT greater than or equal to 60°C, and, if the

substance is a liquid mixture that contains a solvent or desensitising agent, that solvent or desensitising agent is an organic liquid that has a boiling point greater than or equal to 150°C .

These classification criteria are summarised in Table 7.9. A substance is assigned to this category if it meets all of the criteria in the row comprising the table.

Table 7.9: Criteria for allocation to organic peroxide category G

| | | | | | | |
|----|--|--|---|--|-------------------------------------|---|
| i. | Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* |
|----|--|--|---|--|-------------------------------------|---|

Note

* The self-accelerating thermal decomposition temperature from Test Series H is greater than or equal to 60°C for a 50 kg quantity of the substance, and ,if the substance is a liquid mixture containing a solvent or desensitising agent, that solvent or desensitising agent is an organic liquid with a boiling point greater than or equal to 150°C.

7.7. Discussion

Classification of category E, F, or G is provided only for substances that in response to Test Series A do not detonate, and in response to Test Series C do not deflagrate, and in response to Test Series E show either a low or no effect of heating under confinement. These classifications determine the degree to which the explosive power or heating under confinement may be related to quantities in excess of 50 kg. Where this data is not sought, classification of category D is sufficient.

That is, if a substance does not meet the criteria for a 5.2A, 5.2B, or 5.2C hazard classification, then a 5.2D hazard classification applies, unless sufficient data is provided that show the effects meet the criteria for hazard classification 5.2E, 5.2F, or 5.2G.

7.7.1. Multiple hazards classification

A substance may have more than one hazard classification where this is necessary to indicate different classifications according to different physical forms of the substance, if it is a solid, or different concentrations of the substance, if it is a mixture, or has been desensitised or otherwise chemically altered to modify its oxidising effects.

Where a substance is made up of a mixture of one or more substances above the threshold for an organic peroxide and one or more substances that are not, it may, on testing, fail to meet the threshold criteria for an organic peroxide. In this case, the substance mixture is classified as not hazardous based on the oxidising property of the mixture.

References

ASTM 2006. *Test Method for Determining Whether a Material is a Liquid or a Solid*, ASTM D4359-90. American Society for Testing and Materials, Philadelphia, PA.

ISO 1996. *Gases and Gas Mixtures: Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets*, ISO 10156:1996. International Organization for Standardization, Geneva.

United Nations 1994. *European Agreement Concerning the International Carriage of Dangerous Goods by Road*. United Nations, Geneva.

United Nations 1999a. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria*, 3rd revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 1999b. *Recommendations on the Transport of Dangerous Goods Model Regulations*, 11th revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 15th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

Appendix 7A: Organic peroxide classification: Subclassifications

The classification steps are complex although methodical. The decision chart in Figure 7.1 identifies these steps, which are also summarised in Table 7.2 and set out in detail in Tables 7A.2–7A.7. These indicate some subtle but important differences in how a substance may end up with an overall classification such as category B (7 paths) or category C (11 paths). Because of this, each of these separate paths is identified.

This subclassification is not expected to affect packaging or transport labelling controls. It is expected this will assist users to identify innate hazards and precautions that should be taken.

Each of the broad classifications category A to G is assigned a classification category suffix to indicate the response of the substance within the broad classification, to advise the hazardous effects of the substance. The classification suffixes and test result sequences are summarised in Table 7A.1, and shown for each classification category in Tables 7A.2–7A.7. While not part of the regulated classification scheme, these ‘subclassifications’ provide useful information for assessment purposes.

In the tables below, a substance is assigned to a category if it meets all of the criteria in any of the rows comprising the table relevant to that category.

Table 7A.1: Classification as category A (four possible combination of test results)

←Test response----->> Secondary classification

| | | | | |
|--|--|--|--|----------------|
| Propagates a detonation UN Test Series A | Propagates a detonation as confined UN Test Series B | | | A ₁ |
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | A ₂ |
| Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | | A ₃ |
| Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Propagates a rapid deflagration as confined UN Test Series D | | A ₄ |

Table 7A.2: Classification as category B (seven possible combinations of test results)

←Test response-----> Secondary classification

| | | | | | | |
|--|--|---|---|---|--|----------------|
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | B ₁ |
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | B ₂ |
| Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | B ₃ |
| Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | B ₄ |
| Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | B ₅ |
| Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | | B ₆ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Undergoes a thermal explosion as confined UN Test Series G | | | B ₇ |

Table 7A.3: Classification as category C (11 possible combinations of test results)

←Test response----->> Secondary classification

| | | | | | | |
|--|--|--|---|---|---|----------------|
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | C ₁ |
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | | C ₂ |
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | | C ₃ |
| Propagates a detonation UN Test Series A | Does not propagate a detonation as confined UN Test Series B | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E | | | C ₄ |
| Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | | C ₅ |
| Propagates a partial detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | | | C ₆ |
| Propagates a partial detonation UN Test Series A | Propagates a slow or no deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series | Does not undergo a thermal explosion as confined UN | | | C ₇ |

| | | E | Test Series G | | | |
|--|--|---|---|---|--|-----------------|
| Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | | C ₈ |
| Does not propagate a detonation UN Test Series A | Propagates a rapid deflagration UN Test Series C | Does not propagate a rapid deflagration as confined UN Test Series D | Medium, low or no effect when heated under defined confinement UN Test Series E | | | C ₉ |
| Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | | | C ₁₀ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Violent effect when heated under defined confinement UN Test Series E | Does not undergo a thermal explosion as confined UN Test Series G | | | C ₁₁ |

Table 7A.4: Classification as category D (three possible combinations of test results)

← Test response----->> Secondary classification

| | | | |
|--|--|---|----------------|
| Propagates a partial detonation UN Test Series A | Propagates a slow deflagration or no deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E | D ₁ |
| Does not propagate a detonation UN Test Series A | Propagates a slow deflagration UN Test Series C | Medium, low or no effect when heated under defined confinement UN Test Series E | D ₂ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Medium effect when heated under defined confinement UN Test Series E | D ₃ |

Table 7A.5: Classification as category E (two possible combinations of test results)

←Test response----->> Secondary classification

| | | | | | |
|--|--|---|--|--|----------------|
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Not intended to be stored or transported in bulk | | E ₁ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power not low UN Test Series F | E ₂ |

Table 7A.6: Classification as category F (three possible combinations of test results)

←Test response----->> Secondary classification

| | | | | | | |
|--|--|---|--|--------------------------------------|---|----------------|
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | Low effect when heated under defined confinement UN Test Series E | F ₁ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | Explosive power low UN Test Series F | | F ₂ |
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* | F ₃ |

Note

* The self-accelerating thermal decomposition temperature for a 50 kg quantity of the substance in Test Series H is less than 60°C, or, if the substance is a mixture containing a solvent or desensitising agent, that solvent or desensitising agent is not an organic liquid with a boiling point greater than or equal to 150°C

Table 7A.7: Classification as category G (one possible combination of test results)

| ← Test response----->> Secondary classification | | | | | | |
|--|--|---|--|-------------------------------------|---|----------------|
| Does not propagate a detonation UN Test Series A | Does not propagate a deflagration UN Test Series C | Low or no effect when heated under defined confinement UN Test Series E | Intended to be stored or transported in bulk | No explosive power UN Test Series F | No effect when heated under defined confinement UN Test Series E* | G ₁ |

Note

* The self-accelerating thermal decomposition temperature from Test Series H is greater than or equal to 60°C for a 50 kg quantity of the substance, and , if the substance is a liquid mixture containing a solvent or desensitising agent, that solvent or desensitising agent is an organic liquid with a boiling point greater than or equal to 150°C

8. Corrosive Properties – Class 8

8.1. Introduction

The three subclasses under the corrosive property defined in the Hazardous Substances and New Organisms Act 1996 (HSNO Act) are:

- subclass 8.1 – substances corrosive to metals (see section 8.2);
- subclass 8.2 – substance corrosive to skin (see chapter 10 [below](#)); and
- subclass 8.3 – substances corrosive to eyes (see chapter 11 [below](#)).

The two subclasses that deal with corrosion of skin and eyes are addressed in chapters 10 and 11 respectively, because they are an extension of the skin and eye irritancy subclasses.

8.2. Corrosive to metals – subclass 8.1

8.2.1. Corrosive to metals hazard and classification criteria

Corrosive to metals threshold criteria

Schedule 5 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 Minimum degrees of hazard

A substance with corrosive properties is not hazardous for the purposes of the Act unless—

- (a) the substance corrodes, at a rate exceeding 6.25 millimetres per year at a test temperature of 55°C,—*
- steel type P235 (ISO 9328 (II): 1991); or*
 - steel type SAE 1020 (Society of Automotive Engineers); or*
 - non-clad aluminium types SAE 7075-T6 or AZ5GU-T6.*

Corrosive to metals classification criteria

Schedule 5 to the Hazardous Substances (Classification) Regulations 2001 identifies one classification subclass for substances that are corrosive to metals (subclass 8.1).

- Subclass 8.1 – substances that are corrosive to metals

A subclass 8.1 classification and the subsequent category apply to any substance that meets the following criteria.

- a. A substance that corrodes steel type P235 (ISO 9328 (II):1991), or steel type SAE 1020, or non-clad aluminium types SAE 7075-T6 or AZ5GU-T6 at a rate exceeding 6.25 millimetres per year at a test temperature of 55°C.

8.2.2. Acceptable test methodology

The test methodology deemed to meet the requirements for testing the corrosion to metals threshold is Standard Practice for Laboratory Immersion Corrosion Testing of Metals (ASTM, 2004).

References

ASTM 2004. *Standard Practice for Laboratory Immersion Corrosion Testing of Metals*, Standard G31-72. American Society for Testing and Materials, Philadelphia, PA

9. Introduction to Toxicity – Class 6

9.1. Introduction

The eight subclasses under the toxicity property in the Hazardous Substances and New Organisms Act 1996 (HSNO Act) are:

- subclass 6.1 – substances that are acutely toxic (see Chapter 10 [below](#))
- subclass 6.3 – substances that are skin irritants (see Chapter 11 [below](#))
- subclass 6.4 – substances that are eye irritants (see Chapter 12 [below](#))
- subclass 6.5 – substances that are sensitisers (see Chapter 13 [below](#))
- subclass 6.6 – substances that are mutagenic (see Chapter 14 [below](#))
- subclass 6.7 – substances that are carcinogenic (see Chapter 15 [below](#))
- subclass 6.8 – substances that are reproductive or developmental toxicants (see Chapter 16 [below](#))
- and subclass 6.9 – substances that are specific target organ toxicants (see Chapter 17 [below](#))

Note that class 6.2 (infectious substances) is not included in the above list as those substances are not captured under the HSNO Act.

Two subclasses (relevant to toxicity) are also defined under the corrosive property in the HSNO Act. They are:

- subclass 8.2 – substances that are corrosive to skin (see Chapter 11 [below](#));
- and subclass 8.3 – substances that are corrosive to eyes (see Chapter 12 [below](#)).

9.2. Classification of substances

In each of the following sections, guidance is provided on how to classify a substance for each of the 10 subclasses. Each section outlines the key considerations required to assign a classification to a substance and acceptable test methods for deriving data for classification purposes. Additional guidance is provided where there may be difficulties in interpretation of the regulations or more complex types of data.

9.3. Classification of mixtures: generic guidance

Once a substance triggers a threshold, it is then classified. While this is relatively straightforward for single substances, substances as mixtures are more complex.

The general process for classification of toxicity hazards is as follows.

- When toxicity test data are available for the complete substance (or mixture) then classification is based on the test results.
- When test data are not available for the mixture itself, then bridging principles should be considered to see whether they permit classification of the mixture.

- When test data are not available for the mixture (for example, formulation test data), and the available information is not sufficient to allow application of the bridging principles, the method described in each chapter for estimating the hazards of the mixture is based on information on the components, which is used to derive the classification of the mixture.

See the specific toxicity chapters for more details.

9.3.1. Synergistic and antagonistic effects

If there is information about possible synergistic effects that may enhance the toxicity of the substance as a mixture, this must be considered when classifying the substance.

If there is information that antagonistic effects may occur such that the mixture classification is lower than indicated from the calculated value, this should be noted.

9.4. Data requirements and data quality

9.4.1. Minimum data sets

The HSNO Act covers many types of substances with varying degrees of hazardous properties. These substances also have different uses and circumstances of use. The risk associated with a hazardous substance is a function of the degrees of hazard of the substance and the level and duration of exposure to these hazards.

Different types of hazardous substances present different levels of risk, so require different types and levels of information to be considered in applications for approval. Different levels of information could relate to the quantity, extent, or degree of detail of information, as applicable to the substance and type of approval involved.

Further guidance on the likely information requirements (for example, minimum data sets) for applications for approval of hazardous substances is in the user guides to the HSNO Act application forms.

9.4.2. Data quality

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

9.4.3. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under each subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development Test Guidelines or equivalent, according to the principles of Good Laboratory Practice (GLP). When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

9.4.4. Absence of measured data

The EPA recognises that measured data may not be available for all hazard endpoints for all substances. The Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 also acknowledges that:

data includes values that are directly measured, calculated, or estimated for any of the measures given.

Therefore, while it should be noted that where no measured data are available, classification of a substance into a HSNO Act hazard classification category can still occur, using a weight-of-evidence approach that acknowledges all other data that is available on the substance or closely related substances. If this approach is used, any assumptions made and the weight-of-evidence approach for hazard classification should be clearly documented.

If there are no measured (that is, direct) data or indirect data on the substance, the substance cannot be assigned a hazard classification.

9.5. Data sources

The information sources in Tables 9.1–9.20 (toxicity) and 9.21 (physico-chemical) are provided as a starting point only, they are not exhaustive. As noted in section 1.3 in chapter 1, the quality of data is highly variable within and between various sources. It is the user's responsibility to ensure that the data used for classification meet the criteria of reliability, relevance, and adequacy.

Some of the sources listed in the tables may require a subscription, but most are free.

See also Tables 18.1 and 18.2 in chapter 18 [below](#) for data sources for ecotoxicity and environmental fate.

Table 9.1: Information sources for toxicity – environmental organisations: United States

| Information source | URL |
|--|---|
| Agency for Toxic Substances and Disease Registry (ATSDR) | http://www.atsdr.cdc.gov |
| American Chemistry Council (ACC) | http://www.americanchemistry.com/s_acc/index.asp |
| American Industrial Hygiene Association (AIHA) | http://www.aiha.org |
| California Office for Environmental Health Hazard Assessment (OEHHA) | http://www.oehha.ca.gov |
| Centers for Disease Control and Prevention (CDC)/Department of Health and Human Services (HHS) | http://www.cdc.gov |

| | |
|--|---|
| National Cancer Institute (NCI)/National Institutes of Health (NIH) | http://www.cancer.gov |
| National Institute for Occupational Safety and Health (NIOSH) | http://www.cdc.gov/niosh/homepage.html |
| National Toxicology Programme (NTP)/Department of Health and Human Services | http://ntp-server.niehs.nih.gov |
| United States (US) Department of Labor/Occupational Safety & Health Administration | http://www.osha.gov |
| US Department of Transportation/Office of Hazardous Materials Safety | http://hazmat.dot.gov/index.html |
| US Environmental Protection Agency (USEPA) | http://www.epa.gov |

Note: These URLs may not be the only routes to the information.

Table 9.2: Information sources for toxicity – environmental organisations: European Union

| Information source | URL |
|---|--|
| European Agency for Safety and Health at Work | http://osha.eu.int/OSHA |
| European Agency for Safety and Health at Work/Dangerous Substances | http://europe.osha.eu.int/good_practice/risks/dangerous_substances |
| European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) | http://www.ecetoc.org/ |
| European Chemical Industry Council (CEFIC) | http://www.cefic.org |
| European Chemicals Agency (ECHA) | http://echa.europa.eu/ |
| European Environment Agency | http://themes.eea.europa.eu |
| European Food Safety Authority (EFSA) | http://www.efsa.europa.eu/ |
| European Union (EU) Dangerous Goods (DG) Sanco (health and consumer protection) | http://ec.europa.eu/comm/dgs/health_consumer/index_en.htm |
| EU research DG | http://ec.europa.eu/dgs/research/index_en.html |
| Joint Research Centre (JRC) | http://irmm.jrc.ec.europa.eu/ |
| Scientific Institute of Public Health | www.pasteur-international.org/.../scientific-institute-of-public-health |

Note: These URLs may not be the only routes to the information.

Table 9.3: Information sources for toxicity – environmental organisations: International

| Information source | URL |
|--|---|
| Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) | http://www.ospar.org/ |

| | |
|---|---|
| Convention) | |
| Food and Agriculture Organization of the United Nations (FAO) | http://www.fao.org |
| Intergovernmental Forum on Chemical Safety (IFCS) | http://www.who.int/ifcs/en |
| International Labor Organization (ILO) | http://www.ilo.org/public/english |
| International Programme on Chemical Safety (IPCS/WHO) | http://www.who.int/ipcs/en/ |
| International Union of Pure and Applied Chemistry (IUPAC) | http://www.iupac.org/index_to.html |
| Organisation for Economic Co-operation and Development (OECD) | http://www.oecd.org/home |
| Stockholm Convention on Persistent Organic Pollutants (POPs) | http://www.pops.int/documents/press/EIF |
| United Nations Economic Commission for Europe (UNECE) – Dangerous Goods | http://www.unece.org/trans/danger/danger.htm |
| UN Environment Programme (UNEP) | http://www.unep.org |
| UNEP Chemicals | http://www.chem.unep.ch |
| UN Institute for Training and Research (UNITAR) | http://www.unitar.org |
| UNITAR/Chemicals, Waste and Environmental Governance | http://www.unitar.org/cwg/index.html |
| World Health Organization (WHO) | http://www.who.int/en |

Note: These URLs may not be the only routes to the information.

Table 9.4: Information sources for toxicity – environmental organisations: Australia

| Information source | URL |
|---|---|
| Australian Pesticides and Veterinary Medicines Authority (APVMA) | http://www.apvma.gov.au |
| Environmental Protection and Heritage Council | www.ephc.gov.au/ |
| National Industrial Chemicals Notification and Assessment Scheme (NICNAS) | http://www.nicnas.gov.au/default.asp |
| Occupational Health and Safety (OHS) | http://www.business.gov.au/BusinessTopics/Occupationalhealthandsafety/Pages/default.aspx |

Note: These URLs may not be the only routes to the information.

Table 9.5: Information sources for toxicity – environmental organisations: United Kingdom

| Information source | URL |
|--|---|
| Health and Safety Commission (HSC) Advisory Committee on Toxic Substances (ACTS) | http://www.hse.gov.uk/aboutus/meetings/iacs/acts/ |
| Health and Safety Executive (HSE) | http://www.hse.gov.uk/index.htm |
| Chemical Regulation Directorate | http://www.pesticides.gov.uk |
| The Royal Society | http://www.royalsoc.ac.uk |

Note: These URLs may not be the only routes to the information.

Table 9.6: Information sources for toxicity – chemicals databases

| Information source | URL |
|--|---|
| Australian Inventory of Chemical Substances (AICS) | http://www.nicnas.gov.au/Industry/AICS/Search.asp |
| Australian Hazardous Substances Register | http://www.nohsc.gov.au/applications/hsis/searchhs.aspx |
| ChemFinder.com | http://chemfinder.cambridgesoft.com |
| ChemIndustry.com | http://www.chemindustry.com/index.html |
| Chemical toxicity database – Japan | http://wwwdb.mhlw.go.jp/ginc/html/db1.html |
| Chemicals database Japan | www.safe.nite.go.jp/jcheck/english/ |
| Chemicals profiles scorecard | http://www.scorecard.org/chemical-profiles/index.tcl |
| Chemicals Screening Information Data Sets (SIDs) | http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html |
| ChemIDplus | http://chem.sis.nlm.nih.gov/chemidplus |
| ClassLab database | http://echa.europa.eu/ |
| European Chemical Substances Information System (ESIS) | http://echa.europa.eu/ |
| European Union Risk Assessment reports online | http://echa.europa.eu/ |
| International Chemical Safety Cards (ICSCs) – International Programme on Chemical Safety | http://www.cdc.gov/niosh/ipcs/icstart.html |
| International Programme on Chemical Safety (IPCS) INCHEM – search across all collections | http://www.inchem.org/pages/search.html |
| IPCS INCHEM/chemical substances information from intergovernmental organisations | http://www.inchem.org |
| IPCS INTOX databank | http://www.intox.org/databank/index.htm |
| N-class database on environmental hazard | http://apps.kemi.se/nclass/default.asp |

| | |
|---|---|
| classifications | |
| National Institute for Occupational Safety and Health (NIOSH) databases | http://www.cdc.gov/niosh/topics/chemical-safety/default.html |
| NIOSH Pocket Guide to Chemical Hazards | http://www.cdc.gov/niosh/npg |
| Nordic food additives database | http://www.foodcomp.dk/foodadd/GeneralInformation.html |
| NZ TOXINS database | http://www.toxinz.com |
| Organisation for Economic Co-operation and Development (OECD) database on use and release of industrial chemicals | http://webdomino1.oecd.org/ehs/urchem.nsf |
| OECD HPV database | http://cs3-hq.oecd.org/scripts/hpv/index.asp |
| Office of Pesticide Programs - pesticide ecotoxicity database | http://www.ipmcenters.org/Ecotox/DataAccess.cfm |
| Pesticides Action Network North America (PAN Pesticides) databank | http://www.pesticideinfo.org/Index.html |
| Solvents database | http://solvdb.ncms.org/solvdb.htm |
| TOXNET/Toxicology Data Network | http://toxnet.nlm.nih.gov/index.html |
| United Nations Environment Programme (UNEP) legal file (IRPTC) | http://dbserver.irptc.unep.ch:8887/irptc/owa/lg.search_for?iscas=&iarea=&isubject=&ispec= |
| United States Environmental Protection Agency (USEPA)/ECOTOX database | http://mountain.epa.gov/ecotox http://cfpub.epa.gov/ecotox |
| USEPA Envirofacts Master Chemicals Integrator | http://www.epa.gov/enviro/html/emci/emci_query.html |

Note: These URLs may not be the only routes to the information.

Table 9.7: Information sources for toxicity – chemicals lists

| Information source | URL |
|---|---|
| Agency for Toxic Substances and Disease Registry (ATSDR) – Medical Management Guidelines for Acute Chemical Exposures | http://www.atsdr.cdc.gov/mmg.html |
| ATSDR – toxicology profiles | http://www.atsdr.cdc.gov/toxpro2.html |
| Australian National Pollutant Inventory – substance information | http://www.npi.gov.au/cgi-bin/npisubstance.pl |
| Australian National Pollutant Inventory – chemicals information | http://www.npi.gov.au/cgi-bin/npisubstance.pl |
| Chemicals Profile Scorecard | http://www.scorecard.org/chemical-profiles/index.tcl |
| European Union Risk Assessment reports online | http://ecb.jrc.it/esis-pgm/orats_IS_reponse.php?TRI=FIC_DRAFT&FROM |

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|---|---|
| | =FORMULAIRE |
| International Chemical Safety Cards (ICSC) cards/International Labor Organization (ILO) | http://www.ilo.org/public/english/protection/safework/cis/products/icsc/dtasht/index.htm |
| International Programme on Chemical Safety (IPCS) INTOX databank | http://www.intox.org/databank/pages/chemical.html |
| Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) – food additives – evaluation reports | http://www.inchem.org/pages/jecfa.html |
| National Institute for Occupational Safety and Health (NIOSH) 1988 Occupational Safety & Health Administration (OSHA) Permissible Exposure Limits (PEL) project | http://www.cdc.gov/niosh/pel88/npelname.html |
| NIOSH - occupational health guidelines for chemicals hazards | http://www.cdc.gov/niosh/81-123.html |
| NIOSH - Immediately Dangerous to Life and Health (IDLH) | http://www.cdc.gov/niosh/idlh/intridl4.html |
| Nordic food additives database | http://www.foodcomp.dk/foodadd/GeneralInformation.html |
| Organisation for Economic Co-operation and Development (OECD) Screening information data sets (SIDs) in International Uniform Chemical Information Database (IUCLID) | www.oecd.org/ |
| California Office for Environmental Health Hazard Assessment (OEHHA) – acute recommended exposure limits (RELs) air | http://www.oehha.ca.gov/air/acute_rels |
| OEHHA – chronic RELs – air | http://www.oehha.ca.gov/air/chronic_rels/index.html |
| Right to Know, New Jersey Department of Health and Senior Services, Hazardous Substances Factsheets | http://web.doh.state.nj.us/rtkhsfs/indexfs.aspx |
| UNEP OECD SIDs | http://www.chem.unep.ch/irptc/sids/OECDSEIDS/indexcasnumb.htm |
| United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPT) – Chemicals factsheet | http://www.epa.gov/docs/opptintr/chemfact/index.html |
| World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) – chemicals assessment – Environmental Health Criteria, Concise International Chemical Assessment Documents | http://www.who.int/ipcs/assessment/en |

Note: These URLs may not be the only routes to the information.

Table 9.8: Information sources for toxicity – medical data sources

| Information source | URL |
|---|---|
| Agency for Toxic Substances and Disease Registry (ATSDR) Medical Management Guidelines for Acute Exposure | http://www.atsdr.cdc.gov/mmg.html |
| E-medicine | http://www.emedicine.com/specialties.htm |
| Free medical journals online list | http://www.gfmer.ch/Medical_journals/Free_medical.php |
| Medline Plus med glossary | http://www.nlm.nih.gov/medlineplus/plusdictionary.html |
| MEDLINE/PubMed | http://www.ncbi.nlm.nih.gov/entrez/query.fcgi |
| NZ TOXINS database | http://www.toxinz.com |

Note: These URLs may not be the only routes to the information.

Table 9.9: Information sources for toxicity – United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

| Information source | URL |
|--|---|
| GHS first revision (2011) | http://www.unece.org/index.php?id=24056 |
| Official GHS website/United Nations Economic Commission for Europe (UNECE) | http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html |
| UNECE – Transport of Dangerous Goods (TDG)/GHS | http://www.unece.org/trans/danger/danger.htm |

Note: These URLs may not be the only routes to the information.

Table 9.10: Information sources for toxicity – literature sources, reports, and libraries

| Information source | URL |
|---|---|
| Centers for Disease Control and Prevention (CDC) chemicals reports | http://www.cdc.gov/exposurereport/chemicallist.htm |
| <i>Chemical & Engineering News</i> : Journal of the American Chemical Society | http://pubs.acs.org/cen/index.html http://pubs.acs.org/cen/government/84/8409gov1b.html |
| <i>Environmental Health Perspectives</i> (EHP) | http://www.ehponline.org |
| Environmental health, public health, and toxicology journals | http://www.gfmer.ch/Medical_journals/Environmental_and_occupational_health_sciences_toxicology.htm |
| EPA publications | http://www.epa.govt.nz/resources |
| Free medical journals online list | http://www.gfmer.ch/Medical_journals/Free_medical.php |
| Free medical journals site | http://www.freemedicaljournals.com |

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|--|---|
| Health Effects Institute (HEI) research reports | http://www.healtheffects.org/pubs-research.htm |
| Human and Environmental Risk Assessment (HERA) reports | http://www.heraproject.com/RiskAssessment.cfm?SUBID=38 |
| HighWire journals | http://www.pnas.org/searchall |
| HighWire search | http://highwire.stanford.edu/cgi/search |
| <i>Human and Ecological Risk Assessment</i> – journal | http://ejournals.ebsco.com/journal2.asp?journalid=105863&show=105863_11 |
| INGENTA Home | http://www.ingentaconnect.com/;jsessionid=cvkd0r6jkg81.victoria |
| Institute of Environment and Health (Cranfield University, United Kingdom) | http://www.silsoe.cranfield.ac.uk/ieh/publications/seriesorder.html |
| Look Smart Findarticles | http://www.findarticles.com |
| MEDLINE/PubMed | http://www.ncbi.nlm.nih.gov/entrez/query.fcgi |
| National Academies Press | http://www.nap.edu/index.html |
| National Library of Medicine (NLM) Gateway search | http://gateway.nlm.nih.gov/gw/Cmd |
| Patty's toxicology | http://www.mrw.interscience.wiley.com/pattys/pattys_search_fs.html |
| Public Health Resources on the Internet | http://www.lib.berkeley.edu/PUBL/internet.html |
| PubMed Central home | http://pubmedcentral.com |
| PubMed Central journals list | http://www.pubmedcentral.nih.gov/front-page/fp.fcgi?cmd=full_view |
| Regulatory toxicology and pharmacology | http://www.ingentaconnect.com/content/ap/rt |
| <i>Science Daily</i> – an internet online magazine from the US | http://www.sciencedaily.com |
| Ullmann's Encyclopedia of Industrial Chemistry | http://www.mrw.interscience.wiley.com/ueic/ueic_search_fs.html |
| United States PubMed Central literature search | http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pmc |

Note: These URLs may not be the only routes to the information.

Table 9.11: Information sources for toxicity – pesticide information

| Information source | URL |
|--|---|
| Agriculture Canada | http://www.agr.gc.ca/cb/apf/index_e.php |
| Australian Pesticides and Veterinary Medicines Authority (APVMA) reports | http://www.apvma.gov.au/ |

| | |
|---|---|
| Canada Pest Management Regulatory reports | http://www.pmr-arla.gc.ca/english/pubs/pubs-e.html |
| ClassLab database | http://echa.europa.eu/ |
| Dermal exposure from liquid contamination – UK Health and Safety Executive (HSE) | http://www.hse.gov.uk/research/rrhtm/rr004.htm |
| Directory of microbial pesticides for agricultural crops in Organisation for Economic Co-operation and Development (OECD) countries | http://www.agr.gc.ca/env/pdf/cat_e.pdf |
| European Chemical Substances Information System (ESIS) | http://echa.europa.eu/ |
| European Food Safety Authority (EFSA) – existing active substances draft review reports | http://www.efsa.europa.eu/DAR/displaySubstance.cfm?provision=1 |
| European Union (EU) International Portal on food safety, animal and plant health | http://www.ipfsaph.org/id/cthttpwwwfaoorgaosipsaphinformatiionsourcejefca?language=en |
| EU plant protection | http://ec.europa.eu/food/plant/protection/evaluation/framework_en.htm |
| EU plant protection documents and reports for implementation of directive 91/414/EEC | http://ec.europa.eu/food/plant/protection/resources/publications_en.htm |
| EU plant protection products – existing active substances decision and review reports | http://ec.europa.eu/food/plant/protection/evaluation/exist_subs_rep_en.htm |
| EU plant protection products –new active substances decision and review reports | http://ec.europa.eu/food/plant/protection/evaluation/new_subs_rep_en.htm |
| Evaluation reports on pesticides – United Kingdom | http://www.pesticides.gov.uk/psd_evaluation_all.asp |
| International Programme on Chemical Safety (IPCS) INCHEM/ – search across all collections | http://www.inchem.org/pages/search.html |
| Japan Chemicals Evaluation and Research Institute (CERI) | http://www.cerij.or.jp/ceri_en/index_e4.shtml |
| New Zealand Food Safety Authority (NZFSA) Agricultural Compounds and Veterinary Medicines (ACVM) database | http://www.nzfsa.govt.nz/acvm/registers-lists/acvm-register/index.htm |
| OECD guidance documents on pesticide registration | http://www.oecd.org/document/48/0,2340,en_2649_201185_2085104_1_1_1_1,00.html |
| OPP pesticide ecotoxicity database | http://www.ipmcenters.org/Ecotox/DataAccess.cfm |
| Pesticides Safety Directorate (PSD) evaluation documents | http://www.pesticides.gov.uk/publications.asp?id=202 |
| Toxicology Summaries –USEPA California | http://www.cdpr.ca.gov/docs/toxsums/toxsumlist.htm |
| US Pesticide Chemicals in food tolerances | http://www.access.gpo.gov/nara/cfr/waisidx_03/40cfr180_03.html |
| United States Environmental Protection Agency | http://www.epa.gov/pesticides/biopesticides/ingredient |

| | |
|--|---|
| (USEPA) biopesticide active ingredients factsheets | s/index.htm |
| USEPA factsheets on new actives | http://www.epa.gov/opprd001/factsheets/factsht2.htm |
| USEPA high production volume (HPV) database | http://cfpub.epa.gov/hpv-s |
| USEPA pesticide fate database | http://cfpub.epa.gov/pfate/Home.cfm |
| USEPA pesticides Reregistration Eligibility Documents (REDs) | http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg |
| USEPA pesticides – science & policies: human studies | http://www.epa.gov/oppfead1/guidance/human-test.htm |

Note: These URLs may not be the only routes to the information.

Table 9.12: Information sources for toxicity – veterinary medicines

| Information source | URL |
|---|---|
| European Medicines Agency (EMA) veterinary medicines reports | http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fmedicines%2Flanding%2Fvet_mrl_search.jsp&menu=menus%2Fmedicines%2Fmedicines.jsp&mid=WC0b01ac058008d7ad&docType=epmar&searchkwByEnter=false&alreadyLoaded=true&startLetter=L&keyword=Enter+keywords&jsenabled=true |
| European Union (EU) guidelines on veterinary medicines | http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev7.htm |
| EU international portal on food safety, animal and plant health | http://www.ipfsaph.org/id/cthttpwwwfaoorgaosipfsaphinformatiionsourcejecfa?language=en |
| Merck veterinary medicines manual | http://www.merckvetmanual.com/mvm/index.jsp |
| United States Food and drug Administration (USFDA) – acceptable daily intakes (ADIs) veterinary medicines | http://www.washingtonwatchdog.org/documents/cfr/title21/part556.html |
| VetGate – animal health United Kingdom | http://vetgate.ac.uk/browse/cabi/0d78e5779bb120dfd6eed338b3e08ff2.html |

Note: These URLs may not be the only routes to the information.

Table 9.13: Information sources for toxicity – cosmetics

| Information source | URL |
|--|---|
| European Union (EU) consolidated version of cosmetics directive 76/768/EEC | http://ec.europa.eu/enterprise/cosmetics/html/consolidated_dir.htm |
| EU scientific committee for cosmetic products | http://ec.europa.eu/health/ph_risk/committees/sccp/sccp_opinions_en.htm |

Note: These URLs may not be the only routes to the information.

Table 9.14: Information sources for toxicity – chemicals risk assessment

| Information source | URL |
|---|--|
| European Union (EU) Risk assessments for existing chemicals | http://ecb.jrc.it/existing-chemicals |
| EU RA reports | http://ecb.jrc.it/esis-pgm/orats_IS_reponse.php?TRI=FIC_DRAFT&FROM=FORMULAIRE |
| EU testing methods | http://ecb.jrc.it/testing-methods |
| Human and Environmental Risk Assessment reports | http://www.heraproject.com/RiskAssessment.cfm?SUBID=38 |
| Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) | http://iccvam.niehs.nih.gov/home.htm http://iccvam.niehs.nih.gov/agencies/regs.htm |
| Organisation for Economic Co-operation and Development database on chemicals risk assessment models | http://webdomino1.oecd.org/comnet/env/models.nsf |
| ToxTutor | http://sis.nlm.nih.gov/enviro/toxtutor.html |
| United Kingdom (UK) Health and Safety Executive (HSE) dermal exposure from liquid contamination | http://www.hse.gov.uk/research/rrhtm/rr004.htm |
| UK Institute of Environment and Health | http://www.silsoe.cranfield.ac.uk/ieh |
| United States Environmental Protection Agency (USEPA) human health toxicity (hazard and dose-response) | http://www.epa.gov/oswer/riskassessment/human_health_toxicity.htm |
| USEPA environmental training | http://www.epa.gov/air/oaqps/eog/index.html |
| USEPA risk assessment guidelines (Health risk assessments of chemical mixtures) | http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=20533 |
| USEPA superfund risk assessment | http://www.epa.gov/superfund |
| USEPA test guidelines | http://www.epa.gov/pesticides/science/guidelines.htm |

Note: These URLs may not be the only routes to the information.

Table 9.15: Information sources for toxicity – carcinogens

| Information source | URL |
|--|---|
| International Agency for Research on Cancer (IARC) | http://www.iarc.fr/index.html |
| United States National Toxicology Programme (NTP) 11th report on carcinogens | http://ntp.niehs.nih.gov/?objectid=03C9B512-ACF8-C1F3-ADBA53CAE848F635 |

Note: These URLs may not be the only routes to the information.

Table 9.16: Information sources for toxicity – nanoparticles

| Information source | URL |
|-----------------------------------|---|
| The Royal Society – nanoparticles | http://www.nanotec.org.uk/finalReport.htm |

Note: These URLs may not be the only routes to the information.

Table 9.17: Information sources for toxicity – chemical safety

| Information source | URL |
|---|---|
| Australian Occupational Health and Safety (OSH) reports | http://australia.gov.au/topics/employment-and-workplace/ohs-workplace-health-and-safety |
| ChemGuide – helping to understand chemistry | http://www.chemguide.co.uk/index.html#top |
| Chemicals screening information data sets (SIDs) | http://www.chem.unep.ch/irptc/sids/OECDsids/sidspub.html |
| Danish Environmental Protection Agency (EPA) health costs chemicals | http://www.mst.dk/homepage/default.asp?Sub=http://www.mst.dk/udgiv/publications/2004/87-7614-295-7/html/default_eng.htm |
| International Chemicals Control Tool Kit – International Labor Organization (ILO) | http://www.ilo.org/public/english/protection/safework/control_banding/toolkit/main_guide.pdf |
| Organisation for Economic Co-operation and Development (OECD) chemical safety topic reports | http://www.oecd.org/topic/0,2686,en_2649_34365_1_1_1_1_37465,00.html |
| OECD chemical assessments | http://www.oecd.org/document/63/0,2340,en_2649_34373_1897983_1_1_1_1,00.html |
| OECD chemical-testing guidelines | http://www.oecd.org/document/30/0,2340,en_2649_34377_1916638_1_1_1_1,00.html |

Note: These URLs may not be the only routes to the information.

Table 9.18: Information sources for toxicity – glossaries

| Information source | URL |
|--|---|
| Agency for Toxic Substances and Disease Registry glossary | http://www.atsdr.cdc.gov/glossary.html |
| Medline Plus Med glossary | http://www.nlm.nih.gov/medlineplus/medlineplusdictionary.html |
| United States Environmental Protection Agency (USEPA) Terms of Environment | http://www.epa.gov/OCEPAterms |

Note: These URLs may not be the only routes to the information.

Table 9.19: Information sources for toxicity – occupational exposure

| Information source | URL |
|--------------------|-----|
|--------------------|-----|

| | |
|---|---|
| European Agency for Safety and Health at Work occupational exposure limits | http://europe.osha.eu.int/good_practice/risks/ds/oel |
| United States (US) National Institute for Occupational Safety and Health (NIOSH) /Immediately Dangerous to Life and Health (IDLH) | http://www.cdc.gov/niosh/idlh/intridl4.html |
| US NIOSH occupational health guidelines | http://www.cdc.gov/niosh/81-123.html |
| US NIOSH Occupational Safety & Health Administration (OSHA) Permissible Exposure Limits (PELs) | http://www.cdc.gov/niosh/pel88/npelname.html |

Note: These URLs may not be the only routes to the information.

Table 9.20: Information sources for toxicity – material safety data sheets (MSDS)

| Information source | URL |
|--|---|
| MSDS hyper-glossary | http://www.ilpi.com/msds/ref/index.html |
| Cheminfo MSDS | http://intox.org/databank/documents/chemical/lactacid/cie794.htm |
| SIRI (Safety Information Resources Inc) MSDS | http://hazard.com/msds/index.php |

Note: These URLs may not be the only routes to the information.

Table 9.21: Information sources – physico-chemical properties

| Information source | URL |
|---|---|
| Beilstein handbook | http://www.beilstein-online.de/frameset.htm |
| Biodegradation and bioaccumulation database on existing chemicals, Japan (METI) | http://www.safe.nite.go.jp/english/db.html |
| ChemFinder | http://chemfinder.cambridgesoft.com |
| Chemical Evaluation Search and Retrieval System (CESARS) | http://www.ccohs.ca/products/databases/cesars.html |
| Hazardous Substances Data Bank (HSDB) | http://toxnet.nlm.nih.gov |
| ISHOW | http://www.nisc.com/cis |
| International Uniform Chemical Information Database (IUCLID) (part of European Chemical Substances Information System (ESIS)) | http://echa.europa.eu/ |
| International Union of Pure and Applied Chemistry (IUPAC) solubility data series | http://www.iupac.org/publications/sds |
| Merck Index | http://library.dialog.com/bluesheets/html/bl0304.html |
| Organisation for Economic Co-operation and Development (OECD) screening information data sets | http://cs3-hq.oecd.org/scripts/hpv |

| | |
|---|---|
| (SIDs) | |
| Searchable physics information notices (SPIN) | http://www.scitation.aip.org/spinweb |
| Syracuse Research Corporation (SRC) environmental fate database (BIOLOG, BIODEG, CHEMFATE, DATALOG) | http://www.syrres.com/esc/efdb.htm |
| SRC PhysProp | http://www.syrres.com/esc/default1.htm |

Note: These URLs may not be the only routes to the information.

9.6. Definitions

The following terms and definitions are particularly relevant to chapters 9–17 about toxicity.

| Term | Definition |
|----------------------|--|
| acute toxicity | Adverse effects occurring following the oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of four hours. See United Nations (2007). |
| aspiration | The entry of a liquid or solid chemical product directly through the oral or nasal cavity, or indirectly from vomiting, into the trachea and lower respiratory system. Aspiration toxicity includes severe acute effects such as chemical pneumonia, varying degrees of pulmonary injury or death following aspiration. |
| ATE | acute toxicity estimates |
| bw | bodyweight |
| carcinogen | A chemical substance or mixture of chemical substances that induce cancer or increase its incidence. Substances that have induced benign and malignant tumours in well-performed experimental studies on animals are considered to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. See United Nations (2007). |
| conjunctival oedema | A chemically induced swelling around the eye. See IUPAC (2007). |
| conjunctival redness | A chemically induced redness around the eye. |
| corneal opacity | When the cornea becomes cloudy because of scar tissue, an injury, or infection. |
| data | Values that are directly measured, calculated, or estimated for any of the measures given. See the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |

| Term | Definition |
|-------------------------|--|
| developmental effect | <p>In relation to an organism, includes structural abnormality, altered growth, functional deficiency, or interference with the normal development of the organism (including the death of a developing organism), that is:</p> <ol style="list-style-type: none"> a. manifested at any point in the organism's lifespan; and b. caused by the exposure of: <ol style="list-style-type: none"> i. a parent to the substance before conception; or ii. the developing offspring to the substance during prenatal development or postnatal development up to the time of sexual maturation. <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| dust or mist | <p>In relation to a substance in the atmosphere, means 90% of the substance is in the form of particles with an aerodynamic diameter of less than 10 µm.</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| elicitation | <p>The production of a cell-mediated or antibody-mediated response by a sensitised individual exposed to an allergen that is sufficient to elicit the response.</p> <p>See United Nations (2007).</p> |
| erythema | <p>Redness of the skin produced by congestion of the capillaries.</p> <p>See IUPAC (2007).</p> |
| eschar | <p>Slough or dry scab on an area of skin that has been chemically burnt.</p> <p>See IUPAC (2007).</p> |
| expert | <p>A person who is:</p> <ol style="list-style-type: none"> a. a member of a scientific committee set up by an international, national, or professional scientific body to review scientific data; or b. considered by his or her scientific peers to be an expert in the relevant field of scientific study. <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| eye corrosion | <p>The production of tissue damage in the eye, or serious physical decay of vision, following the application of a test substance to the anterior surface of the eye, that is not fully reversible within 21 days of application.</p> <p>See United Nations (2007)</p> |
| eye irritation | <p>The production of changes in the eye following the application of a test substance to the anterior surface of the eye that are fully reversible within 21 days of application.</p> <p>See United Nations (2007).</p> |
| genotoxic, genotoxicity | <p>Agents or processes that cause a genotoxic effect.</p> <p>See United Nations (2007).</p> |

| Term | Definition |
|-----------------------------|--|
| genotoxic effect | <p>Alterations to the structure, information content, or segregation of DNA, including:</p> <ol style="list-style-type: none"> DNA damage caused by interference with its normal replication processes; and temporary non-physiological alterations to its replication. <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| GHS | Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007). |
| iritis | Inflammation of the iris |
| kg | kilogram(s) |
| L | litre(s) |
| LC ₅₀ | <p>The median lethal concentration, being a statistically derived concentration of a substance that can be expected to cause death in 50% of animals.</p> <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| LD ₅₀ | <p>The median lethal dose, being a statistically derived single dose of a substance that can be expected to cause death in 50% of animals.</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| LD _{Lo} | The lowest lethal dose, that is, the minimum amount of a substance that is lethal to a specified type of animal. |
| limited evidence in animals | <p>In relation to a substance, data that indicate a carcinogenic effect after exposure to the substance, but that are limited because:</p> <ol style="list-style-type: none"> the evidence of carcinogenicity is restricted to a single experiment; or questions are unresolved about the adequacy of the design or the conduct or interpretation of the study; or the substance increases the incidence only of benign tumours, or of lesions of uncertain neoplastic potential, or of tumours that may occur spontaneously in high incidence in certain strains of animal. <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| limited evidence in humans | <p>In relation to a substance, means a positive correlation has been observed between exposure to the substance and the development of human cancer, where a causal relationship is credible, but where chance, bias, or confounding cannot be ruled out with reasonable confidence.</p> <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| mean Draize score | In relation to acute skin irritation tests, the mean value in at least two of three tested animals: |

| Term | Definition |
|----------------------|---|
| | <p>a. from Draize grades measured at intervals of 24 hours, 48 hours, and 72 hours after the patch is removed; or</p> <p>b. where reactions are delayed, from Draize grades on three consecutive days after the onset of dermal reactions.</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| mg | milligram(s) |
| mL | millilitre(s) |
| mutagen | <p>Agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.</p> <p>See United Nations (2007).</p> |
| mutagenic effect | <p>A permanent change in the amount or structure of the genetic material in a cell, being a permanent change that is:</p> <p>a. manifested at the phenotypic level; or</p> <p>b. an underlying DNA modification (including specific base pair changes and chromosomal translocations).</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| oedema | <p>The presence of abnormally large amounts of fluid in intercellular spaces of body tissues (visible as swelling).</p> <p>See IUPAC (2007).</p> |
| outlier | An observation that is numerically distant from the rest of the data. |
| photosensitisation | <p>Photosensitisation includes:</p> <p>a. photoirritation, which is a light-induced skin response to a photoreactive chemical; and</p> <p>b. photoallergy, which is an immunologically mediated reaction to a chemical initiated by the formation of photoproducts (for example, the photoproducts produce an antigen).</p> <p>See EMEA (2002).</p> |
| ppm | parts per million |
| ppmV | parts per million by volume |
| reliable information | <p>Information that is derived from:</p> <p>a. a valid and relevant animal study conducted in accordance with internationally accepted test guidelines and principles of good laboratory practice; or</p> <p>b. an epidemiological study in humans that is statistically sound and has undergone peer review; or</p> <p>c. any other study whose relevance and validity can be demonstrated</p> |

| Term | Definition |
|---------------------------------------|--|
| | <p>according to internationally accepted criteria and scientific practice.</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| reproductive effect | <p>Includes:</p> <ol style="list-style-type: none"> a. interference with reproductive ability or capacity, including alteration to the male or female reproductive system; or b. an effect on the onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, or premature reproductive senescence; or c. an effect on or through lactation; or d. modifications in other functions that are dependent on the integrity of the reproductive system. <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| respiratory sensitiser | <p>A substance that will induce hypersensitivity of the airways following inhalation of a substance.</p> <p>See United Nations (2007).</p> |
| sensitisation | <p>An immunologically mediated reaction where, after exposure to a substance to which an organism or a human being has been previously exposed, the organism or human being is, or one or more organs in an organism or a human being are, more readily and adversely affected by that substance.</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| significant adverse biological effect | <p>A toxicologically significant change in an organ or animal observed during the study where the probability that the change is different from any recognised background history of change or from the value in a recognised unexposed control organ or animal group in the test animal strain is greater than 0.95 (equivalent to a probability of 0.05 or less).</p> <p>See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| skin corrosion | <p>The production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.</p> <p>See United Nations (2007).</p> |
| skin irritation | <p>The production of reversible damage to the skin following the application of a test substance for up to four hours.</p> <p>See United Nations (2007).</p> |
| skin sensitiser | <p>A substance that will induce an allergic response following skin contact.</p> |

| Term | Definition |
|---------------------------------|---|
| | See United Nations (2007). |
| sufficient evidence in animals | <p>In relation to a substance, data that indicate a causal relationship between exposure to the substance and:</p> <ol style="list-style-type: none"> a. an increased incidence of malignant tumours, or of a combination of benign and malignant tumours, in: <ol style="list-style-type: none"> i. two or more species of animal; or ii. two or more independent studies in one species carried out at different times, in different laboratories, or under different protocols; or b. malignant tumours that occur to an unusual degree, having regard to incidence, site, type of tumour, or age at onset in a single study in one species. <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| sufficient evidence in humans | <p>In relation to a substance, a causal relationship that has been established between exposure to the substance and the development of human cancer, from which chance, bias, and confounding can be ruled out with reasonable confidence.</p> <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| target organ, systemic toxicity | <p>Toxicologically significant effects on the function or morphology of an organ or on the biochemistry or haematology of a human.</p> <p>See Schedule 4 to the Hazardous Substances (Classification) Regulations 2001.</p> |
| TD _{Lo} | The lowest toxic dose, that is, the minimum amount of a substance that is toxic to a specified type of animal. |
| teratogenicity | <p>The potential to cause the production of non-heritable structural malformations or defects in offspring.</p> <p>See IUPAC (2007).</p> |
| toxicodynamics | <p>The process of interaction of potentially toxic substances with target sites, and the biochemical and physiological consequences leading to adverse effects.</p> <p>See IUPAC (2007).</p> |
| toxicokinetics | <p>Generally, the overall process of the absorption (uptake) of potentially toxic substances by the body, the distribution of the substances and their metabolites in tissues and organs, their metabolism (biotransformation), and the elimination of the substances and their metabolites from the body.</p> <p>See IUPAC (2007).</p> |
| µm | micron(s) |
| urticaria | A vascular reaction of the skin marked by the transient appearance of smooth, slightly elevated patches (for example, wheals and hives) that are redder or paler than the surrounding skin and often attended by severe itching. |

| Term | Definition |
|-------|--|
| | See IUPAC (2007). |
| valid | In relation to a study, means the: a. design of the study methodology accurately reflects the matters the study seeks to measure; and b. study findings can be extrapolated from the sample used in the study to a broader population. See Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |

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10. Acute Toxicity – Subclass 6.1

10.1. General considerations

10.1.1. Acute toxicity

Acute toxicity refers to those adverse effects occurring following the oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of four hours.

See section 9.6 in chapter 9 for definitions of the key terms used in this chapter.

10.1.2. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Preferably, data should preferably be derived using Organisation for Economic Co-operation and Development (OECD) Test Guidelines or equivalent according to the principles of Good Laboratory Practice (GLP). Where such data are not available classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

See Appendix 10A [below](#) for a detailed list of acceptable test methods for acute toxicity.

10.2. Acute toxicity hazard and classification criteria

10.2.1. Acute toxicity hazard and threshold criteria

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 Minimum degrees of hazard

- (1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*
- (a) *data for the substance indicates a LD50 of 5000 milligrams or less of the substance per kilogram of bodyweight as a result of acute exposure of animals to the substance by oral or dermal routes; or*
 - (b) *data for the substance indicates any mortality, as a result of acute exposure of animals by—*
 - (i) *oral or dermal routes to 2000 milligrams or less of the substance per kilogram of bodyweight; or*
 - (ii) *the inhalation route to—*
 - (A) *5000 parts or less of the substance per million in air, if the substance is a gas;*
- or*

- (B) 20 milligrams or less of the substance per litre of air, if the substance is a vapour; or
- (C) 5 milligrams or less of the substance per litre of air, if the substance is a dust or mist; or
- (c) clinical signs (other than diarrhoea, piloerection, or an ungroomed appearance) indicate to an expert a significant adverse biological effect as a result of acute exposure of animals by—
- (i) oral or dermal routes to 2000 milligrams or less of the substance per kilogram of bodyweight; or
- (ii) the inhalation route to—
- (A) 5000 parts or less of the substance per million in air, if the substance is a gas; or
- (B) 20 milligrams or less of the substance per litre of air, if the substance is a vapour; or
- (C) 5 milligrams or less of the substance per litre of air, if the substance is a dust or mist; or
- (d) reliable information for the substance, including reliable information from animal studies other than those from which LD50 data was obtained, where exposure was by a route other than oral, dermal, or inhalation, indicates to an expert the potential for significant acute toxic effects in humans after exposure to the substance; or
- (e) data for the substance, in the opinion of an expert, indicates evidence in humans of significant acute toxic effects as a result of exposure to the substance
- ...
- (2) A substance is not required to be tested in accordance with subclause (1)(a) if the substance—
- (a) has been tested in accordance with subclause (1)(b); and
- (b) does not meet the minimum degree of hazard specified in subclause (1)(b).

10.2.2. Acute toxicity hazard classification criteria for substances

Schedule 4 of the Hazardous Substances Classification Regulations 2001 identifies five classification categories for substances that are acutely toxic (subclass 6.1). These categories are based on the Globally Harmonised System for Classification and Labelling of Chemicals (GHS) (United Nations, 2007) acute toxicity criteria (see Appendix 10C for a comparison with the GHS categories, see Appendix 10D for a comparison of the HSNO acute toxicity categories with the equivalent EU risk phrases).

- Category 6.1A
 - a. A substance for which data indicate an oral median lethal dose (LD₅₀) less than or equal to 5 mg of the substance per kilogram of bodyweight (mg/kg bw) as a result of acute exposure of animals to the substance by the oral route.

- b. A substance for which data indicate a dermal LD₅₀ less than or equal to 50 mg/kg bw as a result of acute exposure of animals to the substance by the dermal route.
 - c. A substance for which data indicate an inhalation median lethal concentration (LC₅₀) less than or equal to 100 ppm of the substance in air as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a gas.
 - d. A substance for which data indicate an inhalation LC₅₀ less than or equal to 0.5 mg of the substance per litre (mg/L) of air as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a vapour.
 - e. A substance for which data indicate an inhalation LC₅₀ less than or equal to 0.05 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a dust or mist.
- Category 6.1B
 - a. A substance for which data indicate an oral LD₅₀ greater than 5 mg/kg bw but less than or equal to 50 mg/kg bw, as a result of acute exposure of animals to the substance by the oral route.
 - b. A substance for which data indicate a dermal LD₅₀ greater than 50 mg/kg bw but less than or equal to 200 mg/kg bw, as a result of acute exposure of animals to the substance by the dermal route.
 - c. A substance for which data indicate an inhalation LC₅₀ greater than 100 ppm in air but less than or equal to 500 ppm in air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a gas.
 - d. A substance for which data indicate an inhalation LC₅₀ greater than 0.5 mg/L of air but less than or equal to 2.0 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a vapour.
 - e. A substance for which data indicate an inhalation LC₅₀ greater than 0.05 mg/L of air but less than or equal to 0.5 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a dust or mist.
- Category 6.1C
 - a. A substance for which data indicate an oral LD₅₀ greater than 50 mg/kg bw but less than or equal to 300 mg/kg bw, as a result of acute exposure of animals to the substance by the oral route.
 - b. A substance for which data indicate a dermal LD₅₀ greater than 200 mg/kg bw but less than or equal to 1,000 mg/kg bw, as a result of acute exposure of animals to the substance by the dermal route.
 - c. A substance for which data indicate an inhalation LC₅₀ greater than 500 ppm in air but less than or equal to 2,500 ppm in air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a gas.

- d. A substance for which data indicate an inhalation LC₅₀ greater than 2.0 mg/L of air but less than or equal to 10 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a vapour.
- e. A substance for which data indicate an inhalation LC₅₀ greater than 0.5 mg/L of air but less than or equal to 1.0 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a dust or mist.
- Category 6.1D
 - a. A substance for which data indicate an oral LD₅₀ greater than 300 mg/kg bw but less than or equal to 2,000 mg/kg bw, as a result of acute exposure of animals to the substance by the oral route.
 - b. A substance for which data indicate a dermal LD₅₀ greater than 1,000 mg/kg bw but less than or equal to 2,000 mg/kg bw, as a result of acute exposure of animals to the substance by the dermal route.
 - c. A substance for which data indicate an inhalation LC₅₀ greater than 2,500 ppm in air but less than or equal to 5,000 ppm in air as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a gas.
 - d. A substance for which data indicate an inhalation LC₅₀ greater than 10 mg/L of air but less than or equal to 20 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a vapour.
 - e. A substance for which data indicate an inhalation LC₅₀ greater than 1.0 mg/L of air but less than or equal to 5 mg/L of air, as a result of acute exposure of animals to the substance by the inhalation route, where the substance is a dust or mist.
- Category 6.1E
 - a. A substance for which data indicate an LD₅₀ greater than 2,000 mg/kg bw, but less than or equal to 5,000 mg/kg bw, as a result of acute exposure of animals to the substance by oral or dermal routes.
 - b. A substance for which assignment to a more hazardous category is not warranted, and:
 - i. data for the substance indicate to an expert evidence in humans of significant acute toxic effects as a result of acute exposure to the substance; or
 - ii. data indicate any mortality when tested up to category D values by the oral, inhalation, or dermal routes as a result of acute exposure to the substance; or
 - iii. clinical signs, other than diarrhoea, piloerection, or an ungroomed appearance, indicate to an expert a significant adverse biological effect when tested up to category D values by the oral, dermal or inhalation routes as a result of acute exposure to the substance; or
 - iv. reliable information, including reliable information from animal studies other than those from which LD50 data were obtained to classify the substance in hazard classification 6.1E, indicates

to an expert the potential for significant acute toxic effects in humans as a result of acute exposure to the substance.

Substances can be allocated to one of five toxicity categories based on acute toxicity by the oral, dermal, or inhalation route according to the numeric cut-off criteria as shown in Table 10.1, and discussed in detail above. Acute toxicity values are expressed as (approximate) LD₅₀ (oral, dermal) or LC₅₀ (inhalation) values or as acute toxicity estimates (ATE). Explanatory notes follow Table 10.1.

The classification scheme for acute oral, dermal, and inhalation toxicity outlined above is presented in Table 10.1.

Table 10.1: Acute toxicity hazard categories and acute toxicity estimates defining the respective categories

| Exposure route | Category | | | | |
|--|----------|-------|---------|---------|---|
| | 6.1A | 6.1B | 6.1C | 6.1D | 6.1E |
| Oral LD ₅₀ (mg/kg bw) ^{a,e} | ≤ 5 | ≤ 50 | ≤ 300 | ≤ 2,000 | ≤ 5,000 and criteria for 6.1E(b)(i)–(iv) ^d |
| Dermal LD ₅₀ (mg/kg bw) ^{a,f} | ≤ 50 | ≤ 200 | ≤ 1,000 | ≤ 2,000 | ≤ 5,000 and criteria for 6.1E(b)(i) – (iv) ^d |
| Gases 4-hour LC ₅₀ (ppmV) ^{a,b,g} | ≤ 100 | ≤ 500 | ≤ 2,500 | ≤ 5,000 | criteria for 6.1E(b)(i) – (iv) ^d |
| Vapours 4-hour LC ₅₀ (mg/L in air) ^{a,b,c,g} | ≤ 0.5 | ≤ 2.0 | ≤ 10 | ≤ 20 | criteria for 6.1E(b)(i) – (iv) ^d |
| Dusts and mists 4-hour LC ₅₀ (mg/L in air) ^{a,b,g} | ≤ 0.05 | ≤ 0.5 | ≤ 1.0 | ≤ 5 | criteria for 6.1E(b)(i) – (iv) ^d |

Notes:

Gas concentrations are expressed in parts per million by volume (ppmV); LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; mg/kg bw = milligrams per kilogram of bodyweight; mg/L = milligrams per litre.

- a. The acute toxicity estimate (ATE) for the classification of a substance or ingredient in a mixture is derived using the:
 - LD₅₀ or LC₅₀, where available;
 - appropriate conversion value from Table 10.2 that relates to the results of a range test; or
 - appropriate conversion value from Table 10.2 that relates to a classification category.
- b. Inhalation cut-off values are based on four-hour testing exposures. Conversion of existing inhalation toxicity data that have been generated according to one-hour exposures should be by dividing by a factor of 2 for gases and vapours and 4 for dusts and mists (see 'Conversions' in section 10.2.7).
- c. For some chemicals, the test atmosphere will not just be a vapour but will consist of a mixture of liquid and vapour phases. For other chemicals, the test atmosphere may consist of a vapour that is near the gaseous phase. In these latter cases, classification should be based on ppmV as follows: category 1 (100 ppmV); category 2 (500 ppmV); category 3 (2,500 ppmV); and category 4 (5,000 ppmV).
Dust is generally formed by mechanical processes. Mist is generally formed by condensation of supersaturated vapours or by physical shearing of liquids. Dusts and mists are defined in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001: "in relation to a substance in the atmosphere, means 90% of the substance is in the form of particles with an aerodynamic diameter of less than 10 microns".

- d. See the relevant part of section 10.2.2.

Criteria for category 6.1E are intended to enable the identification of substances that are of relatively low acute toxicity hazard but that under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD₅₀ in the range of 2,000–5,000 mg/kg bw and equivalent doses for inhalation exposure. Recognising the need to protect animal welfare, testing in animals in category 5 ranges is discouraged and should be considered only when there is a strong likelihood that results of such a test would directly relevant to protecting human health.

- e. The acute oral toxicity classification may not be appropriate if the substance (single component or mixture) is highly volatile or is a gas.

- f. The acute dermal toxicity classification may not be appropriate if the substance (single component or mixture) has a pH ≤ 2 or ≥ 11.5, or if the substance is highly volatile or a gas.

- g. The acute inhalation toxicity classification should be considered if the substance (single component or mixture) meets the following criteria. Substances that do not meet these criteria are not considered to be of toxicological concern via the inhalation route and no classification should be considered.

Single component – A classification for acute inhalation toxicity should be considered where the single component:

- is a gas or liquified gas;
- is to be used as a fumigant;
- is to be included in a smoke generating, aerosol or vapour releasing preparation;
- is to be used with fogging equipment;
- has a vapour pressure >1 x 10⁻² Pa and is to be included in preparations to be used in enclosed spaces such as warehouse or glasshouses;
- is to be included in preparations which are powders containing a significant proportion of particles of diameter <50 µm (>1% on a weight basis); or
- is to be included in preparations to be applied in a manner which generates a significant proportion of particles or droplets of diameter <50 µm (>1% on a weight basis).

Mixtures – A classification for acute inhalation toxicity should be considered where the mixture:

- is used with fogging equipment;
- is an aerosol;
- is a powder containing a significant proportion of particles of diameter <50 µm (>1% on a weight basis);
- is to be applied from aircraft in cases where inhalation exposure is relevant;
- is to be applied in a manner which generates a significant proportion of particles or droplets of diameter <50 µm (>1% on a weight basis); or
- contains a volatile component >10%.

Selecting the most appropriate LD₅₀ value

When experimental data for acute toxicity are available in several animal species, scientific judgement should be used when selecting the most appropriate LD₅₀ value from among valid, well-performed tests. Consideration should therefore be given to the following.

- Reliability
 - Does the selected value meet the definition of 'reliable information'?
 - What was the date of reference that supports the acute toxicity value? Studies conducted before the advent of GLP and internationally accepted test guidelines may not be of acceptable quality.
 - Are the acute toxicity values cited from an adequate source that has been peer reviewed?

- Are any of the acute toxicity values 'outliers'? Acute toxicity values can vary from study to study, between species, within a species, or between sexes.
- Relevance
 - Is the route of exposure tested relevant to likely human exposure to the substance?
 - Were the acute toxicity values reported in preferred laboratory species? (The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat, while the rat or rabbit is preferred for evaluation of acute dermal toxicity.)
 - If there are acute toxicity values in animal species that are not preferred laboratory species, is the animal used considered to be a relevant indicator of acute toxicity exposure in humans?
 - The appropriate LD₅₀ value should, therefore, be the most reliable and relevant LD₅₀ value.

10.2.3. Reliable information

The EPA acknowledges that the use of reliable information to determine threshold within clause 2(1)(d) of Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 should be linked to evident toxicity. Evident toxicity means clear signs of toxicity following the administration of a test substance sufficient for hazard assessment and such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.

It is also important to note that an epidemiological study in humans that shows evident toxicity has not occurred is also important information that should be considered when determining whether classification of a substance is necessary (provided it is well established that human exposure did occur).

10.2.4. Lowest toxic doses and lowest lethal doses

Some acute toxicity databases give information on the lowest lethal doses (LD_{LO}) or the lowest toxic doses (TD_{LO}). Similarly inhalation concentrations are sometimes found (LC_{LO} and TC_{LO}). This information can be derived from an animal study or human exposure. These values in humans tend to be based on anecdotal exposure to a single dose or occupational exposure, so uncertainty may exist about the actual dose taken.

If an LD_{LO} is available, this may be used directly in the calculations for the toxicity of a mixture. However, the resultant value may result in an overly conservative classification. How to calculate the toxicity of a mixture is described in section 10.3.

If a TD_{LO} is available, then a general indication of acute toxicity may be inferred from the value. The TD_{LO} is the lowest dose known to cause a toxic effect, as opposed to a result from a standardised acute toxicity testing method that gives rise to the lethal dose capable of killing 50% of the test animals (LD₅₀). It can, therefore, be assumed that the LD₅₀ for the substance would be greater (a larger amount) than the TD_{LO}.

10.2.5. Precedence of human data over animal data

When direct human data show an acute effect (for example, the LD_{LO} is established or clinical signs of acute toxicity in humans are observed), this effect takes precedence over negative results from animal studies.

10.2.6. Low viscosity substances with an aspiration hazard



Some liquid substances and preparations present an aspiration hazard in humans because of their low viscosity.

The 6.1E acute oral toxicity classification is triggered if the substance has the following physical properties or has known aspiration hazards in humans.

- The 6.1E acute oral toxicity classification is triggered if:
 - a. The substance is a hydrocarbon with a kinematic viscosity of $\leq 20.5 \text{ mm}^2/\text{s}$ measured at 40°C or there is reliable and good quality human evidence to indicate a human aspiration (note this is essentially the same as the GHS category 1); or
 - b. The substance has a kinematic viscosity $\leq 14 \text{ mm}^2/\text{s}$ at 40°C , with evidence from existing animal studies, and expert judgment which takes into account surface tension, water solubility, boiling point and volatility (note this is essentially the same as the GHS category 2).

The following formula provides a conversion between dynamic and kinematic viscosity:

$$\frac{\text{Dynamic viscosity (mPa.s)}}{\text{Density (g/cm}^3\text{)}} = \text{kinematic viscosity (mm}^2/\text{s)}$$

A mixture is classified as 6.1E acute oral toxicity (aspiration hazard) if it contains:

- $\geq 10\%$ of a substance classified under criterion 1, and has a kinematic viscosity of viscosity of $\leq 20.5 \text{ mm}^2/\text{s}$ measured at 40°C (GHS Category 1); or
- $\geq 10\%$ of a substance classified under criterion 1, and has a kinematic viscosity of viscosity of $\leq 14 \text{ mm}^2/\text{s}$ measured at 40°C (GHS Category 2)

A mixture which separates into two or more distinct layers, one of which contains $\geq 10\%$ of an ingredient classified according to either criterion, then the entire mixture is classified accordingly.

The latest revision to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) has included two separate classifications for substances that present an aspiration hazard. The EPA is reviewing the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and the Hazardous Substances (Classification) Regulations 2001 in light of this revision to determine whether these regulations should be amended.

10.2.7. Specific considerations for inhalation toxicity

Conversions

Values for inhalation toxicity are based on four-hour tests in laboratory animals. For conversion of existing inhalation toxicity data generated from exposures other than four hours, the following formulae should be used.

- Dusts and mists: $\text{LC}_{50} (4 \text{ hours}) = \text{LC}_{50} (x \text{ hours}) \times (x/4)$
- Vapours and gases: $\text{LC}_{50} (4 \text{ hours}) = \text{LC}_{50} (1 \text{ hour}) \times (1/2)$

Conversion of inhalation data for vapours, dusts, or mists specified as milligrams per cubic metre (mg/m^3) to mg/L : $\text{mg}/\text{L} = \frac{\text{mg}/\text{m}^3}{1000}$

1,000

- Conversion of inhalation data for gases specified as mg/m³ to ppm:

$$\text{ppm} = \frac{\text{mg/m}^3 \times 24.45}{\text{gram molecular}}$$

weight of substance

(Note: 24.45 = the molar volume of air in litres at normal temperature (25°C) and pressure (760 torr).)

- Conversion of oral or dermal data specified as mL/kg to mg/kg:

$$\text{mass (g)} = \text{volume (mL)} \times \text{density (g/mL)}$$

Other considerations

Units for inhalation toxicity are a function of the form of the inhaled material. Values for dusts and mists are expressed in mg/L. Values for gases are expressed in parts per million by volume (ppmV). Table 10.1 acknowledges the difficulties in testing vapours, some of which consist of mixtures of liquid and vapour phases, and provides values in units of mg/L. However, for those vapours that are near the gaseous phase, classification should be based on ppmV. As inhalation test methods are updated, the OECD and other test guideline programmes will need to define vapours in relation to mists for greater clarity.

Vapour inhalation values are intended for use in the classification of acute toxicity for all sectors. It is also recognised that the saturated vapour concentration of a chemical is used by the transport sector as an additional element in classifying chemicals for packing groups.

Of particular importance is the use of well-articulated values in the high toxicity categories for dusts and mists. Inhaled particles between 1 and 4 µm in mean mass aerodynamic diameter (MMAD) will deposit in all regions of the rat respiratory tract. This particle size range corresponds to a maximum dose of about 2 mg/L. To achieve applicability of animal experiments to human exposure, dusts and mists are ideally tested in this range in rats.

The cut-off values in Table 10.1 for dusts and mists allow clear distinctions to be made for materials with a wide range of toxicities measured under varying test conditions.

10.2.8. Synergistic and antagonistic effects

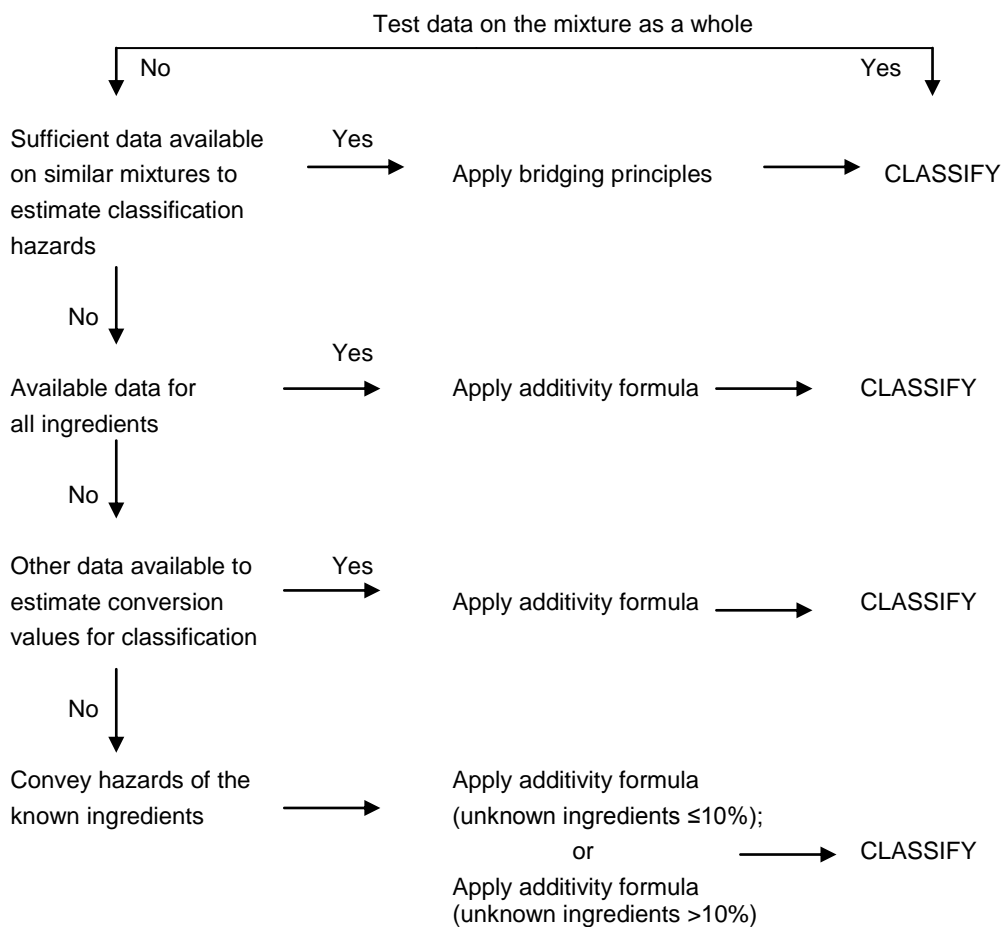
If the applicant is aware of any available information about possible synergistic effects that may enhance the toxicity of the substance as a mixture, this must be considered.

If the applicant is aware of any available information that antagonistic effects may occur such that the substance as a mixture classification is lower than indicated from the calculated value, this should be noted. For example, the encapsulation of a substance as a mixture can lower the toxicity of the substance.

10.3. Classification of mixtures

The criteria for substances classify acute toxicity by use of lethal dose data (tested or derived). For mixtures, it is necessary to obtain or derive information that allows the criteria to be applied to the mixture for the purpose of classification. The approach to classification for acute toxicity is tiered, and depends on the amount of information available for the mixture itself and for its ingredients. The flow chart in Figure 10.1 outlines the process to be followed.

Figure 10.1: Tiered approach to classification of mixtures for acute toxicity



To make use of all available data to classify the hazards of mixtures, certain assumptions have been made and are applied where appropriate in the tiered approach.

- a. The 'relevant ingredients' of a mixture are those that are present in concentrations of 1% (by weight for solids, liquids, dusts, mists, and vapours and by volume for gases) or greater, unless there is a reason to suspect that an ingredient present at a concentration of less than 1% is still relevant for classifying the mixture for acute toxicity. This point is particularly relevant when classifying untested mixtures that contain ingredients that are classified as 6.1A or 6.1B.

When a classified mixture is used as an ingredient of another mixture, the actual or derived ATE for that mixture may be used when calculating the classification of the new mixture using the formulas in 'Data available for all ingredients' and 'Mixture with an ingredient with unknown acute toxicity' in section 10.3.3.

10.3.1. Classification of mixtures where acute toxicity test data are available for the complete mixture

When the mixture itself has been tested to determine its acute toxicity, it will be classified according to the criteria presented in Table 10.1.¹ If test data for the mixture are not available, the procedures presented in section 10.3.2 should be followed.

10.3.2. Classification of mixtures where acute toxicity test data are not available for the complete mixture: Bridging principles

When the mixture itself has not been tested to determine its acute toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data are used in accordance with the following agreed bridging rules. This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without the necessity for additional testing in animals.

a. Dilution

If a substance as a mixture is diluted with a substance that has an equivalent or lower toxicity classification than the least toxic original component, and that is not expected to affect the toxicity of other components, then the new mixture may be classified as equivalent to the original mixture.

If a substance as a mixture is diluted with water or other totally non-toxic material, the toxicity of the mixture can be calculated from test data on the undiluted substance as a mixture. For example, if a substance as a mixture has an LD₅₀ of 1,000 mg/kg bw and is diluted with an equal volume of water, then the subsequent LD₅₀ of the diluted substance as a mixture would be 2,000 mg/kg bw.

b. Batching

The toxicity of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another batch of the same commercial product, which is produced by or under the control of the

¹ Note that if the ATE_{mix} (LD₅₀) from the oral or dermal route is greater than 5,000 mg/kg bw but human exposure shows acute toxic effects to the substance as a mixture, then the substance as a mixture still triggers the threshold.

same manufacturer, unless there is reason to believe there is significant variation such that the toxicity of the batch has changed. If the latter occurs, new classification is necessary.

c. Concentration of highly toxic mixtures

If a substance as a mixture is classified as category 6.1A, and the concentration of the components of the mixture that are 6.1A are increased, the new substance as a mixture should be classified as category 6.1A without additional testing.

d. Interpolation within one toxicity class

If mixtures A and B are in the same toxicity category, and mixture C includes toxicologically active components with concentrations intermediate to those in mixtures A and B, then mixture C is assumed to be in the same toxicity category as mixtures A and B.

e. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of ingredient B is essentially the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
- iv. data on toxicity for ingredients A and C are available and substantially equivalent; that is, they are in the same hazard category and are not expected to affect the toxicity of B; then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

f. Aerosols

i. Aspiration hazard

A hazard classification relating to aspiration hazards will not generally be applicable for aerosol products. The key consideration for aspiration hazards is whether a pool may be formed in the mouth that can then be aspirated. For aerosol products it is unlikely that a pool in the mouth will be formed (unless deliberate misuse occurs), and the exposure necessary for the hazard to present will, therefore, be unlikely to occur.

ii. Acute oral toxicity

A hazard classification relating to acute oral toxicity will not generally be applicable for aerosol products. The exposure necessary for an acute oral toxicity hazard to present is unlikely to occur.

iii. Acute dermal toxicity

A hazard classification may be assigned for acute dermal toxicity for aerosol products. However, the propellant should generally not be taken into account when classifying aerosols, as the gaseous propellant will not be present in the liquid that comes into contact with the skin.

iv. Acute inhalation toxicity

A hazard classification may be assigned for acute inhalation toxicity for aerosol products. The classification assigned should also take into account the propellant in the aerosol.

10.3.3. Classification of mixtures based on ingredients of the mixture (additivity formula)

Data available for all ingredients

The ATE of ingredients should be considered in the following way.

- Include ingredients (including impurities and additives) with a known acute toxicity, that fall into any of the HSNO Act acute toxicity categories.
- Ignore ingredients that are presumed not acutely toxic (for example, water and sugar).
- Ignore ingredients if the oral limit test does not show acute toxicity at 2,000 mg/kg bw.

Ingredients that fall within the scope of this paragraph are considered to be ingredients with a known ATE. The ATE of the mixture is determined using the ATE values for all relevant ingredients, according to the following formula for oral, dermal, or inhalation toxicity:

$$C_a/ATE_a + C_b/ATE_b + \dots + C_z/ATE_z = 100/ATE_{mix}$$

Where:

C_a = percentage of the component in the substance as a mixture

ATE_a = acute toxicity estimate of component

ATE_{mix} = estimated LD_{50} of the mixture

See the worked examples in Appendix 10B.

Data are not available for one or more ingredients of the mixture

When an ATE is not available for an individual ingredient of the mixture, but information such as that listed below can provide a derived conversion value (see Table 10.2) the formula above may be applied.

This may include evaluating:

- the extrapolation between oral, dermal, and inhalation ATEs,² which could require appropriate pharmacodynamic and pharmacokinetic data;
- evidence from human exposure that indicates toxic effects but does not provide lethal dose data;
- evidence from any other toxicity tests and assays available on the substance that indicates toxic acute effects but does not necessarily provide lethal dose data; or
- data from closely analogous substances using structure activity relationships.

This approach generally requires substantial supplemental technical information and a highly trained and experienced expert to reliably estimate acute toxicity. If such information is not available, follow the provisions below.

² For ingredients with ATEs available for other than the most appropriate exposure route, values may be extrapolated from the available exposure route to the most relevant route.

No useable information about an ingredient that is present at concentrations of 1% or greater

When there is no useable information about an ingredient and it is present in a mixture at concentrations of 1% or greater, the mixture cannot be assigned a definitive LD₅₀ or LC₅₀. The mixture is, therefore, classified based on the known ingredients only, and an additional statement is attached to the classification that 'x percent of the mixture consists of a component of unknown toxicity'.

Table 10.2: Conversion from experimentally obtained acute toxicity range values (or acute toxicity hazard categories) to acute toxicity point estimates for classification for the respective routes of exposure

| Exposure routes | Experimentally obtained or derived LD ₅₀ or LC ₅₀ range estimate ^a | Converted LD ₅₀ or LC ₅₀ ^b |
|-------------------------|---|---|
| Oral (mg/kg bw) | 0 < Category 1 ≤ 5 | 0.5 |
| | 5 < Category 2 ≤ 50 | 5 |
| | 50 < Category 3 ≤ 300 | 100 |
| | 300 < Category 4 ≤ 2,000 | 500 |
| | 2,000 < Category 5 ≤ 5,000 | 2,500 |
| Dermal (mg/kg bw) | 0 < Category 1 ≤ 50 | 5 |
| | 50 < Category 2 ≤ 200 | 50 |
| | 200 < Category 3 ≤ 1,000 | 300 |
| | 1,000 < Category 4 ≤ 2,000 | 1,100 |
| | 2000 < Category 5 ≤ 5,000 | 2,500 |
| Gases (ppm in air) | 0 < Category 1 ≤ 100 | 10 |
| | 100 < Category 2 ≤ 500 | 100 |
| | 500 < Category 3 ≤ 2,500 | 700 |
| | 2,500 < Category 4 ≤ 5,000 | 3,000 |
| | Category 5 | |
| Vapours (mg/L in air) | 0 < Category 1 ≤ 0.5 | 0.05 |
| | 0.5 < Category 2 ≤ 2.0 | 0.5 |
| | 2.0 < Category 3 ≤ 10.0 | 3.0 |
| | 10.0 < Category 4 ≤ 20.0 | 11.0 |
| | Category 5 | |
| Dust/mist (mg/L in air) | 0 < Category 1 ≤ 0.05 | 0.005 |
| | 0.05 < Category 2 ≤ 0.5 | 0.05 |
| | 0.5 < Category 3 ≤ 1.0 | 0.5 |

| | | |
|--|------------------------|-----|
| | 1.0 < Category 4 ≤ 5.0 | 1.5 |
| | Category 5 | |

Notes: Gases concentration are expressed in parts per million by volume (ppmV); LD₅₀ = median lethal dose; median lethal concentration = LC₅₀; mg/kg bw = milligrams per kilogram of bodyweight; mg/L = milligrams per litre.

- a. Criteria for 6.1E are intended to enable the identification of substances that are of relatively low acute toxicity hazard but that under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD₅₀ in the range of 2,000 – 5,000 mg/kg bw and equivalent doses for inhalation exposure. Recognising the need to protect animal welfare, testing in animals in category 5 ranges is discouraged and should be considered only when there is a strong likelihood that the results of such a test would have a direct relevance for protecting human health.
- b. The converted LD₅₀ and LC₅₀ values are designed to be used in the calculation of the acute toxicity estimate for classifying a mixture based on its components, and do not represent test results. The values are conservatively set at the lower end of the range of categories 6.1A and 6.1B, and at a point approximately one-tenth from the lower end of the range for categories 6.1C–6.1E.

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 10A: Acceptable test methods for acute toxicity

10A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. Harmonized Test Guidelines. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.

10A.2 Acute toxicity test guidelines

The guidelines in Table 10A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.
http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series Retrieved 14 August 2007.

See also Table 10A.1.

Table 10A.1: Acute toxicity test guidelines for chemicals, including mixtures

| Test protocols | OECD | EC | USEPA OPPTS |
|--|------|---|-------------|
| Acute oral toxicity | | | |
| Acute oral toxicity – fixed dose method | 420 | EC B.1 bis acute oral toxicity – fixed dose procedure | 870.1100 |
| Acute oral toxicity – acute toxic class method | 423 | EC B.1 tris – acute toxic class method | 870.1100 |
| Acute oral toxicity – up and down procedure | 425 | – | 870.1100 |
| Acute dermal toxicity | | | |
| Acute dermal toxicity | 402 | EC B.3 Acute toxicity (dermal) | 870.1200 |
| Acute inhalation toxicity | | | |
| Acute inhalation toxicity | 403 | EC B.2 Acute toxicity (inhalation) | 870.1300 |
| Acute inhalation toxicity with histopathology | – | | 870.1350 |

OECD Test Guideline 401 (acute oral toxicity) was deleted from the OECD manual of internationally accepted test guidelines on 17 December 2002. Acute oral toxicity studies conducted after this date should now adhere to one of the three alternative methods (OECD Guidelines 420, 423, or 425 or equivalent).

Appendix 10B: Calculating acute toxicity – examples

10B.1 Example 1

A mixture contains 10% of component A with an oral median lethal dose (LD₅₀) of 2,000 milligrams per kilogram of bodyweight (mg/kg bw) and 30% of component B with an oral LD₅₀ of 1,500 mg/kg bw.

The toxicity of the substance as a mixture expressed as an LD₅₀ would be as follows.

$$C_a/ATE_a + C_b/ATE_b + \dots + C_z/ATE_z = 100/ATE_{mix}$$

Where:

C_a = percentage of the component in the substance as a mixture

ATE_a = acute toxicity estimate of component

ATE_{mix} = estimated LD₅₀ of the mixture

Therefore, example 1:

$$10/2,000 + 30/1,500 = 100/LD_{50} \text{ (mixture)}$$

$$LD_{50} \text{ (mixture)} = 4,000 \text{ mg/kg bw}$$

The calculated LD₅₀ of the mixture of 4000 mg/kg bw is less than the acute oral toxicity threshold of 5000 mg/kg bw and so triggers the threshold. The mixture would be classified as 6.1E.

10B.1 Example 2

A mixture contains 10% of component A, which is classified as a category 6.1C toxicant, and 30% of component B with an oral LD₅₀ of 1,500 mg/kg bw. Using the formula in example 1, the toxicity of the substance as a mixture, expressed as an LD₅₀, would be as follows.

Using the range conversion table (see Table 10.2), a category 6.1C classification is equivalent to an LD₅₀ of 100 mg/kg bw.

$$C_A/T_A + C_B/T_B = 100/T_M$$

$$10/100 + 30/1,500 = 100/LD_{50} \text{ (mixture)}$$

$$LD_{50} \text{ (mixture)} = 833 \text{ mg/kg bw}$$

The calculated LD₅₀ of the mixture of 833 mg/kg bw is less than the acute oral toxicity threshold of 5,000 mg/kg bw, so triggers the threshold. The mixture would be classified as a 6.1D.

Appendix 10C: Globally Harmonized System of Classification and Labelling of Chemicals acute toxicity hazard classification

This appendix displays the toxicity categories from the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) based on acute toxicity by the oral, dermal, and inhalation routes. (See Table 10C.1)

The GHS acute toxicity categories are generally comparable with the acute toxicity categories in the Hazardous Substances and New Organisms Act 1996 (HSNO Act) (see Table 10.1 and Table 10.2). However some of the key differences are as follows.

- The upper limit for gases under HSNO Act category 6.1D is 5,000 parts per million by volume (ppmV). Under the GHS, the upper limit for gases for category 4 (which is comparable to 6.1D) is 20,000 ppmV.
- The HSNO Act definition of dust and mist differs to that in the GHS.

Table 10.1 has provided additional guidance as to when an acute inhalation toxicity classification should be assigned.

Table 10C.1: Acute toxicity hazard categories and acute toxicity estimate values defining the respective categories

| Exposure route | Category 1 HSNO Act 6.1A | Category 2 HSNO Act 6.1B | Category 3 HSNO Act 6.1E | Category 4 HSNO Act 6.1D | Category 5 HSNO Act 6.1E |
|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Oral (mg/kg bw) ^a | 5 | 50 | 300 | 2,000 | 5000 ^f |
| Dermal LD ₅₀ (mg/kg bw) ^a | 50 | 200 | 1,000 | 2,000 | |
| Gases (ppmV) ^{a,b} | 100 | 500 | 2,500 | 20,000 | |
| Vapours (mg/L) ^{a,b,c,d} | 0.5 | 2.0 | 10 | 20 | |
| Dusts and Mists (mg/L) ^{a,b,e} | 0.05 | 0.5 | 1.0 | 5 | |

Notes: Gases concentration are expressed in ppm by volume (ppmV); LD₅₀ = median lethal dose; median lethal concentration = LC₅₀; mg/kg bw = milligrams of the substance per kilogram of bodyweight; mg/L = milligrams of the substance per litre

- The acute toxicity estimate (ATE) for the classification of a substance or ingredient in a mixture is derived using the:
 - LD₅₀/LC₅₀ where available;
 - appropriate conversion value from Table 10C.2 that relates to the results of a range test; or
 - appropriate conversion value from Table 10C.2 that relates to a classification category;
- Inhalation cut-off values in the table are based on four-hour testing exposures. Conversion of existing inhalation toxicity data that has been generated according to one-hour exposures should be by dividing by a factor of 2 for gases and vapours and 4 for dusts and mists.

- Saturated vapour concentration may be used as an additional element by some regulatory systems to provide for specific health and safety protection (for example, the United Nations Recommendations for the Transport of Dangerous Goods (United Nations, 1999).
- For some chemicals the test atmosphere will not just be a vapour but will consist of a mixture of liquid and vapour phases. For other chemicals, the test atmosphere may consist of a vapour that is near the gaseous phase. In these latter cases, classification should be based on ppmV as follows: category 1 (100 ppmV); category 2 (500 ppmV); category 3 (2,500 ppmV); and category 4 (5,000 ppmV).
 - a. The terms 'dust', 'mist', and 'vapour' are defined as follows.
 - Dust means solid particles of a substance or mixture suspended in a gas (usually air).
 - Mist means liquid droplets of a substance or mixture suspended in a gas (usually air).
 - Vapour means the gaseous form of a substance or mixture released from its liquid or solid state.
 - b. Dust is generally formed by mechanical processes. Mist is generally formed by the condensation of supersaturated vapours or physical shearing of liquids. Dusts and mists generally have sizes ranging from less than 1 µm to about 100 µm;
- The values for dusts and mists should be reviewed to adapt to any future changes to OECD Test Guidelines with respect to technical limitation in generating, maintaining, and measuring dust and mist concentrations in respirable form.
- Criteria for category 5 are intended to enable the identification of substances that are of relatively low acute toxicity hazard, but that under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD₅₀ in the range of 2,000–5,000 mg/kg bw and equivalent doses for inhalation. The specific criteria for category 5 are:
 - i. the substance is classified in this category if reliable evidence is already available that indicates the LD₅₀ (or LC₅₀) to be in the range of category 5 values or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature;
 - ii. the substance is classified in this category through extrapolation, estimation, or measurement of data, if assignment to a more hazardous category is not warranted, and:
 - A reliable information is available indicating significant toxic effects in humans; or
 - B any mortality is observed when tested up to category 4 values by the oral, inhalation, or dermal routes; or
 - C where expert judgement confirms significant clinical signs of toxicity, when tested up to category 4 values, except for diarrhoea, piloerection, or an ungroomed appearance; or
 - D where expert judgement confirms reliable information indicating the potential for significant acute effects from other animal studies.

Recognising the need to protect animal welfare, testing in animals in category 5 ranges is discouraged and should be considered only when there is a strong likelihood that results of such a test would be directly relevant to protecting human health.

Table 10C.2: Conversion from experimentally obtained acute toxicity range values (or acute toxicity hazard categories) to acute toxicity point estimates for classification for the respective routes of exposure

| Exposure routes | Classification category or experimentally obtained acute toxicity range estimate ^a | Converted acute toxicity point estimate ^b |
|-----------------|---|--|
| Oral (mg/kg bw) | 0 < Category 1 ≤ 5 | 0.5 |
| | 5 < Category 2 ≤ 50 | 5 |
| | 50 < Category 3 ≤ 300 | 100 |

| | | |
|-------------------------|-----------------------------|-------|
| | 300 < Category 4 ≤ 2,000 | 500 |
| | 2,000 < Category 5 ≤ 5,000 | 2,500 |
| Dermal (mg/kg bw) | 0 < Category 1 ≤ 50 | 5 |
| | 50 < Category 2 ≤ 200 | 50 |
| | 200 < Category 3 ≤ 1000 | 300 |
| | 1,000 < Category 4 ≤ 2,000 | 1,100 |
| | 2,000 < Category 5 ≤ 5,000 | 2,500 |
| Gases (ppm in air) | 0 < Category 1 ≤ 100 | 10 |
| | 100 < Category 2 ≤ 500 | 100 |
| | 500 < Category 3 ≤ 2,500 | 700 |
| | 2,500 < Category 4 ≤ 20,000 | 4,500 |
| | Category 5 ^c | |
| Vapours (mg/L in air) | 0 < Category 1 ≤ 0.5 | 0.05 |
| | 0.5 < Category 2 ≤ 2.0 | 0.5 |
| | 2.0 < Category 3 ≤ 10.0 | 3.0 |
| | 10.0 < Category 4 ≤ 20.0 | 11.0 |
| | Category 5 ^c | |
| Dust/mist (mg/L in air) | 0 < Category 1 ≤ 0.05 | 0.005 |
| | 0.05 < Category 2 ≤ 0.5 | 0.05 |
| | 0.5 < Category 3 ≤ 1.0 | 0.5 |
| | 1.0 < Category 4 ≤ 5.0 | 1.5 |
| | Category 5 ^c | |

Notes: Gas concentrations are expressed in parts per million by volume (ppmV); mg/kg bw = milligrams per kilogram bodyweight; mg/L = milligrams per litre.

- a. Category 5 is for mixtures that are of relatively low acute toxicity, but that under certain circumstances may pose a hazard to vulnerable populations. These mixtures are expected to have an oral or dermal LD50 value in the range of 2,000–5,000 mg/kg bw or equivalent dose for other routes of exposure. In light of animal welfare considerations, testing in animals in category 5 ranges is discouraged and should be considered only when there is a strong likelihood that results of such testing would have direct relevance for protecting human health.
- b. These values are designed to be used in the calculation of the acute toxicity estimate for classification of a mixture based on its components and do not represent test results. The values are conservatively set at the lower end of the range of categories 1 and 2, and at a point approximately one-tenth from the lower end of the range for categories 3–5.

- c. The OECD Task Force on Harmonisation of Classification and Labelling did not include values in Table 10C.1 and Table 10C.2 for acute inhalation toxicity category 5 but instead specified doses 'equivalent' to the range 2,000–5,000 mg/kg bw by oral or dermal exposure. (See note f to Table 10C.1.)

References

United Nations 1999. *Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria*, 3rd revised edition. United Nations, New York and Geneva. (Note: Equivalent material can be found in more recent versions of this document, for example, the 4th revised edition. Some material is available for purchasing and downloading from <http://www.unece.org/trans/danger/danger.htm>.)

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 10D: Comparison of European Union acute toxicity risk phrases with HSNO Act acute toxicity classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification categories in Table 10D.1. Note that some cut-off values are not totally aligned with HSNO Act classification categories. This is noted in Table 10D.1 and for HSNO Act classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 10D.1: European Union risk phrases compared with HSNO Act acute toxicity classifications

| European Union risk phrases | HSNO Act equivalent category |
|--|--|
| Very Toxic (T+) A substance is determined to be hazardous and classified as Very Toxic (T+) and assigned one of the following risk phrases in accordance with the criteria given below. | 6.1A |
| R26 Very toxic by inhalation Acute toxicity results: LC ₅₀ inhalation, rat, for aerosols or particulates: ≤ 0.25 mg/L over 4 hours; LC ₅₀ inhalation, rat, for gases and vapours: ≤ 0.5 mg/L over 4 hours. | 6.1A 6.1A |
| R27 Very toxic in contact with skin Acute toxicity results: LD ₅₀ dermal, rat or rabbit: ≤ 50 mg/kg. | 6.1A |
| R28 Very toxic if swallowed Acute toxicity results: LD ₅₀ oral, rat: ≤25 mg/kg; less than 100% survival at 5 mg/kg oral, rat, by the fixed dose procedure. | 6.1A Note this cut-off crosses into 6.1B |
| Toxic (T) A substance is determined to be hazardous and classified as Toxic (T) and assigned one or more of the following risk phrase in accordance with the criteria given below. | 6.1B and 6.1C |
| R23 Toxic by inhalation Acute toxicity results: LC ₅₀ inhalation, rat, for aerosols or particulates: 0.25 < LC ₅₀ ≤ 1 mg/L over 4 hours; LC ₅₀ inhalation, rat, for gases and vapours: 0.5 < LC ₅₀ ≤ 2 mg/L over 4 hours. | 6.1B and 6.1C 6.1B |
| R24 Toxic in contact with skin Acute toxicity results: LD ₅₀ dermal, rat or rabbit: 50 < LD ₅₀ ≤ 400 mg/kg. | 6.1B Note this cut-off crosses into 6.1C. |
| R25 Toxic if swallowed | 6.1B |

| | |
|--|--------------------------------------|
| Acute toxicity results: LD ₅₀ oral, rat: 25 < LD ₅₀ ≤ 200 mg/kg. | Note this cut-off crosses into 6.1C. |
| Harmful (Xn) A substance is determined to be hazardous and classified as Harmful (Xn) and assigned one or more of the following risk phrases in accordance with the criteria given below. | |
| R20 Harmful by inhalation Acute toxicity results: LC ₅₀ inhalation, rat, for aerosols or particulates: 1 < LC ₅₀ ≤ 5 mg/L over 4 hours; LC ₅₀ inhalation, rat, for gases or vapours: 2 < LC ₅₀ ≤ 20 mg/L over 4 hours. | 6.1D |
| R65 Harmful: May cause lung damage if swallowed Liquid substances and preparations presenting an aspiration hazard in humans because of their low viscosity: | 6.1E (aspiration hazard) |
| R21 Harmful in contact with skin Acute toxicity results: LD ₅₀ dermal, rat or rabbit: 400 < LD ₅₀ ≤ 2,000 mg/kg. | 6.1D |
| R22 Harmful if swallowed Acute toxicity results: LD ₅₀ per oral, rat 200 ≤ LD ₅₀ < 2,000 mg/kg; discriminating dose, oral, rat, 50 mg/kg: 100% survival but evident toxicity; less than 100% survival at 500 mg/kg, rat oral by the fixed dose procedure | 6.1D |

Note: LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; mg/kg = milligrams per kilogram; mg/L = milligrams per litre

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

11. Skin Corrosion and Irritation – Subclass 6.3 (8.2)

11.1. General considerations

11.1.1. Skin corrosion or irritation overview

See section 9.6 in chapter 9, for definitions of the key terms used in this chapter.

Several factors should be considered when determining the corrosive and irritant potential of substances before testing is undertaken. Solid substances (powders) may become corrosive or irritant when moistened or in contact with moist skin or mucous membranes. Existing human experience and data, including from single or repeated exposure, and animal observations and data should be analysed first, as they give information directly relevant to effects on the skin. In some cases enough information may be available from structurally related compounds to make classification decisions.

Likewise, pH extremes like ≤ 2 and ≥ 11.5 may produce skin effects, especially when buffering capacity is known, although the correlation is not perfect. Generally, such substances are expected to produce significant effects on the skin.

If a substance is highly toxic by the dermal route, a skin corrosion or irritation study on animals may not be practicable, since the amount of test substance to be applied would considerably exceed the toxic dose, so would result in the death of the animals. When observations are made of skin corrosion or irritation in acute toxicity studies and are observed up through the limit dose, additional testing is not needed, provided the dilutions used and species tested are equivalent. *In vitro* alternatives that have been validated and accepted may also be used to help make classification decisions.

Although information might be gained from the evaluation of single parameters within a tier, for example, caustic alkalis with extreme pH are considered skin corrosives, there is merit in considering the totality of existing information and making an overall weight-of-evidence determination. This is especially true when there is information available on only some parameters. Generally, primary emphasis should be placed on existing human experience and data, followed by animal experience and testing data, followed by other sources of information, but case-by-case determinations are necessary.

A tiered approach to the evaluation of initial information should be considered, where applicable (Table 11.1), recognising that all elements may not be relevant in certain cases.

Table 11.1: Tiered testing and evaluation of skin corrosion and irritation potential

| Step | Parameter | Finding | Conclusion |
|------|--|-----------|------------------------------------|
| 1a | Existing human or animal experience ^g ⇒ ↓ Not corrosive or no data ↓ | Corrosive | Classify as corrosive ^a |
| 1b | Existing human or animal experience ^g ⇒ | Irritant | Classify as irritant ^a |

| | | | |
|----|---|-----------------------------------|---|
| | <p>⇓</p> <p>Not irritant or no data</p> <p>⇓</p> | | |
| 1c | <p>Existing human or animal experience ⇒</p> <p>⇓</p> <p>No data</p> <p>⇓</p> | Not corrosive or irritant | No further testing, not classified |
| 2a | <p>Structure-activity relationships or ⇒</p> <p>Structure-property relationships^b</p> <p>⇓</p> <p>Not corrosive or no data</p> <p>⇓</p> | Corrosive | Classify as corrosive ^a |
| 2b | <p>Structure activity relationships or ⇒</p> <p>Structure-property relationships^b</p> <p>⇓</p> <p>Not irritating or no data</p> <p>⇓</p> | Irritant | Classify as irritant ^a |
| 3 | <p>pH with buffering^c ⇒</p> <p>⇓</p> <p>Not pH extreme or no data</p> <p>⇓</p> | $\text{pH} \leq 2$ or ≥ 11.5 | Classify as corrosive ^a |
| 4 | <p>Existing skin data in animals indicate no need for animal testing^d ⇒</p> <p>⇓</p> <p>No indication or no data</p> <p>⇓</p> | Yes | Possibly no further testing may be deemed corrosive or irritant |
| 5 | <p>Valid and accepted <i>in vitro</i> skin corrosion test^e ⇒</p> <p>⇓</p> <p>Negative response or no data</p> | Positive response | Classify as corrosive ^a |
| 6 | <p>Valid and accepted <i>in vitro</i> skin irritation test^f ⇒</p> <p>⇓</p> <p>Negative response or no data</p> <p>⇓</p> | Positive response | Classify as irritant ^a |
| 7 | <p><i>In vivo</i> skin corrosion ⇒</p> | Positive response | Classify as corrosive ^a |

| | | | | |
|---|--|--|---|---|
| | test (one animal) ⇓ Negative response ⇓ | | | |
| 8 | <i>In vivo</i> skin irritation ⇒ test (three animals total) ^h ⇓ Negative response ⇒ ⇓ | | Positive response No further testing | Classify as irritant ^a |
| 9 | When it is ethical to perform ⇒ human patch testing ^g ⇓ Not as above ⇒ | | Positive response Negative response | Classify as irritant ^a No further testing, not classified |

Notes

- Classify in the HSNO Act classification scheme.
- Structure-activity and structure-property relationships are presented separately but would be conducted in parallel.
- Measurement of pH alone may be adequate, but assessment of acid or alkali reserve is preferable; methods are needed to assess buffering capacity.
- Pre-existing animal data should be carefully reviewed to determine if *in vivo* skin corrosion or irritation testing is needed. For example, testing may not be needed when a test material has not produced any dermal irritation in an acute dermal toxicity test at the limit dose, or produces very toxic effects in an acute dermal toxicity test. In the latter case, the material would be classed as being very hazardous by the dermal route for acute toxicity; it is moot whether the material is also corrosive or irritating on the skin. It should be kept in mind when evaluating acute dermal toxicity information that the reporting of skin lesions may be incomplete, testing and observations may be made on a species other than the rabbit, and species may differ in sensitivity in their responses.
- Examples of internationally accepted validated *in vitro* test methods for skin corrosion are OECD Test Guidelines 430 and 431.
- There are no validated and internationally accepted *in vitro* test methods for dermal irritation.
- This evidence could be derived from single or repeated exposures. There is no internationally accepted test method for human dermal irritation testing, but an OECD guideline has been proposed.
- Testing is usually conducted in three animals, one coming from the negative corrosion test.

11.1.2. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development Test

Guidelines or equivalent according to the principles of Good Laboratory Practice. Where such data are not available classification should be based on the best available data using a weight of evidence approach.

See section 1.3 in chapter 1 for information about assessing data quality.

See Appendix 11A for a detailed list of acceptable test methods for skin corrosion or irritancy.

11.1.3. Synergistic and antagonistic effects

If the applicant is aware of any available information about possible synergistic effects that may enhance the irritancy of the substance as a mixture, this must be considered. If a substance contains a component that has defatting properties, this component may enhance the irritant properties of the substance. (Note that substances that have defatting properties are not considered skin irritants in their own right.)

If the applicant is aware of any available information that antagonistic effects may occur such that the substance as a mixture classification is lower than indicated from the calculated value, this should be noted. For example, encapsulation of a substance as a mixture can lower the corrosivity or irritancy of the substance.

11.2. Skin corrosion or irritation hazard and classification criteria

11.2.1. Skin corrosion or irritation threshold criteria

Skin corrosion

Schedule 5 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with corrosive properties is not hazardous for the purposes of the Act unless—*

...

(b) *data for the substance indicates that the substance has a pH level of 2 or less, or 11.5 or more; or*

(c) *data for the substance indicates destruction of dermal tissue, being visible necrosis through the epidermis and into the dermis, as a result of exposure to the substance, that has not fully reversed within an observation period of 14 days.*

Skin irritation

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*

...

(f) *data for the substance indicates a mean Draize score of 1.5 or more for either of the skin irritation effects known as erythema or oedema, as a result of exposure to the substance.*

11.2.2. Skin corrosion or irritation classification criteria for substances

Skin corrosion

Schedule 5 to the Hazardous Substances (Classification) Regulations 2001 identifies three classification subclasses for substances that are corrosive to skin (subclass 8.2) as follows.

- Subclass 8.2 – substances that are corrosive to dermal tissue
A subclass 8.2 classification and one of the subsequent three categories apply to any substance that meets the following criteria.
 - a. Category 8.2A
A substance for which data indicate irreversible destruction of dermal tissue, which destruction is visible necrosis through the epidermis and into the dermis, within 1 hour following exposure to the substance for less than or equal to 3 minutes in greater than or equal to 33% of exposures as a result of exposure to the substance.
 - b. Category 8.2B
A substance for which data indicate irreversible destruction of dermal tissue, which destruction is visible necrosis through the epidermis and into the dermis, within 14 days following exposure to the substance for greater than 3 minutes, but not more than 1 hour, in greater than or equal to 33% of exposures as a result of exposure to the substance.
 - c. Category 8.2C
A substance for which data indicate irreversible destruction of dermal tissue, which destruction is visible necrosis through the epidermis and into the dermis, within 14 days following exposure to the substance for greater than 1 hour, but not more than 4 hours, in greater than or equal to 33% of exposures as a result of exposure to the substance.

Skin irritation

Schedule 4 to the Hazardous Substances Classification Regulations 2001 identifies two classification categories for substances that are skin irritants (subclass 6.3) as follows.

- Category 6.3A – substances that are irritating to the skin
 - a. A substance for which reversible adverse effects on dermal tissue are evidenced by data indicating a mean Draize score greater than or equal to 2.3, but less than or equal to 4.0, for either erythema or eschar or oedema, as a result of exposure to the substance.
 - b. A substance for which data indicate skin inflammation, including alopecia over a limited area, hyperkeratosis, hyperplasia, and scaling, that persists for 14 days following exposure to the substance in at least 66% of exposures, as a result of exposure to the substance.
 - c. A substance for which data indicate a pronounced variability of adverse effects between and within test exposures, even though the effects of exposure to the substance do not meet the criteria in (a) or (b), or for hazard classification 6.3B.

- Category 6.3B – substances that are mildly irritating to the skin

A substance for which reversible adverse effects on dermal tissue are evidenced by data indicating a mean Draize score greater than or equal to 1.5, but less than 2.3, for either of the skin irritation effects known as erythema or oedema, as a result of exposure to the substance.

The classification criteria above are based on the Globally Harmonized System for Classification and Labelling of Chemicals (GHS) (United Nations, 2007) criteria for skin irritation and corrosion. See Appendix 11C [below](#) for a comparison of the HSNO Act criteria with the GHS and Appendix 11D [below](#) for a comparison with the European Union risk phrases.

Animal irritant responses within a test can be quite variable, as they are with corrosion. The main criterion for classification of a substance as irritant to skin, as shown above, is the mean value of the Draize scores for either erythema, eschar, or oedema calculated over all the animals tested (See Appendix 11B [below](#) for grading Draize scores and calculating mean Draize scores.)

Reversibility of skin lesions is another consideration in evaluating irritant responses

When inflammation persists to the end of the observation period in two or more test animals, taking into consideration a limited degree of alopecia, hyperkeratosis, hyperplasia, and scaling, then a material is considered an irritant.

11.3. Classification of mixtures

11.3.1. Classification of mixtures when data are available for the complete mixture

The mixture is classified using the criteria in sections 11.2.1 and 11.2.2, and taking into account the testing and evaluation strategies to develop data for these hazard classes.

Unlike other hazard classes, alternative tests are available for the skin corrosivity of certain types of substances and mixtures that can give an accurate result for classification purposes, as well as being simple and relatively inexpensive to perform. When considering testing the mixture, classifiers are encouraged to use a tiered weight-of-evidence strategy (as included in the criteria for the classification of substances for skin corrosion or irritation) to help ensure an accurate classification and avoid unnecessary animal testing.

A mixture is considered corrosive to skin (subclass 8.2) if it has a pH of 2 or less or 11.5 or greater. If consideration of the alkali/acid reserve suggests the substance or mixture may not be corrosive despite the low or high pH value, then further testing needs to be carried out to confirm this, preferably by using an appropriate validated *in vitro* test.

11.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its skin corrosion or irritation properties, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data should be used in accordance with the bridging principles set out below.

This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that has an equivalent or lower corrosivity or irritancy classification than the least corrosive or irritant original ingredient and that is not expected to affect the corrosivity or irritancy of other ingredients, then the new mixture may be classified as equivalent to the original mixture. Alternatively, the method explained in section 11.3.3 could be applied.

b. Batching

The corrosion or irritation potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product, which is produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the toxicity of the batch has changed. If the latter occurs, new classification is necessary.

c. Concentration of mixtures of the highest corrosion or irritation category

If a tested mixture classified in the highest subcategory for corrosion is concentrated, a more concentrated mixture should be classified in the highest corrosion subcategory without additional testing. If a tested mixture classified in the highest category for skin irritation is concentrated and does not contain corrosive ingredients, a more concentrated mixture should be classified in the highest irritation category without additional testing.

d. Interpolation within one toxicity category

For three mixtures with identical ingredients, where mixtures A and B are in the same corrosion or irritation toxicity category and mixture C has the same toxicologically active ingredients with concentrations intermediate to the concentrations of those ingredients in mixtures A and B, then mixture C is assumed to be in the same corrosion or irritation category as are A and B.

e. Substantially similar mixtures

Given the following:

- i. two mixtures: (A + B) and (C + B);
 - ii. the concentration of ingredient B is essentially the same in both mixtures;
 - iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
 - iv. data on corrosion or irritation for ingredients A and C are available and substantially equivalent; that is, they are in the same hazard category and are not expected to affect the toxicity of ingredient B;
- then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

f. Aerosols

A hazard classification may be assigned for skin corrosion or skin irritation for aerosol products.

Generally, however, the propellant should not be taken into account when classifying aerosols, as the gaseous propellant will not be present in the liquid that comes into contact with the skin.

11.3.3. Classification of mixtures when data are available for all or some components of the mixture

To make use of all available data for when classifying the skin corrosion or irritation hazards of mixtures, the following assumption has been made and is applied where appropriate in the tiered approach.

The 'relevant ingredients' of a mixture are those that are present in concentrations (including impurities and additives) of 1% (by weight for solids, liquids, dusts, mists, and vapours, and by volume for gases) or greater, unless there is a presumption (for example, in the case of corrosive ingredients) that an ingredient present at a concentration of less than 1% can still be relevant for classifying the mixture for skin corrosion or irritation.

In general, the approach to classifying mixtures as corrosive or irritant to skin when data are available on the components, but not on the mixture as a whole, is based on the theory of additivity. In additivity, for subclass 6.3 (8.2), each corrosive or irritant component contributes to the overall corrosive or irritant properties of the mixture in proportion to its potency and concentration. A weighting factor of 10 is used for corrosive components when they are present at a concentration below the generic concentration limit for classification with an 8.2 classification, but are at a concentration that will contribute to the classification of the mixture as an irritant. The mixture is classified as corrosive or irritant when the sum of the concentrations of such components exceeds a concentration limit.

Table 11.2 provides the generic concentration limits to be used to determine if the mixture is considered to be an irritant or a corrosive to the skin.

Particular care must be taken when classifying certain types of chemicals such as acids, bases, inorganic salts, aldehydes, phenols, and surfactants. Many of these substances are corrosive or irritant at concentrations < 1%. For mixtures containing strong acids or bases, the pH should be used as classification criteria since pH will be a better indicator of corrosion than will the concentration limits in Table 11.2. A mixture containing corrosive or irritant ingredients that cannot be classified based on the additivity approach, because chemical characteristics make this approach unworkable, should be classified as 8.2A, 8.2B, or 8.2C if the mixture contains $\geq 1\%$ of a corrosive ingredient(s) or as 6.3A or 6.3B if the mixture contains $\geq 3\%$ of an irritant ingredient(s). Table 11.3 outlines the approach for substances where additivity does not work.

On occasion, reliable data may show that the skin corrosion or irritation hazard of an ingredient will not be evident when present at a level above the generic concentration limits mentioned in Table 11.2 and Table 11.3. In these cases, the mixture is classified according to that data. On occasion, when it is expected that the skin corrosion or irritation of an ingredient is not evident when present at a level above the generic concentration limits mentioned in Table 11.2 and Table 11.3, testing of the mixture may be considered. In those cases the tiered weight-of-evidence strategy should be applied, as described in section 11.3.3 and illustrated in Table 11.1.

If any data show that an ingredient(s) may be corrosive or irritant at a concentration of < 1% (corrosive) or < 3% (irritant), the mixture should be classified accordingly.

Table 11.2: Skin corrosive or irritant classifications for mixtures using additivity

| Sum of concentrations of ingredients classified as category | Classification of a mixture as category | | |
|---|---|---------------|----------------|
| | 8.2A, 8.2B, or 8.2C | 6.3A | 6.3B |
| 8.2A, 8.2B, or 8.2C (alone or sum) | ≥ 5% | ≥ 1% but < 5% | |
| 6.3A (alone or sum) | | ≥ 10% | ≥ 1% but < 10% |
| 6.3B (alone or sum) | | | ≥ 10% |
| (8.2A, 8.2B, and 8.2C × 10) + 6.3A | | ≥ 10% | ≥ 1% but < 10% |
| (8.2A, 8.2B, and 8.2C × 10) + 6.3A + 6.3B | | | ≥ 10% |

Notes

- Determine whether a classification should be assigned by starting at the top left column of the table and working down.
- The percentage of each component(s) that triggers a particular classification (multiplied by a factor of 10 where indicated) is compared against the concentration cut-offs required to trigger a classification in the mixture.
- When the sum of all ingredients classified as category 8.2A, 8.2B, or 8.2C is:
 - each ≥ 5%, then the mixture is classified in the same subclass (for example, if the sum of 8.2A ≥ 5% then classify as 8.2A);
 - if the sum of 8.2A is < 5% but the sum of 8.2A + 8.2B is ≥ 5%, then classify the mixture as 8.2B; and
 - if the sum of 8.2A + 8.2B is < 5% but the sum of 8.2A + 8.2B + 8.2C is ≥ 5%, then classify the mixture as 8.2C.

Table 11.3: Classification of substances where additivity does not apply

| Ingredient | Concentration | Mixture classified as category |
|---|---------------|--------------------------------|
| Acid with pH ≤ 2 | ≥ 1% | 8.2A, 8.2B, or 8.2C |
| Base with pH ≥ 11.5 | ≥ 1% | 8.2A, 8.2B, or 8.2C |
| Other corrosive (8.2A, 8.2B, or 8.2C) ingredients for which additivity does not apply | ≥ 1% | 8.2A, 8.2B, or 8.2C |
| Other irritant (6.3A) ingredients for which additivity does not apply | ≥ 3% | 6.3A |

It may be possible to calculate the molar balance of an acid and base in a neutralisation reaction. This would allow the determination of residual acid or base (after neutralisation) and whether the residual acid or base should trigger a classification in the mixture.

Appendix 11A: Acceptable test methods for skin corrosion or irritancy

11A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union*. Part 2 – Testing Methods. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.

11A.2 Skin corrosion or irritancy test guidelines

The guidelines in Table 11A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.
http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series Retrieved 14 August 2007.

See also Table 11A.1.

Table 11A.1: Skin corrosion or irritancy toxicity test guidelines for chemicals, including mixtures

| Test | Test guideline number | | |
|---|-----------------------|--|-------------|
| | OECD | EC | USEPA OPPTS |
| Acute dermal corrosion or irritation | 404 | EC B.4: Acute toxicity: dermal corrosion or irritation | 870.2500 |
| <i>In vitro</i> skin corrosion: transcutaneous electrical resistance test (TER) | 430 | EC B.40: Skin corrosion (<i>in vitro</i>) | – |
| <i>In vitro</i> skin corrosion: human skin model test | 431 | EC B.40: Skin corrosion (<i>in vitro</i>) | – |
| <i>In vitro</i> membrane barrier test method for skin corrosion | 435 | – | – |

Appendix 11B: Grading skin reactions and calculating mean Draize scores

1. Grading skin reactions

Table 11B.1 displays the grading scores for skin reactions. This is sourced from Organisation for Economic Co-operation and Development Test Guideline 404.

Table 11B.1: Grading scores for skin reactions

| Score | Description | |
|-------|---|---|
| | Erythema | Oedema |
| 0 | No erythema | No oedema |
| 1 | Very slight erythema (barely perceptible) | Very slight oedema (barely perceptible) |
| 2 | Well-defined erythema | Slight oedema (edges of area well defined by definite raising) |
| 3 | Moderate to severe erythema | Moderate oedema (raised approximately 1 mm) |
| 4 | Severe erythema (beet redness) to eschar formation preventing grading of erythema | Severe oedema (raised more than 1 mm and extending beyond the area of exposure) |

2. Calculating mean Draize scores

An example (using substance X) for calculating the mean Draize score is in Table 11B.2.

Table 11B.2: Calculating mean Draize scores for erythema and oedema for substance X

| Animal number/sex Bodyweight (kg) | Scoring interval | Erythema | Oedema |
|--------------------------------------|------------------|----------|--------|
| 1/M 2.634 | 1 hour | 2 | 1 |
| | 24 hours | 2 | 0 |
| | 48 hours | 2 | 0 |
| | 72 hours | 1 | 0 |
| | 7 days | 1 | 0 |
| 2/M 2.754 | 1 hour | 2 | 1 |
| | 24 hours | 1 | 0 |
| | 48 hours | 1 | 0 |
| | 72 hours | 0 | 0 |
| | 7 days | 0 | 0 |

| | | | |
|--------------|----------|---|---|
| 3/M 2.591 | 1 hour | 2 | 1 |
| | 24 hours | 2 | 1 |
| | 48 hours | 2 | 1 |
| | 72 hours | 2 | 1 |
| | 7 days | 1 | 0 |
| 4/F 2.932 | 1 hour | 2 | 1 |
| | 24 hours | 2 | 1 |
| | 48 hours | 2 | 0 |
| | 72 hours | 2 | 0 |
| | 7 days | 2 | 0 |
| 5/F 2.284 | 1 hour | 2 | 1 |
| | 24 hours | 2 | 1 |
| | 48 hours | 1 | 1 |
| | 72 hours | 1 | 0 |
| | 7 days | 0 | 0 |
| 6/F 2.719 | 1 hour | 3 | 1 |
| | 24 hours | 3 | 1 |
| | 48 hours | 3 | 1 |
| | 72 hours | 2 | 1 |
| | 7 days | 2 | 0 |

Note:

Mean Draize scores:

Erythema = $\frac{\text{Total of 24-, 48-, and 72-hour Draize scores for all six animals}}{\text{Total number of 24-, 48-, and 72-hour readings for all six animals}}$

Erythema = $31/18 = 1.72$

Oedema = $\frac{\text{Total of 24-, 48-, and 72-hour Draize scores for all six animals}}{\text{Total number of 24-, 48-, and 72-hour readings for all six animals}}$

Oedema = $9/18 = 0.5$

Substance X is thus classified as 6.3B for skin irritancy based on a mean Draize score (erythema) of 1.72.

Appendix 11C: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act skin corrosion/irritation hazard classification criteria

Table 11C.1 and Table 11C.2 display the skin corrosion categories from the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 11C.1: Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act skin corrosion criteria

| GHS corrosive subcategories | HSNO Act equivalent category | Corrosive in \geq one of three animals | |
|-----------------------------|------------------------------|--|----------------|
| | | Exposure | Observation |
| 1A | 8.2A | \leq 3 minutes | \leq 1 hour |
| 1B | 8.2B | $>$ 3 minutes \leq 1 hour | \leq 14 days |
| 1C | 8.2C | $>$ 1 hour \leq 4 hours | \leq 14 days |

Table 11C.2: Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act skin irritation criteria

| GHS irritation subcategory | Criteria | HSNO Act equivalent category |
|----------------------------|---|------------------------------|
| Irritant Category 2 | <p>One of the following.</p> <ul style="list-style-type: none"> • Mean value of $\geq 2.3 < 4.0$ for erythema/eschar or for oedema in at least two of three tested animals from gradings at 24, 48, and 72 hours after patch removal or, if reactions are delayed, from grades on three consecutive days after the onset of skin reactions. • Inflammation that persists to the end of the observation period normally 14 days in at least two animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling. • In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above. | 6.3A |
| Mild Irritant Category 3 | <p>Mean value of $\geq 1.5 < 2.3$ for erythema/eschar or for oedema from gradings in at least two of three tested animals from grades at 24, 48 and 72 hours or, if reactions are delayed, from grades on three consecutive days after the onset of skin reactions (when not included in the irritant category above).</p> | 6.3B |

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 11D: Comparison of European Union skin corrosion/irritancy risk phrases with HSNO Act skin corrosion/irritancy classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 11D.1. Note that some cut-off values are not totally aligned with HSNO Act classification categories. This is noted in the table and for classification purposes. A precautionary approach is advocated, so the higher hazard category is assigned.

Table 11D.1: Comparison of European Union skin corrosion/irritancy risk phrases with equivalent HSNO Act classifications

| European Union risk phrases | HSNO Act equivalent category |
|---|---|
| Skin corrosion | |
| <p>Corrosive (C)</p> <p>A substance is considered to be Corrosive (C) if, when applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal during the test for skin irritation or if the results can be predicted, for example from strongly acid or alkaline reactions (demonstrated pH of ≤ 2 or ≥ 11.5. Alkaline or acidic reserves should also be taken into account).</p> <p>Classification can be based on the results of validated <i>in vitro</i> tests.</p> <p>A substance is determined to be hazardous and classified as Corrosive (C) and assigned either risk phrase R34 or R35 in accordance with the criteria below.</p> | |
| <p>R35 Causes severe burns</p> <p>If when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to three minutes exposure, or if this result can be predicted.</p> | 8.2A |
| <p>R34 Causes burns</p> <p>If when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to four hours exposure, or if this result can be predicted.</p> <p>Organic hydroperoxides, except where evidence to the contrary is available.</p> | 8.2B and 8.2C |
| Skin irritancy | |
| <p>Irritant (Xi)</p> <p>A substance is determined to be hazardous and classified as Irritant (Xi) if it causes:</p> <ol style="list-style-type: none"> inflammation of the skin; eye irritation; serious eye effects; or irritation to the respiratory system. | |
| <p>R38 Irritating to skin</p> <p>Organic peroxides, except where evidence to the contrary is available.</p> <p>Substances that cause significant inflammation of the skin, based on practical observation in</p> | <p>6.3A</p> <p>Note there is some overlap</p> |

humans.

Substances that cause significant inflammation of the skin that persists for at least 24 hours after an exposure period of up to four hours determined on the rabbit according to a test method analogous to OECD Test Guidelines 404.

Inflammation of the skin is significant if:

- e. the mean value of the scores for either erythema and eschar formation or oedema formation, calculated over all the animals tested, is 2 or more; or
- f. in the case where the test has been completed using three animals, either erythema and eschar formation or oedema formation equivalent to a mean value of 2 or more calculated for each animal separately has been observed in two or more animals.

Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time.

with 6.3B

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

12. Eye Corrosion and Irritation – Subclass 6.4 (8.3)

12.1. General considerations

12.1.1. Eye corrosion or irritation overview

See section 9.6 in chapter 9 for definitions of the key terms used in this chapter.

Before there is any *in vivo* testing for eye corrosion or eye irritation, all existing information on a substance should be reviewed. Preliminary decisions can often be made from existing data as to whether a substance causes corrosive (that is, irreversible) damage to the eyes. If, based on this information, a substance can be classified, no testing is required. A highly recommended way of evaluating information on existing substances or of approaching new uninvestigated substances is to use a tiered testing strategy for eye corrosion and eye irritation.

Several factors should be considered in determining the eye corrosive or irritation potential of a substance before testing is undertaken. Accumulated human and animal experience should be analysed first, as it gives information directly relevant to effects on the eye. In some cases, enough information may be available from structurally related compounds to classify the substance. Likewise, pH extremes (≤ 2 and ≥ 11.5), may produce eye corrosion, especially when associated with significant buffering capacity. Such substances are expected to produce significant effects on the eyes. Possible skin corrosion must be evaluated before eye corrosion or irritation is considered in order to avoid testing for local effects on eyes with skin corrosive substances. *In vitro* alternatives that have been validated and accepted may be used to make classification decisions.

Although information might be gained from the evaluation of single parameters within a tier (for example, caustic alkalis with extreme pH should be considered as local corrosives), there is merit in considering the totality of existing information and making an overall weight-of-evidence determination. This is especially true when there is information available on only some parameters. Generally, primary emphasis should be placed on expert judgement, considering human experience with the substance, followed by the outcome of skin irritation testing and well-validated alternative methods. Animal testing with corrosive substances should be avoided whenever possible.

A tiered approach to the evaluation of initial information should be considered where applicable (Table 12.1), recognising that all elements may not be relevant in certain cases. The tiered testing approach provides good guidance on how to organise existing information on a test material and to make a weight-of-evidence decision about hazard assessment and hazard classification – ideally, without conducting new animal tests.

Table 12.1: Testing and evaluation strategy for eye corrosivity and eye irritation

| Step | Parameter | Findings | Conclusions |
|------|---|--|---|
| 1a | Data relating to historical human or animal experience ↓ | <pre> graph TD A[Data relating to historical human or animal experience] --> B[Eye corrosive] A --> C[Eye irritant] </pre> | <pre> graph TD B --> D[Category 8.3A] C --> E[Category 6.4A] </pre> |

| Step | Parameter | Findings | Conclusions |
|------|--|--|--|
| | No or don't know ↓ | | |
| 1b | Data relating to historical human or animal experience ↓ No or don't know ↓ | → Skin corrosive | → No evaluation of effects on eyes; deemed to be Category 8.3A |
| 1c | Data relating to historical human or animal experience ↓ No or don't know ↓ | → Skin irritant | → No evaluation of effects on eyes; deemed to be Category 6.4A |
| 2a | Structure activity relationships/ structure property relationships (SARs/SPRs) ↓ No or don't know ↓ | → Eye corrosive | → Category 8.3A |
| 2b | SARs/SPRs ↓ No or don't know ↓ | Eye irritant | → No evaluation of effects on eyes; deemed to be Category 6.4A |
| 2c | SARs/SPRs ↓ No or don't know ↓ | Skin corrosive | → No evaluation of effects on eyes; deemed to be category 8.3A |
| 3a | pH/acid or alkaline reserve ↓ | → pH ≥ 11.5 or pH ≤ 2 (considering acid or alkaline reserve) | → Category 8.3A |
| 3b | 2 < pH < 11.5 (no buffering potential) ↓ | | |
| 4 | Other information indicating the material is a skin corrosive ↓ | → Yes | → No evaluation of effects on eyes; deemed to be category 8.3A |

| Step | Parameter | Findings | Conclusions |
|------|--|--|--|
| | No ↓ | | |
| 5a | Is a valid <i>in vitro</i> test available to assess severe damage to eyes ↓ | → No | → Go to step 6 |
| 5b | <i>In vitro</i> test for severe eye irritation ↓ Not a severe eye irritant ↓ | → Eye corrosive | → Category 8.3A |
| 6 | Is a valid <i>in vitro</i> test for eye irritation available No → ↓ Yes → ↓ | → But <i>in vitro</i> test for severe eye irritancy was negative → In the absence of any <i>in vitro</i> test | → Go to step 8 → Go to step 7 |
| 6a | <i>In vitro</i> eye irritation test ↓ No indication of eye irritant properties ↓ | → Eye irritant | → Category 6.4A |
| 7 | Experimentally assess skin corrosion potential (see Table 11.1 in chapter 11) ↓ Not corrosive ↓ | → Skin corrosive | → No evaluation of effects on eyes, deemed to be category 8.3A |
| 8 | One-rabbit eye test ↓ Not corrosive ↓ | → Eye corrosive | → Category 8.3A |
| 9 | One or two further rabbits ↓ | → Eye irritant → Not an eye irritant | → Category 6.4A → Not classified |

Notes: See also Table 11.1 in chapter 11.

- Step 1: Data relating to historical human or animal experience: pre-existing information on eye irritation and skin corrosion are shown separately because evaluation of skin corrosion has to be considered if there is no information on local effects on eyes. Analysis of pre-existing experience with the chemical may identify serious eye damage, corrosion, and irritation potential for both skin and eye effects.
- i Step 1a: Reliable determination of eye irritancy based on human or animal experience – Depends on expert judgement. In most cases human experience is based on accidental events, so the local effects detected after an accident have to be compared with classification criteria created for evaluation of animal test data.
 - ii Step 1b: Evaluation of data on skin corrosivity – Skin corrosive substances should not be instilled into the eyes of animals; such substances should be considered as leading to eye corrosion as well (category 8.3A).
- Step 2: Structure activity relationships (SARs)/structure property relationships (SPRs) for eye irritation and skin corrosion are shown separately but in reality would probably be done in parallel. This stage should be completed using validated and accepted SAR/SPR approaches. The SAR/SPR analysis may identify serious eye damage, corrosion, and irritation potential for both skin and eye effects.
- i Step 2a: Reliable determination of eye irritancy only by theoretical evaluations – In most cases it will be appropriate only for substances that are homologous to agents with very well-known properties.
 - ii Step 2c: Theoretical evaluation of skin corrosivity – Skin corrosive substances should not be instilled into the eyes of animals; such substances should be considered as leading to eye corrosion as well (category 8.3A).
- Step 3: pH extremes (≤ 2 and ≥ 11.5) may indicate strong local effects, especially in combination with assessment of acid or alkaline reserve, substances exhibiting such physico-chemical properties should be considered as leading to eye corrosion (category 8.3A).
- Step 4: All attainable information should be used, including human experience. But this information should be restricted to that which pre-exists (for example, the results of a skin median lethal dose (LD50) test or historical information on skin corrosion).
- Step 5: These must be alternative methods for the assessment of eye irritation or corrosion (for example, irreversible corneal opacity) that have been validated in accordance with internationally agreed principles and criteria.
- Step 6: This step seems not to be achievable in the near future. Validated alternative methods for the reliable assessment of (reversible) eye irritation need to be developed.
- Step 7: In the absence of any other relevant information, it is essential to obtain this using an internationally recognised corrosion or irritation test before proceeding to a rabbit eye irritation test. This must be conducted in a staged manner. If possible, this should be achieved using a validated, accepted *in vitro* skin corrosivity assay. If this is not available, then the assessment should be completed using animal tests (see section 11.1 in chapter 11).
- Step 8: Staged assessment of eye irritation *in vivo* – If in a limit test with one rabbit eye corrosion is detected, no further testing is needed
- Step 9: Only two animals may be used for irritation testing (including the one used for evaluating possible serious effects), if these two animals give concordant clearly irritant or clearly non-irritant responses. In the case of different or borderline responses, a third animal is needed. Depending on the result of this three-animal test, classification may be required or not.

12.1.2. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions are clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Preferably, data should be derived using Organisation for Economic Co-operation and Development Test Guidelines or equivalent according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 [above](#) in chapter 1 for information about assessing data quality.

See Appendix 12A [below](#) for a detailed list of acceptable test methods for eye corrosion or irritancy.

12.1.3. Synergistic and antagonistic effects

If the applicant is aware of any available information about possible synergistic effects that may enhance the irritancy of the substance as a mixture, this must be considered.

If the applicant is aware of any available information that antagonistic effects may occur such that the substance as a mixture classification is lower than indicated from the calculated value, this should be noted. For example, the encapsulation of a substance as a mixture can lower the corrosivity or irritancy of the substance.

12.2. Eye corrosion or irritation hazard and classification criteria

12.2.1. Eye corrosion or irritation threshold criteria

Eye corrosion

Schedule 5 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with corrosive properties is not hazardous for the purposes of the Act unless—*

...

(b) data for the substance indicates that the substance has a pH level of 2 or less, or 11.5 or more; or

(c) data for the substance indicates destruction of dermal tissue, being visible necrosis through the epidermis and into the dermis, as a result of exposure to the substance, that has not fully reversed within an observation period of 14 days; or

(d) data for the substance indicates destruction of ocular tissue being adverse effects on the cornea, iris, or conjunctiva, as a result of exposure to the substance, that has not fully reversed within an observation period of 21 days; or

(e) data for the substance indicates a mean Draize score of 3 or more for the eye irritation effect known as corneal opacity, as a result of exposure to the substance; or

(f) data for the substance indicates a mean Draize score of 1.5 or more for the eye irritation effect known as iritis, as a result of exposure to the substance.

Eye irritation

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*

...

(g) *data for the substance indicates a mean Draize score of 1 or more for either of the eye irritation effects known as corneal opacity or iritis, as a result of exposure to the substance;*
or

(h) *data for the substance indicates a mean Draize score of 2 or more for either of the eye irritation effects known as conjunctival redness or chemosis, as a result of exposure to the substance.*

12.2.2. Eye corrosion or irritation classification criteria for substances

Eye corrosion

Schedule 5 to the Hazardous Substances (Classification) Regulations 2001 identifies one classification subclass for substances that are corrosive to eyes (subclass 8.3) as follows.

- Subclass 8.3 – substances that are corrosive to ocular tissue

A subclass 8.3 classification and the subsequent category apply to any substance that meets one of the following criteria.

- a. A substance for which data indicate evidence in at least 33% of exposures of destruction of ocular tissue, being adverse effects on the cornea, iris or conjunctiva as a result of exposure to the substance that are not expected to reverse or have not fully reversed within 21 days of exposure to the substance.
- b. A substance for which data indicate a mean Draize score greater than or equal to 3 for corneal opacity as a result of exposure to the substance.
- c. A substance for which data indicate a mean Draize score greater than 1.5 for iritis as a result of exposure to the substance.

These observations include animals with grade 4 cornea lesions and other severe reactions (for example, destruction of cornea) observed at any time during the test, as well as persistent corneal opacity, discoloration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight. In this context, persistent lesions are considered those that are not fully reversible within an observation period of normally 21 days.

Eye irritation

Schedule 4 to the Hazardous Substances Classification Regulations 2001 identifies one classification category for substances that are eye irritants (subclass 6.4).

- Category 6.4A – substances that are irritating to the eye

A substance for which adverse effects on ocular tissue, as a result of exposure to the substance, are evidenced by data indicating a mean Draize score:

- a. ≥ 1 , but < 3 , for corneal opacity, where the effects reverse within 21 days after exposure to the substance; or
- b. ≥ 1 , but < 1.5 , for iritis, where the effects reverse within 21 days after exposure to the substance; or
- c. ≥ 2 , for conjunctival redness, where the effects reverse within 21 days after exposure to the substance; or
- d. ≥ 2 , for conjunctival oedema (chemosis), where the effects reverse within 21 days after exposure to the substance.

The classification criteria above are based on the Globally Harmonised System for Classification and Labelling of Chemicals (GHS) (United Nations, 2007). See Appendix 12C for a comparison of the HSNO Act criteria with the GHS criteria and Appendix 12D for comparisons with the EU risk phrases of effects on eyes.

See Table 12.6 in Appendix 12B for an example of calculating a mean Draize score from an acute eye irritation study.

Substances mildly irritating to the eye

The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) acknowledges that substances classified as irritating to the eye may be only mildly irritating when the effect is fully reversible within 7 days. The Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification system does not specifically mention this. However, this is a lesser degree of hazard. Therefore, a substance whose irritating effect fully reverses within 7 days is classified as category 6.4A, but the identification of this hazard may state 'mildly irritating to the eye' as opposed to 'irritating to the eye'.

12.3. Classification of mixtures

12.3.1. Classification of mixtures when data are available for the complete mixture

The mixture will be classified using the criteria in sections 12.2.1 and 12.2.2, and taking into account the testing and evaluation strategies used to develop data for these hazard classes.

Unlike other hazard classes, alternative tests are available for skin corrosivity of certain types of chemicals that can give an accurate result for classification purposes, as well as being simple and relatively inexpensive to perform. When considering testing the mixture manufacturers are encouraged to use a tiered weight-of-evidence strategy as included in the criteria for classification of substances for skin corrosion, eye corrosion, and eye irritation to help ensure an accurate classification, as well as to avoid unnecessary animal testing.

A mixture is considered to cause eye corrosion (category 8.3A) if it has a pH of 2 or less or 11.5 or greater. If consideration of the alkali/acid reserve suggests the substance or preparation may not have the potential to cause eye corrosion despite the low or high pH value, then further testing needs to be carried out to confirm this, preferably by using an appropriate validated *in vitro* test.

12.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its skin corrosivity or potential to cause eye corrosivity or irritation, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data should be used in accordance with the following agreed bridging rules. This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that has an equivalent or lower classification for eye corrosivity or irritancy than the least corrosive or irritant original component, and that is not expected to affect the corrosivity or irritancy of other components, then the mixture may be classified as equivalent to the original mixture. Alternatively, the method in section 12.3.3 could be applied.

b. Batching

The irritation or corrosion potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product, which is produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the toxicity of the batch has changed. If the latter occurs, a new classification is necessary.

c. Concentration of mixtures of the highest eye corrosion or irritation category

If a tested mixture classified in the highest category for eye corrosion (8.3A) is concentrated, a more concentrated mixture should be classified in the highest eye corrosion category without additional testing. If a tested mixture classified in the highest subcategory for eye irritation (6.4A) is concentrated and does not contain eye corrosive ingredients, then the new mixture should be classified in the highest eye irritation category without additional testing.

d. Interpolation within one irritation or corrosion class

For three mixtures with identical ingredients, where mixtures A and B are in the same eye irritation or corrosion toxicity category and mixture C has the same toxicologically active ingredients with concentrations intermediate to the concentrations of those ingredients in mixtures A and B, then mixture C is assumed to be in the same eye irritation or corrosion category as are mixtures A and B.

e. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
 - ii. the concentration of ingredient B is essentially the same in both mixtures;
 - iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B); and
 - iv. data on eye irritation or corrosion for ingredients A and C are available and substantially equivalent;
- then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

f. Aerosols

A hazard classification may be assigned for eye corrosion or irritation for aerosol products. However, the propellant should generally not be taken into account when classifying aerosols, as the gaseous propellant will not be present in the liquid that comes into contact with the eyes.

12.3.3. Classification of mixtures when data are available for all or some ingredients of the mixture

To make use of all available data for purposes of classifying the eye irritation or corrosive properties of the mixtures, the following assumption has been made and is applied where appropriate in the tiered approach.

The 'relevant ingredients' of a mixture are those that are present in concentrations of 1% (by weight for solids, liquids, dusts, mists, and vapours, and by volume for gases) or greater, unless there is a presumption (for example, in the case of corrosive ingredients) that an ingredient present at a concentration of less than 1% can still be relevant for classifying the mixture for eye irritation or serious eye damage.

In general, the approach to the classification of mixtures as eye irritant or corrosive to the eye when data are available on the components, but not on the mixture as a whole, is based on the theory of additivity. In additivity for subclass 6.4 (8.3), each corrosive or irritant component contributes to the overall irritant or corrosive properties of the mixture in proportion to its potency and concentration. A weighting factor of 10 is used for corrosive components when they are present at a concentration below the concentration limit for classification with 8.3A, but are at a concentration that will contribute to the classification of the mixture as an irritant. The mixture is classified as corrosive to the eye or an eye irritant when the sum of the concentrations of such components exceeds a threshold cut-off value or concentration limit.

Table 12.2 provides the cut-off value or concentration limits to be used to determine if the mixture should be classified an irritant or corrosive to the eye.

Particular care must be taken when classifying certain types of chemicals such as acids and bases, inorganic salts, aldehydes, phenols, and surfactants. The approach explained above might not work because many of such substances are corrosive or irritant at concentrations < 1%. For mixtures containing strong acids or bases, the pH should be used as classification criteria (see section 12.3.1) since pH will be a better indicator of serious eye damage than will the concentration limits in Table 12.2. A mixture containing corrosive or irritant ingredients that cannot be classified based on the additivity approach applied in Table 12.2 because of chemical characteristics that make this approach unworkable, should be classified as 8.3A, if it contains $\geq 1\%$ of a corrosive ingredient and as 6.4A when it contains $\geq 3\%$ of an irritant ingredient. Classification of mixtures with ingredients for which the approach in Table 12.2 does not apply is summarised in Table 12.3.

On occasion, reliable data may show that the reversible and irreversible eye effects of an ingredient are not evident when present at a level above the generic cut-off values or concentration limits mentioned in Table

12.2 and Table 12.3. In these cases the mixture could be classified according to those data. On occasion, when it is expected that the skin corrosion or irritation or the reversible or irreversible eye effects of an ingredient will not be evident when present at a level above the generic concentration or cut-off values mentioned in Table 12.2 and Table 12.3, testing of the mixture may be considered. In those cases, the tiered weight of evidence strategy should be applied as referred to in section 12.3 and Table 12.1, and explained in detail in this chapter.

If data show that an ingredient(s) may be corrosive or irritant at a concentration of <1% (corrosive) or <3% (irritant), the mixture should be classified accordingly.

Table 12.2: Eye corrosive or irritancy classifications for mixtures using additivity

| Sum of concentrations of ingredients classified as category | Classification of a mixture as category | |
|---|---|--------------|
| | 8.3A | 6.4A |
| 8.3A or (8.2A, 8.2B, or 8.2C) (alone or sum) | ≥ 3% | ≥ 1% but <3% |
| 6.4A | | ≥ 10% |
| (8.3A × 10) + 6.4A | | ≥ 10% |
| 8.2A, 8.2B, and 8.2C + 8.3A | ≥ 3% | ≥ 1% but <3% |
| 10 × (8.2A, 8.2B, and 8.2C + 8.3A) + 6.4A | | ≥ 10% |

Notes

- Determine whether a classification should be assigned by starting at the top left column of the table and working down.
- The percentage of each component(s) that triggers a particular classification (multiplied by a factor of 10 where indicated) is compared against the concentration cut-offs required to trigger a classification in the mixture.
- When 8.3A and 8.2A, 8.2B, and 8.2C components are summed, care should be taken to ensure the same component is not counted twice (that is, the component triggers both skin and eye corrosion classifications).

Table 12.3: Concentration of substances where additivity does not apply

| Ingredient | Concentration | Mixture classified as category |
|--|---------------|--------------------------------|
| Acid with pH ≤ 2 | ≥ 1% | 8.3A |
| Base with pH ≥ 11.5 | ≥ 1% | 8.3A |
| Other corrosive (8.3A) ingredients for which additivity does not apply | ≥ 1% | 8.3A |
| Other irritant (6.4A) ingredients for which additivity does not apply | ≥ 3% | 6.4A |

It may be possible to calculate the molar balance of an acid and base in a neutralisation reaction. This would allow the determination of residual acid or base (after neutralisation) and whether the residual acid or base should trigger a classification in the mixture.

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 12A: Acceptable test methods for eye corrosion or irritancy

12A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.

12A.2 Eye corrosion or irritancy test guidelines

The guidelines in Table 12A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.
http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series Retrieved 14 August 2007.

See also Table 12A.1.

Table 12A.1: Eye corrosion or irritancy toxicity test guidelines for chemicals, including mixtures

| Test | Test guideline number | | |
|----------------------|-----------------------|--|-------------|
| | OECD | EC | USEPA OPPTS |
| Acute eye irritation | 405 | EC B.5: Acute toxicity: Eye irritation or corrosion | 870.2400 |

Appendix 12B: Grading of eye reactions and calculating mean Draize scores

12B.1 Grading eye reactions

Table 12B.1 displays the grading scores for eye reactions. This is sourced from Organisation for Economic Co-operation and Development Test Guideline 405.

Table 12B.1: Grading of eye reactions

| Score | Description | | | |
|-------|---|--|--|---|
| | Corneal opacity | Iritis | Conjunctival redness | Conjunctival oedema (chemosis) |
| 0 | No ulceration or opacity | Normal | Normal | Normal |
| 1 | Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible | Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect) | Some blood vessels hyperaemic (injected) | Some swelling above normal |
| 2 | Easily discernible translucent area; details of iris slightly obscured | Hemorrhage, gross destruction, or no reaction to light | Diffuse, crimson colour; individual vessels not easily discernible | Obvious swelling, with partial eversion of lids |
| 3 | Nacrous area; no details of iris visible; size of pupil barely discernible | – | Diffuse beefy red | Swelling, with lids about half closed |
| 4 | Opaque cornea; iris not discernible through the opacity | – | – | Swelling, with lids more than half closed |

12B.2 Calculating mean Draize scores

An example (using substance X) for calculating the mean Draize score is in Table 12B.2.

Table 12B.2: Calculating mean Draize scores for erythema and oedema for substance x

| Rabbit number/sex (bodyweight kg) | 1/M (3.15) | | | | 2/M (2.97) | | | | 3/M (3.41) | | | | 1/F (2.94) | | | | 2/F (3.12) | | | | 3/F (2.74) | | | |
|--------------------------------------|---------------|----|----|----|---------------|----|----|----|---------------|----|----|----|---------------|----|----|----|---------------|----|----|----|---------------|----|----|----|
| | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 |
| Time after treatment (hrs) | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 |
| Cornea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degree of opacity | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Area of opacity | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Iris | | | | | | | | | | | | | | | | | | | | | | | | |
| Iritis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Conjunctivae | | | | | | | | | | | | | | | | | | | | | | | | |
| Redness | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| Chemosis | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Discharge | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Total score | 10 | 6 | 4 | 4 | 10 | 4 | 4 | 2 | 10 | 6 | 6 | 2 | 8 | 4 | 4 | 2 | 8 | 4 | 2 | 2 | 8 | 2 | 2 | 2 |

Mean Draize scores

Conjunctival redness = $\frac{\text{Total of 24-, 48-, and 72-hour Draize scores for all six animals}}{\text{Total number of 24-, 48-, and 72-hour readings for all six animals}}$

$$\text{Conjunctival redness} = 27/18 = 1.5$$

Conjunctival chemosis = $\frac{\text{Total of 24-, 48-, and 72-hour Draize scores for all six animals}}{\text{Total number of 24-, 48-, and 72-hour readings for all six animals}}$

$$\text{Conjunctival chemosis} = 4/18 = 0.22$$

Under the HSNO Act, substance X would *not* be classified for eye irritancy.

Appendix 12C: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act eye corrosion or irritation hazard classification criteria

Table 12C.1 and Table 12C.2 display the eye corrosion or irritation categories from the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 12C.1: Eye corrosion categories from the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and the HSNO Act equivalent

| GHS irreversible effects on the eye (eye corrosion) categories | Criteria | HSNO Act equivalent category |
|--|--|------------------------------|
| Eye irritant Category 1 (irreversible effects on the eye) | <p>A test material that produces:</p> <ul style="list-style-type: none"> • at least in one animal effects on the cornea, iris, or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or • at least in two of three tested animals, a positive response of: <ol style="list-style-type: none"> i. corneal opacity ≥ 3; and/or ii. iritis > 1.5; <p>calculated as the mean scores following grading at 24, 48, and 72 hours after installation of the test material.</p> | 8.3A |

Table 12C.2: Eye irritant categories from the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and the HSNO Act equivalent

| GHS reversible effects on the eye categories | Criteria | HSNO Act equivalent category |
|---|---|------------------------------|
| Eye irritant Category 2A (irritating to eyes) | <p>A test material that produces at least in two of three tested animals a positive response of:</p> <ul style="list-style-type: none"> • corneal opacity ≥ 1; and/or • (iritis ≥ 1; and/or • conjunctival redness ≥ 2; and/or • conjunctival oedema (chemosis) ≥ 2; <p>calculated as the mean scores following grading at 24, 48, and 72 hours after installation of the test material, and which fully reverses within an observation period of normally 21 days.</p> | 6.4A* |
| Eye irritant Category 2B (mildly irritating to eyes) | <p>Within this category an eye irritant is considered mildly irritating to eyes (2B) when the effects listed above (under 2A) are fully reversible within 7 days of observation.</p> | |

Note

* The GHS acknowledges that substances classified as irritating to the eye may be only mildly irritating when the effect is fully reversible within 7 days. The HSNO Act classification system does not specifically mention this. However, this is a lesser degree of hazard. Therefore, a substance whose irritating effect fully reverses within 7 days is classified category 6.4A, but the identification of this hazard may state 'mildly irritating to the eye' as opposed to 'irritating to the eye'.

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 12D: Comparison of European Union eye corrosion or irritancy risk phrases with HSNO Act eye corrosion or irritancy classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 12D.1. Note that some cut-off values are not totally aligned with HSNO Act classification categories. This is noted in the table, and for classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 12D.1: Comparison of European Union eye corrosion or irritancy risk phrases with equivalent HSNO Act classification

| European Union risk phrases | HSNO Act equivalent category |
|--|------------------------------|
| <p><i>Irritant (Xi)</i> A substance is determined to be hazardous and classified as Irritant (Xi) if it causes:</p> <ul style="list-style-type: none"> • inflammation of the skin; • eye irritation; • serious eye effects; or • irritation to the respiratory system. | |
| <p><i>R41 Risk of serious damage to eyes</i> Substances that cause severe ocular lesions, based on practical experience in humans. Substances that, when applied to the eye of the animal cause severe ocular lesions that occur within 72 hours after exposure and persist for at least 24 hours. Ocular lesions are severe when the results of the standard eye irritation test correspond to:</p> <ul style="list-style-type: none"> • cornea opacity equal to or greater than 3; or • iris lesion greater than 1.5. <p>When three animals are used in the test, the mean values on two or more animals are equivalent to:</p> <ul style="list-style-type: none"> • cornea opacity equal to or greater than 3; or • iris lesion equal to 2. <p>In both cases all scores at each of the reading times (24, 48, and 72 hours) for an effect should be used in calculating the respective mean values. Ocular lesions are also severe:</p> <ul style="list-style-type: none"> • when they are still present at the end of the observation time. • if the substance causes irreversible coloration of the eyes. | 8.3A |
| <p><i>R36 Irritating to eyes</i> Organic peroxides except where evidence to the contrary is available. Substances that cause significant ocular lesions, based on practical experience in humans. Substances that, when applied to the eye of the animal, cause significant ocular lesions that occur within 72 hours after exposure and persist for at least 24 hours. Ocular lesions are considered significant when the results of tests carried out in accordance with a method analogous to OECD Test Guideline 405 correspond to:</p> <ul style="list-style-type: none"> • cornea opacity equal to or greater than 2 but less than 3; • iris lesion equal to or greater than 1 but not greater than 1.5; • redness of the conjunctivae equal to or greater than 2.5; or • oedema of the conjunctivae (chemosis) equal to or greater than 2, or, when three animals are used in the test, the mean values, on two or more animals, are equivalent | 6.4A |

to:

- cornea opacity equal to or greater than 2 but less than 3;
- iris lesion equal to or greater than 1 but less than 2;
- redness of the conjunctivae equal to or greater than 2.5; or
- oedema of the conjunctivae (chemosis): equal to or greater than 2.

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

13. Respiratory or Contact Sensitisation – Subclass 6.5

13.1. General considerations

13.1.1. Respiratory or contact sensitisation

See section 9.6 in chapter 9 for definitions of the key terms used in this chapter.

Sensitisation includes two phases: the first phase is the induction of specialised immunological memory in an individual by exposure to an allergen; the second phase is the production of a cell-mediated or antibody-mediated response by a sensitised individual exposed to an allergen that is sufficient to elicit the response. This two-phase process applies to both respiratory and contact sensitisation.

For contact sensitisation, an induction phase is required in which the immune system learns to react. Clinical symptoms can then arise when subsequent exposure is sufficient to elicit a visible skin reaction (the elicitation phase). As a consequence, predictive tests usually follow this pattern in which there is an induction phase, the response to which is measured by a standardised elicitation phase, typically involving a patch test. The local lymph node assay, which directly measures the induction response, is the exception.

Evidence of contact sensitisation in humans normally is assessed by a diagnostic patch test.

Usually, for both contact and respiratory sensitisation, lower levels are necessary for elicitation than are required for induction.

13.1.2. Sensitisation by other routes

Sensitisation may occur through routes other than through contact or respiratory exposure (for example, photosensitisation or oral ingestion causing sensitisation). At this stage, sensitisation through these routes will not trigger a 6.5 classification. However, when the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 and Hazardous Substances (Classification) Regulations 2001 are reviewed, the criteria for classifying substances as sensitisers may be revised to capture substances that cause sensitisation through routes other than contact and respiratory sensitisation.

Photosensitisation

Photosensitisation reactions may occur when a substance absorbs ultra-violet (UV) or visible light.

Photosensitisation includes:

- photo-irritation, which is a light-induced skin response to a photo-reactive chemical; and
- photo-allergy, which is an immunologically mediated reaction to a chemical initiated by the formation of photo-products (for example, the photo-products produce an antigen).

Substances that cause photosensitisation (for example, halogenated aromatic hydrocarbons or sunscreen agents can cause photo-contact allergy) should not be classified under subclass 6.5. Photosensitisation is not considered an intrinsic property of a substance as an external stimulus is required (for example, UV or visible light).

Oral ingestion causing sensitisation

Substances that are orally ingested that can cause a systemic allergic response (for example, antibiotics) are not classified under subclass 6.5, because the hazard and classification criteria discussed below relate strictly to contact and respiratory sensitisation.

13.1.3. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development (OECD) Test Guidelines or equivalent according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 for information about assessing data quality.

See Appendix 13A for a detailed list of acceptable test methods for respiratory or contact sensitisation.

13.2. Respiratory or contact sensitisation hazard and classification criteria

13.2.1. Respiratory or contact sensitisation threshold criteria

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 Minimum degrees of hazard

(1) A substance with toxic properties is not hazardous for the purposes of the Act unless—

...

- (i) data for the substance indicates positive evidence of respiratory sensitisation in animals as a result of exposure to the substance; or*
- (j) data for the substance indicates positive evidence of sensitisation by skin contact in animals as a result of exposure to the substance of either—*
 - i. 30% or more sensitisation response in an adjuvant type test method; or*
 - ii. 15% or more sensitisation response in a non-adjuvant type test; or*
- (k) data for the substance, in the opinion of an expert, indicates evidence in humans of specific respiratory hypersensitivity (including asthma, rhinitis and alveolitis) as a result of exposure to the substance; or*
- (l) data for the substance, in the opinion of an expert, indicates evidence in humans of sensitisation by skin contact as a result of exposure to the substance.*

13.2.2. Respiratory or contact sensitisation classification criteria for substances

Schedule 4 to the Hazardous Substances (Classification) Regulations 2001 identifies two classification categories for substances that are sensitisers (subclass 6.5). It should be noted that these two classification categories do not reflect a difference in the magnitude of the effect but reflect the exposure route and nature of effect.

- Category 6.5A – substances that are respiratory sensitisers
 - a. A substance for which data indicate to an expert positive respiratory sensitisation effects in a relevant animal test as a result of exposure to the substance.
 - b. A substance for which data indicate to an expert evidence in humans of specific respiratory hypersensitivity (including asthma, rhinitis, and alveolitis) with the clinical character of an allergic reaction as a result of exposure to the substance.
- Category 6.5B – substances that are contact sensitisers
 - a. A substance for which data indicate to an expert positive contact sensitisation effects in a reliable animal test either:
 - i. equal to or greater than 30% sensitisation response in an adjuvant type test method as a result of exposure to the substance; or
 - ii. equal to or greater than 15% sensitisation response in a non-adjuvant type test method as a result of exposure to the substance; or
 - b. A substance for which data indicate to an expert evidence in humans of sensitisation by skin contact as a result of exposure to the substance.

The classification criteria above are based on the Globally Harmonised System for Classification and Labelling (GHS) (United Nations, 2007) for sensitisation. See Appendix 13B for a comparison of the HSNO Act and GHS criteria and Appendix 13C for a comparison of the HSNO Act criteria with the EU risk phrases for sensitisation.

13.2.3. Using human evidence and animal studies to classify respiratory sensitisers (subclass 6.5A)

Human evidence

Evidence that a substance can induce specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis, conjunctivitis, and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.

When considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases, the:

- size of the population exposed; and
- extent of exposure.

The evidence referred to above could be:

- clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence, which may include:
 - a. an *in vivo* immunological test (for example, the skin prick test);
 - b. an *in vitro* immunological test (for example, serological analysis);
 - c. studies that may indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven (for example, repeated low level irritation or pharmacologically mediated effects);
 - d. a chemical structure related to substances known to cause respiratory hypersensitivity;
- data from positive bronchial challenge tests with the substance, conducted according to accepted guidelines for determining a specific hypersensitivity reaction.

Clinical history should include both medical and occupational history to determine a relationship between exposure to a specific substance and the development of respiratory hypersensitivity. Relevant information includes aggravating factors in the home and workplace, the onset and progress of the disease, and family and medical histories of the patient in question. The medical history should also include a note of other allergic or airway disorders from childhood, and the patient's smoking history.

The results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own. It is, however, recognised that in practice many of the examinations listed above will already have been carried out.

Animal studies

Data from appropriate animal studies³ that may be indicative of the potential of a substance to cause sensitisation by inhalation in humans⁴ may include:

- measurements of Immunoglobulin E (IgE) and other specific immunological parameters (for example, in mice); and
- specific pulmonary responses in guinea pigs.

13.2.4. Contact sensitisation – specific considerations (subclass 6.5B)

For classification of a substance, evidence should include any or all of:

- a. positive data from patch testing, normally obtained in more than one dermatology clinic;

³ Recognised animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, animal testing may be used; for example, a modification of the guinea pig maximisation test for determining the relative allergenicity of proteins. However, these tests still need further validation.

⁴ The mechanisms by which substances induce symptoms of asthma are not yet fully known. For preventative measures, these substances are considered respiratory sensitisers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyperreactivity, they should not be considered respiratory sensitisers.

- b. epidemiological studies showing allergic contact dermatitis caused by the substance (situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small);
- c. positive data from appropriate animal studies;
- d. positive data from experimental studies in humans; and
- e. well-documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic.

Positive effects seen in either humans or animals will normally justify classification.

Evidence from animal studies is usually much more reliable than evidence from human exposure. However, when evidence is available from both sources, but the results conflict, the quality and reliability of the evidence from both sources must be assessed to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm the lack of effects seen in animal tests. Consequently, positive human data on contact sensitisation are usually derived from case-control or other, less-defined, studies. The evaluation of human data must, therefore, be carried out with caution, because the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition, and any preventive measures taken. Negative human data should not normally be used to negate positive results from animal studies.

If none of the above-mentioned conditions is met, the substance need not be classified as a contact sensitiser. However, a combination of two or more indicators of contact sensitisation as listed below may alter the decision. This should be considered on a case-by-case basis. Indicators of contact sensitisation include:

- a. isolated episodes of allergic contact dermatitis;
- b. epidemiological studies of limited power (for example, where chance, bias, or confounders have not been ruled out fully with reasonable confidence);
- c. data from animal tests, performed according to existing guidelines, that do not meet the criteria for a positive result described in sections 13.2.1 and 13.2.2, but which are sufficiently close to the limit to be considered significant;
- d. positive data from non-standard methods; and
- e. positive results from close structural analogues.

13.2.5. Immunological contact urticaria

Substances meeting the criteria for classification as respiratory sensitisers may also cause immunological contact urticaria. Consideration should be given to classifying these substances also as contact sensitisers.

Substances that cause immunological contact urticaria without meeting the criteria for respiratory sensitisers should also be considered for classification as contact sensitisers.

No recognised animal model is available to identify substances that cause immunological contact urticaria. Therefore, classification is normally based on human evidence, which will be similar to that for contact sensitisation.

13.2.6. Using animal studies to classify contact sensitisers

When an adjuvant type test method for contact sensitisation is used, a response in at least 30% of the animals is considered positive. For a non-adjuvant test method a response in at least 15% of the animals is considered positive. Test methods for contact sensitisation are described in the OECD Test Guideline 406 (the guinea pig maximisation test and the Buehler guinea pig test) and Test Guideline 429 (local lymph node assay) (see Appendix 13A). Other methods may be used provided they are well validated and scientific justification is given. The mouse ear swelling test (MEST), appears to be a reliable screening test to detect moderate to strong sensitisers, and can be used as a first stage in the assessment of contact sensitisation potential. If there is a positive result in this latter test, it may not be necessary to conduct a further guinea pig test.

When evaluating animal data generated according to OECD or equivalent guidelines for contact sensitisation, the proportion of sensitised animals may be considered. This reflects the sensitising capacity of a substance in relation to its mildly irritating dose. This dose may vary between substances. A more appropriate evaluation of the sensitising capacity of a substance could be carried out if the dose–response relationship was known for the substance.

Some substances are extremely sensitising at low doses, while others require high doses and prolonged exposure before sensitisation develops. For the purpose of hazard classification it may be considered preferable to distinguish between strong and moderate sensitisers. However, at present, animal or other test systems to subcategorise sensitisers have not been validated and accepted. Therefore, subcategorisation is not currently considered as part of the harmonised classification system.

13.3. Classification of mixtures

13.3.1. Classification of mixtures when data are available for the complete mixture

When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture can be classified by a weight-of-evidence evaluation of these data. Care should be exercised when evaluating data on mixtures that the dose used does not render the results inconclusive.

13.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its sensitising properties, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data will be used in accordance with the following agreed bridging rules. This ensures that the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that is not a sensitiser and is not expected to affect the sensitisation of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

b. Batching

The sensitising properties of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product, which is produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the sensitisation of the batch has changed. If the latter occurs, a new classification is necessary.

c. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of ingredient B is essentially the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
- iv. ingredient B is a sensitiser and ingredients A and C are not sensitisers; and
- v. ingredients A and C are not expected to affect the sensitising properties of ingredient B; then if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

d. Aerosols

i. Respiratory sensitisation

A hazard classification may be assigned for respiratory sensitisation for aerosol products. The classification should also take into account the propellant in the aerosol.

ii. Contact sensitisation

A hazard classification may be assigned for contact sensitisation for aerosol products. However, the propellant should generally not be taken into account when classifying aerosols, as the gaseous propellant will not be present in the liquid that comes into contact with the skin.

13.3.3. Classification of mixtures when data are available for all or some ingredients of the mixture

The mixture should be classified as a respiratory or contact sensitiser when at least one ingredient has been classified as a respiratory or contact sensitiser and is present at or above the appropriate cut-off value or concentration limit for the specific endpoint, as shown in Table 13.1 for solids/liquids and gases respectively.

Table 13.1: Cut-off values or concentration limits of ingredients of a mixture classified as contact sensitiser or respiratory sensitiser that would trigger classification of the mixture

| Ingredient classified as | Classification of a mixture as | | |
|--------------------------|--------------------------------|------------------------|--------|
| | Contact sensitiser | Respiratory sensitiser | |
| | All physical states | Solid/liquid | Gas |
| Contact sensitiser | ≥ 0.1% | – | – |
| Respiratory sensitiser | – | ≥ 0.1% | ≥ 0.1% |

The generic hazard cut-off values or concentration limits do not apply, if it can be shown that the substance causes a sensitisation hazard that will be evident below the generic hazard cut-off values or concentration limits.

Appendix 13A: Acceptable test methods for respiratory or contact sensitisation

13A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.

13A.2 Respiratory or contact sensitisation test guidelines

The guidelines in Table 13A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides which include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011..

See also Table 13A.1.

Table 13A.1: Respiratory* or contact sensitisation toxicity test guidelines for chemicals, including mixtures

| Test | Test guideline number | | |
|--|-----------------------|---|-------------|
| | OECD | EC | USEPA OPPTS |
| Skin sensitisation | 406 | EC B.6: Skin sensitisation | 870.2600 |
| Skin sensitisation: Local lymph node assay | 429 | EC B.42: Skin sensitisation: Local lymph node assay | 870.2600 |

Note

* Recognised animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, animal testing may be used, for example, a modification of the guinea pig maximisation test for determining the relative allergenicity of proteins. However, these tests still need further validation.

Appendix 13B: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act respiratory or contact sensitization hazard classification criteria

Table 13B.1 and Table 13B.2 display the eye corrosion/irritation categories from the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 13B.1: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act respiratory hazard classification criteria

| GHS respiratory sensitisation classification criteria | HSNO Act equivalent category |
|--|------------------------------|
| Substances are classified as respiratory sensitizers (category 1) if: <ol style="list-style-type: none"> there is evidence in humans that the substance can induce specific respiratory hypersensitivity; and/or there are positive results from an appropriate animal test. | 6.5A |

Table 13B.2: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act contact sensitization hazard classification criteria

| GHS contact sensitisation classification criteria | HSNO Act equivalent category |
|--|------------------------------|
| Substances are classified as contact sensitizers (category 1) if: <ol style="list-style-type: none"> there is evidence in humans that the substance can induce sensitisation by skin contact in a substantial number of people; or there are positive results from an appropriate animal test. | 6.5B |

Further details on the GHS classification criteria for respiratory or contact sensitisation are in United Nations (2007, pp 147–150).

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 13C: Comparison of European Union respiratory or skin sensitisation risk phrases with HSNO Act respiratory or skin sensitisation classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 13C.1. Note that some cut-off values are not totally aligned with HSNO Act classification categories. This is noted in the table, and for classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 13C.1: Comparison of European Union acute toxicity risk phrases with HSNO Act respiratory or skin sensitisation classifications

| European Union risk phrases | HSNO Act equivalent category |
|--|------------------------------|
| <p>Sensitisation (Xn)</p> <p>Substances are determined to be hazardous and classified as Harmful (Xn) and assigned one or more of the following risk phrases in accordance with the criteria given below.</p> | |
| <p>R42 May cause sensitisation by inhalation</p> <p>There is evidence that the substance or preparation can induce specific respiratory hypersensitivity.</p> <p>There are positive results from appropriate animal tests.</p> <p>The substance is an isocyanate, unless there is evidence that the substance does not cause respiratory hypersensitivity.</p> | 6.5A |
| <p>R43 May cause sensitisation by skin contact</p> <p>Practical experience shows that the substances are capable of inducing sensitisation by skin contact in a substantial number of people.</p> <p>There are positive results from an appropriate animal test.</p> | 6.5B |

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. <http://ec.europa.eu/>

14. Mutagenicity – Subclass 6.6

14.1. General considerations

14.1.1. Mutagenicity overview

See section 9.6 in chapter 9 [above](#) for definitions of the key terms used in this chapter.

This hazard class is primarily concerned with chemicals that may cause mutagenic effects in the germ cells of humans that can be transmitted to the progeny. However, mutagenicity/genotoxicity tests *in vitro* and in mammalian somatic cells *in vivo* are also considered in classifying substances and mixtures within this hazard class.

A mutagenic effect means a permanent change in the amount or structure of the genetic material in a cell, being a permanent change that is:

- manifested at the phenotypic level; or
- an underlying DNA modification (including specific base pair changes and chromosomal translocations).

The terms ‘mutagenic’ and ‘mutagen’ are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

A genotoxic effect means alterations to the structure, information content, or segregation of DNA, including:

- DNA damage caused by interference with its normal replication processes; and
- temporary non-physiological alterations to its replication.

The terms ‘genotoxic’ and ‘genotoxicity’ refer to those agents or processes that cause a genotoxic effect.

14.1.2. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonized test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development (OECD) Test Guidelines or equivalent according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

See Appendix 14A [below](#) for a detailed list of acceptable test methods for mutagenicity

14.2. Mutagenicity hazard and classification criteria

14.2.1. Mutagenicity threshold criteria

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:



2 Minimum degrees of hazard

(1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*

...

- (m) *data for the substance indicates evidence of mutagenic effects as a result of mammalian in vivo exposure to the substance; or*
- (n) *data for the substance indicates evidence of—*
 - i. *genotoxic effects as a result of mammalian in vivo exposure to the substance; and*
 - ii. *mutagenic effects as a result of in vitro exposure to the substance; or*
- (o) *data for the substance indicates evidence of mutagenic effects as a result of in vitro exposure of mammalian cells to the substance and the substance has a structure-activity relationship to known germ cell mutagens, where—*
 - i. *structure-activity relationship means a significant correlative relationship between the chemical structure of the substance and the chemical structure of a known germ cell mutagen; and*
 - ii. *the relationship relates to that germ cell mutagenic activity.*

14.2.2. Mutagenicity classification criteria for substances

Schedule 4 to the Hazardous Substances Classification Regulations 2001 identifies two classification categories for substances that are mutagenic (subclass 6.6).

- Category 6.6A – substances that are known or presumed human mutagens
 - a. A substance for which data indicate a causal relationship between the exposure of humans to the substance and the induction of heritable mutagenic effects in the germ cells of humans.
 - b. A substance for which data indicate evidence of heritable mutagenic effects in the germ cells of mammals as a result of *in vivo* exposure to the substance.
 - c. A substance for which data indicate, as a result of *in vivo* exposure to the substance:
 - i. evidence of mutagenic effects in the somatic cells of mammals; and
 - ii. evidence that the substance has the potential to cause mutagenic effects in germ cells of mammals (including evidence of genotoxic effects in germ cells or evidence of the ability of the substance or its metabolites to interact with the genetic material of germ cells).
 - d. A substance for which data indicate evidence of mutagenic effects in the germ cells of humans as a result of exposure to the substance without evidence of transmission to progeny (including an increase in the frequency of aneuploidy in sperm cells of exposed humans).
- Category 6.6B – substances that are suspected human mutagens
 - a. A substance for which data indicate evidence of mutagenic effects in the somatic cells of mammals as a result of *in vivo* exposure to the substance.

- b. A substance for which data indicate evidence of genotoxic effects in the somatic cells of mammals as a result of *in vivo* exposure to the substance, and evidence of mutagenic effects as a result of *in vitro* exposure to the substance.
- c. A substance for which data indicate evidence of mutagenic effects as a result of *in vitro* exposure of mammalian cells to the substance, where there is a structure activity relationship to known germ cell mutagens (which relationship is a significant correlative relationship between the chemical structure of the substance and the chemical structure of a known germ cell mutagen, where the relationship relates to that germ cell mutagen activity).

The classification criteria above are based on the Globally Harmonised System for Classification and Labelling of Chemicals (GHS) (United Nations, 2007). See Appendix 14B for a comparison of the HSNO Act criteria with the GHS criteria for mutagenicity and Appendix 14C for a comparison with the equivalent EU risk phrases.

14.2.3. Considerations for mutagenicity classification

To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in *in vitro* tests may also be considered.

The system is hazard based, classifying chemicals on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the quantitative risk assessment of chemical substances.

Classification for heritable effects in human germ cells is made on the basis of well-conducted, sufficiently validated tests, preferably as described in OECD Test Guidelines. Evaluation of the test results should be done using expert judgement and all the available evidence should be weighed for classification.

In vivo heritable germ cell mutagenicity tests include the:

- rodent dominant lethal mutation test (OECD Test Guideline 478);
- mouse heritable translocation assay (OECD Test Guideline 485); and
- mouse specific locus test (United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Test Guidelines 820.5200 and 820.5195).

In vivo somatic cell mutagenicity tests include the:

- mammalian bone marrow chromosome aberration test (OECD Test Guideline 475);
- mouse spot test (OECD Test Guideline 484); and
- mammalian erythrocyte micronucleus test (OECD Test Guideline 474).

Mutagenicity/genotoxicity tests in germ cells include:

- mutagenicity tests, including the:
 - mammalian spermatogonial chromosome aberration test (OECD Test Guideline 483); and
 - spermatid micronucleus assay;
- genotoxicity tests, including the:
 - sister chromatid exchange analysis in spermatogonia; and

- unscheduled DNA synthesis test (UDS) in testicular cells.

Genotoxicity tests in somatic cells include the:

- liver UDS *in vivo* (OECD Test Guideline 486); and
- mammalian bone marrow sister chromatid exchanges (SCE)

In vitro mutagenicity tests include the:

- *in vitro* mammalian chromosome aberration test (OECD Test Guideline 473);
- *in vitro* mammalian cell gene mutation test (OECD Test Guideline 476); and
- bacterial reverse mutation tests (OECD Test Guideline 471).

The classification of individual substances should be based on the total weight of evidence available, using expert judgement. When a single well-conducted test is used for classification, it should provide clear and unambiguously positive results. If new, well-validated tests arise, these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the chemical compared with the route of human exposure should also be taken into account.

14.3. Classification of mixtures

14.3.1. Classification of mixtures when data are available for the complete mixture

The classification of mixtures is based on the available test data for the individual ingredients of the mixture using cut-off values or concentration limits for the ingredients classified as germ cell mutagens.

The classification may be modified on a case-by-case basis based on the available test data for the mixture as a whole. In such cases, the test results for the mixture as a whole must be shown to be conclusive, taking into account dose and other factors such as duration of exposure, observations, and analysis (for example, statistical analysis and test sensitivity) of germ cell mutagenicity test systems.

14.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data will be used in accordance with the following agreed bridging rules. This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that is not expected to affect the germ cell mutagenicity of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

b. Batching

The germ cell mutagenic potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product produced by

and under the control of the same manufacturer, unless there is reason to believe there is significant variation in composition such that the germ cell mutagenic potential of the batch has changed. If the latter occurs, a new classification is necessary.

c. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of mutagen ingredient B is the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
- iv. data on toxicity for ingredients A and C are available and substantially equivalent; that is, they are in the same hazard category and are not expected to affect the germ cell mutagenicity of ingredient B;
then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

d. Aerosols

A hazard classification may be assigned for mutagenicity for aerosol products. The classification should also take into account the propellant in the aerosol.

14.3.3. Classification of mixtures when data are available for all or some ingredients of the mixture

A mixture will be classified as a mutagen when at least one ingredient has been classified as a category 6.6A or 6.6B mutagen and is present at or above the appropriate hazard cut-off value or concentration limit mentioned in Table 14.1 for category 6.6A and 6.6B respectively.

Table 14.1: Cut-off values or concentration limits of ingredients of a mixture classified as mutagenic that would trigger classification of the mixture

| Ingredient classified as category | Cut-off value or concentration limit triggering classification of a mixture as category | |
|-----------------------------------|---|------|
| | 6.6A | 6.6B |
| 6.6A mutagen | ≥ 0.1% | – |
| 6.6B mutagen | – | ≥ 1% |

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

OECD 1998. *The OECD Harmonised Integrated Hazard Classification System*. Organisation for Economic Co-operation and Development, Paris.

Appendix 14A: Acceptable test methods for mutagenicity

14A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. Harmonized Test Guidelines. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.
<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.

14A.2 Mutagenicity test guidelines

The guidelines in Table 14A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 14 August 2007.

See also Table 14A.1.

Table 14A.1: Mutagenicity test guidelines for chemicals, including mixtures

| Test | Test guideline number | | |
|---|-----------------------|--|-------------|
| | OECD | EC | USEPA OPPTS |
| <i>In vivo</i> heritable, germ cell mutation tests | | | |
| Rodent dominant lethal assay | 478 | EC B.22: Rodent dominant lethal test | 870.5450 |
| Rodent heritable translocation assays | 485 | EC B.25 Mouse heritable translocation | 870.5460 |
| Mouse visible specific locus test | – | | 870.5200 |
| Mouse biochemical specific locus test | – | | 870.5195 |
| <i>In vivo</i> somatic cell mutation tests: | | | |
| <i>In vivo</i> mammalian cytogenetics tests: Bone marrow chromosomal analysis | 475 | EC B.11: <i>In vivo</i> Mammalian bone marrow chromosome aberration test | 870.5385 |
| <i>In vivo</i> mammalian cytogenetics tests: Erythrocyte/bone marrow micronucleus assay | 474 | EC B.12 Mammalian erythrocyte micronucleus test | 870.5395 |
| Mouse spot test | 484 | EC B.24: Mouse spot test | – |
| Mutagenicity tests in germ cells | | | |
| <i>In vivo</i> mammalian cytogenetics tests: spermatogonial chromosomal aberrations | 483 | EC B.23: Mammalian spermatogonial chromosome aberration test | 870.5380 |
| Genotoxicity tests in germ cells | | | |
| <i>In vivo</i> sister chromatid exchange assay | – | – | 870.5915 |
| Genotoxicity tests in somatic cells | | | |
| <i>In vivo</i> unscheduled DNA synthesis test with mammalian liver cells | 486 | – | – |
| <i>In vitro</i> mammalian cell mutagenicity tests | | | |
| Detection of gene mutations in somatic cells in | 476 | EC B.17: | 870.5300 |

| Test | Test guideline number | | |
|--|-----------------------|---|-------------|
| | OECD | EC | USEPA OPPTS |
| culture | | Mutagenicity – In vitro cell gene mutation test | |
| <i>In vitro</i> mammalian cytogenetics | 473 | EC B.10: Mutagenicity – In vitro mammalian chromosome aberration test | 870.5375 |
| <i>In vitro</i> mammalian cell transformation tests | – | EC B.21: In vitro mammalian cell transformation test | – |
| Unscheduled DNA synthesis in mammalian cells in culture | 482 | EC B.18: DNA Damage and repair – Unscheduled DNA synthesis – Mammalian cells in vitro | 870.5550 |
| <i>In vitro</i> sister chromatid exchange assay in mammalian cells | 479 | EC B.19: Sister chromatid exchange assay in vitro | 870.5900 |
| <i>In vitro</i> microbial, or insect cell mutation tests | | | |
| <i>Escherichia coli</i> WP2 and WP2 uvrA reverse mutation assays | 472 | EC B.13: Mutagenicity - Reverse mutation test using bacteria | 870.5100 |
| Gene mutation in <i>Aspergillus nidulans</i> | – | – | 870.5140 |
| Gene mutation in <i>Neurospora crassa</i> | – | – | 870.5250 |
| <i>Salmonella typhimurium</i> reverse mutation assay | 471 | EC B.14: Mutagenicity - Reverse mutation test using bacteria | 870.5265 |
| Sex-linked recessive lethal test in <i>Drosophila melanogaster</i> | 477 | EC B.20: Sex-linked recessive lethal test in <i>Drosophila</i> | 870.5275 |

| Test | Test guideline number | | |
|--|-----------------------|--|-------------|
| | OECD | EC | USEPA OPPTS |
| | | melanogaster | |
| Bacterial DNA damage or repair tests | – | – | 870.5500 |
| Gene mutation assay in <i>Saccharomyces cerevisiae</i> | 480 | EC B.15: Gene mutation – Saccharomyces cerevisiae | – |
| Mitotic gene conversion in <i>Saccharomyces cerevisiae</i> | 481 | EC B.16: Mitotic recombination - Saccharomyces cerevisiae | 870.5575 |

Appendix 14B: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act mutagenicity hazard classification

Table 14B.1 displays the mutagenicity categories from the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 14B.1: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act mutagenicity hazard classification

| GHS carcinogenicity classification | HSNO Act equivalent category |
|--|------------------------------|
| <p>Category 1</p> <p>Chemicals known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.</p> <p><i>Category 1A</i></p> <p>Chemicals known to induce heritable mutations in germ cells of humans.</p> <p>Criterion: Positive evidence from human epidemiological studies.</p> <p><i>Category 1B</i></p> <p>Chemicals that should be regarded as if they induce heritable mutations in the germ cells of humans.</p> <p>Criterion:</p> <ul style="list-style-type: none"> • positive result(s) from <i>in vivo</i> heritable germ cell mutagenicity tests in mammals; or • positive result(s) from <i>in vivo</i> somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells; this supporting evidence may, for example, be derived from mutagenicity/genotoxic tests in germ cells <i>in vivo</i>, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or • positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. | 6.6A |
| <p>Category 2</p> <p>Chemicals that cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.</p> <p>Criterion:</p> <ul style="list-style-type: none"> • positive evidence obtained from experiments in mammals and/or in some cases from <i>in vitro</i> experiments, obtained from: <ol style="list-style-type: none"> a. somatic cell mutagenicity tests <i>in vivo</i>, in mammals; or b. other <i>in vivo</i> somatic cell genotoxicity tests that are supported by positive results from <i>in vitro</i> mutagenicity assays. <p>Note: Chemicals that are positive in <i>in vitro</i> mammalian mutagenicity assays, and show a chemical structure activity relationship to known germ cell mutagens, should be considered for classification as category 2 mutagens.</p> | 6.6B |

Note

* The GHS (United Nations, 2007) proposes a distinction between known (class 1A) and regarded (class 1B) human mutagens. The HSNO Act classification system groups these two subclasses under the same category (6.6A).

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 14C: Comparison of European Union acute toxicity risk phrases with HSNO Act mutagenicity classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 14C.1. Note that some cut-off values are not totally aligned with HSNO Act classification categories. This is noted in the table, and for classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 14C.1: Comparison of European Union acute toxicity risk phrases with HSNO Act mutagenicity classifications

| European Union risk phrases | HSNO Act equivalent category |
|---|------------------------------|
| <p>Mutagenic substances</p> <p>Substances are determined to be hazardous due to mutagenic effects if they fall into one of the following categories:</p> <ul style="list-style-type: none"> • Category 1: Substances known to be mutagenic to humans. • Category 2: Substances that should be regarded as if they are mutagenic to humans. • Category 3: Substances that cause concern for humans because of possible mutagenic effects, but in respect of which available information does not satisfactorily demonstrate heritable genetic damage. | |
| <p><i>Category 1</i></p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R46 in accordance with the criteria given below.</p> <p>R46 may cause heritable genetic damage</p> <p>A substance is included in category 1, if sufficient evidence establishes a causal relationship between human exposure to a substance and heritable genetic damage.</p> <p>To place a substance in category 1, positive evidence from human mutation epidemiology studies is needed. Examples of such substances are not known. It is recognised that it is extremely difficult to obtain reliable information from studies on the incidence of mutations in human populations, or on possible increases in their frequencies.</p> | 6.6A |
| <p><i>Category 2</i></p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R46 in accordance with the criteria given below.</p> <p>R46 may cause heritable genetic damage</p> <p>A substance is included in category 2 if there is sufficient evidence to provide a strong presumption that human exposure to the substance may result in the development of heritable genetic damage, generally on the basis of appropriate animal studies and other relevant information.</p> | 6.6A |
| <p><i>Category 3</i></p> <p>Substances are determined to be hazardous and classified as Harmful (Xn) and assigned risk phrase R68 in accordance with the criteria given below.</p> | 6.6B |

R68 Possible risk of irreversible effects

A substance is included in category 3, if there is evidence from appropriate mutagenicity studies that human exposure can result in the development of heritable genetic damage, but this evidence is insufficient to place the substance in category 2.

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

15. Carcinogenic Effects – Subclass 6.7

15.1. General considerations

15.1.1. Carcinogenicity overview

See section 9.6 in chapter 9 [above](#) for definitions of the key terms used in this chapter.

The purpose of carcinogenicity studies is to observe test animals for the development of neoplastic lesions during or after prolonged and repeated exposure to various doses of a test substance. Exposure should occur by an appropriate route and encompass a major portion of the animal's life span. Carcinogenesis is considered a multi-stage phenomenon with direct and indirect effect on the genome leading to the development of cancerous cells. The predominant theory is that 'initiating' events, which directly mutate DNA, are needed to cause cells to become cancerous. This process can be accelerated by promotional factors that increase cell division or decrease the effectiveness of repair mechanisms. The entire phenomenon usually takes considerable time for all the necessary events to occur and the effects to manifest. Chemical carcinogens can have initiating and/or promoting properties.

Chronic studies also observe test animals after prolonged and repeated exposure for a major portion of their life span, but determine effects that require a long latent period or are cumulative to become manifested. These studies generate data to identify the majority of chronic effects and to determine dose–response relationships for general toxicity, including neurological, physiological, and biochemical effects and exposure-related, morphological effects. The endpoints identified in chronic studies are considered as specific target organ effects (see chapter 17).

Some studies are designed to detect both carcinogenic and chronic effect endpoints.

15.1.2. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development Test Guidelines or equivalent, according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 for information about assessing data quality. See Appendix 15A for a detailed list of acceptable test methods for carcinogenicity.

15.2. Carcinogenicity threshold and classification criteria

15.2.1. Carcinogenicity threshold criteria

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*

...

(p) *reliable information for the substance indicates to an expert that exposure to the substance causes the development of cancer or an increase in the incidence of benign or malignant tumours in an organ or an organism.*

15.2.2. Carcinogenicity classification criteria

Schedule 4 to the Hazardous Substances (Classification) Regulations 2001 identifies two classification categories for substances that are carcinogenic (subclass 6.7).

- Category 6.7A – substances that are known or presumed human carcinogens
 - a. A substance for which data indicate sufficient evidence in humans of a causal relationship between exposure to the substance and the development of cancer in humans.
 - b. A substance for which data indicate sufficient evidence in animals of a causal relationship between exposure to the substance and an increased incidence of tumours.
 - c. A substance for which data indicate:
 - i. limited evidence in humans of a positive correlation between exposure to the substance and the development of human cancer; and
 - ii. limited evidence in animals that exposure to the substance may lead to an increased incidence of tumours.
- Category 6.7B – substances that are suspected human carcinogens:

A substance for which data indicate limited evidence in humans or limited evidence in animals that exposure to the substance may lead to the development of cancer or an increased incidence of tumours, where the strength and weight of the evidence indicate to an expert that the evidence is not sufficient to classify the substance in hazard classification 6.7A.

The classification criteria above are based on the Globally Harmonised System for Classification and Labelling of Chemicals (GHS) (United Nations, 2007). See Appendix 15B for a comparison of the HSNO Act criteria with those of the GHS. See Appendix 15C for comparisons with the EU and other jurisdictions' criteria for carcinogenicity.

'Evidence' in carcinogenicity studies involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the agent and an increased incidence of tumours. Limited evidence in humans

is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. See also the definitions in section 9.6 in chapter 9.

Assignment to either category 6.7A or 6.7B depends on the strength of the evidence and the weight of evidence obtained from human and/or animal studies.

Classify as category 6.7A (known human carcinogen), if evidence from human data showing a causal relationship between human exposure and the development of cancer in which chance, bias, and confounding could be ruled out with reasonable confidence. The existence of a causal relationship would be any of:

- an increased incidence of one or more cancer types in an exposed population in comparison with a non-exposed population;
- evidence of dose–time–response relationships; that is, an increased cancer incidence associated with higher exposure levels or with increasing exposure duration;
- an association between exposure and increased risk observed in more than one study;
- a demonstration of a decline in risk after reduction of exposure; and
- the specificity of any association, defined as an increased occurrence of cancer at one target organ or of one morphological type.

Classify as category 6.7A (presumed human carcinogen), if:

- evidence from animal data establishes a causal relationship between the substance and an increased incidence of malignant neoplasms or an appropriate combination of benign and malignant neoplasms, in two or more species of animal or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols; or
- a single study in one animal species establishes that malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour, or age at onset.

Evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

Classify as category 6.7B (suspected human carcinogen), if:

- evidence obtained from human data shows a positive association between exposure to the substance and cancer, but chance, bias, or confounding could not be ruled out with reasonable confidence;
- evidence obtained from animal data suggests a carcinogenic effect, but the evidence is not sufficiently convincing to place the substance in category 6.7A; for example:
 - the evidence of carcinogenicity is restricted to a single experiment;
 - carcinogenic effects occur only at very high dose levels exceeding the maximal tolerated dose (which is characterised by toxic effects that, although not yet reducing lifespan, go along with physical changes such as about a 10% retardation in weight gain);
 - the appearance of tumours, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumour formation; and

- the appearance of tumours only at the site of application in very sensitive test systems (for example, intraperitoneal or subcutaneous application of certain locally active compounds), if the particular target is relevant to humans;
- questions are unresolved regarding the adequacy of the design, conduct, or interpretation of the study; for example:
 - the existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (for example, hormonal effects on target organs or on mechanisms of physiological regulation or chronic stimulation of cell proliferation); and
 - the existence of a species-specific mechanism of tumour formation (for example, by specific metabolic pathways) irrelevant for humans;
- the substance increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms that may occur spontaneously in high incidences in certain strains; or
- there is a lack of genotoxicity in short-term tests *in vivo* and *in vitro*.

Do not assign a classification for carcinogenicity, if the:

- mechanism(s) of experimental tumour formation is/are clearly identified, with good evidence that such mechanism(s) cannot be extrapolated to humans for each tumour;
- only available tumour data are liver tumours in certain sensitive strains of mice (for example, B6C3F1 mice), without any other supplementary evidence; or
- only available tumour data are neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.

15.3. Additional considerations for carcinogenicity classification

Some of the criteria discussed in section 15.2 are complex and require expert judgement and a weight-of-evidence assessment as set out below.

Beyond the determination of the strength of evidence for carcinogenicity, other factors should be considered that influence the overall likelihood that an agent may pose a carcinogenic hazard in humans. The full list of factors that influence this determination is lengthy, but the most important ones are considered here.

The factors can increase or decrease the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends on the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease, rather than increase, the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

Important factors that may be taken into consideration when assessing the overall level of concern include:

- tumour type and background incidence (see section 15.3.5);
- multi-site responses;
- the progression of lesions to malignancy; and
- reduced tumour latency.

Additional factors that may increase or decrease the level of concern include:

- whether responses are in a single sex or both sexes (see section 15.3.3);
- whether responses are in a single or several species (see section 15.3.2);
- whether there is structural similarity with a chemical for which there is good evidence of carcinogenicity;
- the routes of exposure (certain chemicals can cause carcinogenicity through a specific route of exposure; for example, crystalline silica is a known human carcinogen when inhaled as a fine respirable dust);
- a comparison of absorption, distribution, metabolism, and excretion between test animals and humans;
- the possibility of a confounding effect of excessive toxicity at test doses (see section 15.3.4); and
- the mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, and immunosuppression (see section 15.3.6).

15.3.1. Mode of action

Mode of action in and of itself, or a consideration of comparative metabolism, should be evaluated on a case-by-case basis, and is part of an analytic evaluative approach. Any mode of action must be looked at closely in animal experiments, taking into consideration comparative toxicokinetics and toxicodynamics between the animal test species and humans to determine the relevance of the results to humans. This may lead to the possibility of discounting very specific effects of certain types of chemical. Life stage-dependent effects on cellular differentiation may also lead to qualitative differences between animals and humans. Only if a mode of action of tumour development is conclusively determined not to be operative in humans, may the carcinogenic evidence for that tumour be discounted. However, a weight-of-evidence evaluation for a substance calls for any other tumorigenic activity to be evaluated as well.

15.3.2. Response in multiple animal experiments

Positive responses in several species add to the weight of evidence that a chemical is a carcinogen. Taking into account the factors listed in section 15.3, chemicals with positive outcomes in two or more species would provisionally be classified 6.7A, until the human relevance of animal results is assessed. It should be noted, however, that positive results for one species in at least two independent studies, or a single positive study showing unusually strong evidence of malignancy, may also lead to a 6.7A classification. Consideration should also be given to evidence of mutagenic activity *in vivo*.

15.3.3. Responses in one sex or both sexes

Any case of sex-specific tumours should be evaluated in light of the total tumorigenic response to the substance observed at other sites (multi-site responses or incidence above background) in determining the carcinogenic potential of the substance.

If tumours are seen only in one sex of one animal species, the mode of action should be carefully evaluated to see if the response is consistent with the postulated mode of action. Effects seen in only one sex in a test species may be less convincing than effects seen in both sexes, unless there is a clear patho-physiological difference consistent with the mode of action to explain the single-sex response.

15.3.4. Confounding effects of excessive toxicity or localised effects

Tumours occurring only at excessive doses associated with severe toxicity generally have doubtful potential for carcinogenicity in humans. In addition, tumours occurring only at sites of contact and/or only at excessive doses need to be carefully evaluated for human relevance for carcinogenic hazard. For example, forestomach tumours in rats, following administration by gavage of an irritating or corrosive, non-mutagenic chemical may be of questionable relevance. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered.

15.3.5. Tumour type, reduced tumour latency

Unusual tumour types or tumours occurring with reduced latency may add to the weight of evidence for the carcinogenic potential of a substance, even if the tumours are not statistically significant.

Toxicokinetic behaviour is normally assumed to be similar in animals and humans, at least from a qualitative perspective. On the other hand, certain tumour types in animals may be associated with toxicokinetics or toxicodynamics that are unique to the animal species tested and may not be predictive of carcinogenicity in humans. Very few such examples have been agreed internationally. However, one example is the lack of human relevance of kidney tumours in male rats associated with compounds causing α_2 microglobulin nephropathy (Capen et al, 1999). Even when a particular tumour type may be discounted, expert judgement must be used in assessing the total tumour profile in any animal experiment.

15.3.6. Mutagenicity

It is recognised that genetic events are central in the overall process of cancer development. Therefore, evidence of mutagenic activity *in vivo* may indicate that a chemical has a potential for carcinogenic effects.

15.3.7. Other considerations

The following additional considerations apply to classification of chemicals into either category 6.7A or 6.7B. A chemical that has not been tested for carcinogenicity may in certain instances be classified in 6.7A or 6.7B based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, for example, for benzidine congener dyes.

The classification should also take into consideration whether the chemical is absorbed by a given route(s), or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show a lack of carcinogenicity.

It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, that is, the structure activity relationship, is taken into consideration when undertaking classification.

15.4. Classification of mixtures

15.4.1. Classification of mixtures when data are available for the complete mixture

The classification of mixtures is based on the available test data of the individual ingredients of the mixture using cut-off values or concentration limits for those ingredients. The classification may be modified on a case-by case basis based on the available test data for the mixture as a whole. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, and analysis (for example, statistical analysis and test sensitivity) of carcinogenicity test systems.

15.4.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its carcinogenic hazard, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data will be used in accordance with the following agreed bridging rules. This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that is not expected to affect the carcinogenicity of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

b. Batching

The carcinogenic potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product produced by and under the control of the same manufacturer, unless there is reason to believe there is significant variation in composition such that the carcinogenic potential of the batch has changed. If the latter occurs, a new classification is necessary.

c. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of carcinogenic ingredient B is the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
- iv. data on toxicity for ingredients A and C are available and substantially equivalent; that is, they are in the same hazard category and are not expected to affect the carcinogenicity of ingredient B; then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

d. Aerosols

A hazard classification may be assigned for carcinogenicity for aerosol products. The classification should also take into account the propellant in the aerosol.

15.4.3. Classification of mixtures when data are available for all or some ingredients of the mixture

The mixture will be classified as a carcinogen when at least one ingredient has been classified as a 6.7A or 6.7B carcinogen and is present at or above the appropriate cut-off value or concentration limit as shown in Table 15.1 for 6.7A and 6.7B respectively.

Table 15.1: Cut-off values or concentration limits of ingredients

| Ingredient classified as category | Cut-off values or concentration limits triggering classification of a mixture as category | |
|-----------------------------------|---|--------|
| | 6.7A | 6.7B |
| 6.7A carcinogen | ≥ 0.1% | – |
| 6.7B carcinogen | – | ≥ 0.1% |

Note: The hazard cut-off values or concentration limits in the table apply to solids and liquids (by weight) as well as gases (by volume).

The generic hazard cut-off values or concentration limits do not apply if it can be shown that the substance causes a carcinogenic hazard that will be evident below the generic hazard cut-off values or concentration limits.

References

Capen, CC, Dybing, E, Rice, JM, Wilbourn, JD (eds.) 1999. *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis*, Scientific Publication 147. International Agency for Research on Cancer.

Appendix 15A: Acceptable test methods for carcinogenicity

15A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 14 August 2007.

15A.2 Carcinogenicity test guidelines

The guidelines in Table 15A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.

See also Table 15A.1.

Table 15A.1: Carcinogenicity test guidelines for chemicals

| Test protocols | Test guideline | | |
|---|----------------|--|-------------|
| | OECD | EC | USEPA OPPTS |
| Carcinogenicity | 451 | EC B.32 Carcinogenicity test | 870.4200 |
| Combined chronic toxicity and carcinogenicity | 453 | EC B.33 Combined chronic toxicity/carcinogenicity test | 870.4300 |

Appendix 15B: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act carcinogenicity hazard classification

Table 15B.1 displays the carcinogenicity categories from the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 15B.1: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act carcinogenicity hazard classification

| GHS carcinogenicity classification | HSNO Act equivalent category* |
|--|-------------------------------|
| <p>Category 1: Known or presumed human carcinogens</p> <p>The placing of a chemical in category 1 is done on the basis of epidemiological and/or animal data. An individual chemical may be further distinguished.</p> <ul style="list-style-type: none"> • Category 1A: Known to have carcinogenic potential for humans; the placing of a chemical is largely based on human evidence. • Category 1B: Presumed to have carcinogenic potential for humans; the placing of a chemical is largely based on animal evidence. <p>Based on the strength of evidence together with additional considerations, such evidence may be derived from human studies that establish a causal relationship between human exposure to a chemical and the development of cancer (known human carcinogen).</p> <p>Alternatively, evidence may be derived from animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).</p> <p>In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.†</p> <p>Classification: Category 1 (A and B) carcinogen.</p> | 6.7A |
| <p>Category 2: Suspected human carcinogens</p> <p>The placing of a chemical in category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the chemical in category 1. Based on the strength of evidence together with additional considerations, such evidence may be from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.</p> <p>Classification: Category 2 carcinogen</p> | 6.7B |

Notes

* The GHS (United Nations, 2007) proposes a distinction between known (class 1A) and presumed (class 1B) human carcinogens. The HSNO Act classification system groups these two subclasses under the same category (6.7A).

† The GHS (United Nations, 2007) wording differs from that in the regulations made under the HSNO Act in that it assigns classification in this category on a case-by-case basis where expert judgement considers there is limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals. The wording in the HSNO Act regulations separates these two data sources. The GHS wording, based on expert judgement of these two data sources together, will result in a category 6.7A classification. If they occur separately, then the classification is category 6.7B.

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 15C: Comparison of European Union carcinogenicity risk phrases with HSNO Act carcinogenicity classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 15C.1. Note that some cut-off values are not totally aligned with the HSNO Act classification categories. This is noted in the table, and for classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 15C.1: Comparison of European Union acute toxicity risk phrases with HSNO Act carcinogenicity classifications

| European Union risk phrases | HSNO Act equivalent category |
|--|-------------------------------------|
| <p>Carcinogens</p> <p>Substances are determined to be hazardous due to carcinogenic effects if they fall into one of the following categories:</p> <ul style="list-style-type: none"> • Category 1: Substances known to be carcinogenic to humans. • Category 2: Substances that should be regarded as if they are carcinogenic to humans. • Category 3: Substances that cause concern for humans owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment. | <p>6.7A</p> <p>6.7A</p> <p>6.7B</p> |
| <p><i>Category 1</i></p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R45 or R49 in accordance with the criteria given below.</p> <p>R45 May cause cancer</p> <p>R49 May cause cancer by inhalation</p> <p>A substance is included in category 1, if there is sufficient evidence to establish a causal association between human exposure and the development of cancer on the basis of epidemiological data.</p> | <p>6.7A</p> |
| <p><i>Category 2</i></p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R45 or R49 in accordance with the criteria given below.</p> <p>R45 May cause cancer</p> <p>R49 May cause cancer by inhalation</p> <p>A substance is included in category 2, if there is sufficient evidence, on the basis of appropriate long-term animal studies or other relevant information, to provide a strong presumption that human exposure to that substance may result in cancer developing.</p> | <p>6.7A</p> |
| <p><i>Category 3</i></p> <p>Substances are determined to be hazardous and classified as Harmful (Xn) and assigned risk phrase R40 in accordance with the criteria given below.</p> <p>R40 possible risk of irreversible effects</p> | <p>6.7B</p> |

A substance is included in category 3 if there is some evidence from appropriate animal studies that human exposure can result in the development of cancer, but this evidence is insufficient to place the substance in category 2.

Category 3 comprises two subcategories.

- a. Substances that are well investigated, but for which the evidence of a tumour-inducing effect is insufficient for classification in category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.
- b. Substances that are insufficiently investigated. The available data are inadequate, but they raise concern for humans. This classification is provisional; further experiments are necessary before a final decision can be made.

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

Appendix 15D: Comparison of HSNO Act classifications with other carcinogenicity classifications

Table 15D.1 compares Hazardous Substances and New Organisms Act 1996 (HSNO Act) classifications with other carcinogenicity classifications.

Table 15D.1: HSNO Act classifications compared with other carcinogenicity classifications

| HSNO Act category | USEPA | IARC | NTP | OSHA | EU risk phrase |
|-------------------|---|---|---|-------------|----------------|
| 6.7A | (Group A) Human carcinogen | (Group 1) Carcinogenic to humans | Human carcinogen | Category I | R45 R49 |
| | (Group B1, B2) Probable human carcinogen | (Group 2A) Probably carcinogenic to humans | Reasonably anticipated to be a carcinogen | Category II | R45 R49 |
| 6.7B | (Group C) Possible human carcinogen | (Group 2B) Possibly carcinogenic to humans | | | R40 |
| No | (Group D) Not classifiable as to human carcinogenicity | (Group 3) Not classifiable as to human carcinogenicity | | | |
| | (Group E) Evidence of non-carcinogenicity for humans | (Group 4) Probably not carcinogenic to humans | | | |

Notes

EU = European Union; IARC = International Agency for Research on Cancer; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; USEPA = United States Environmental Protection Agency.

This table is only a guideline, so should not be used to overrule a classification based on the best available human or animal data.

16. Reproductive and Developmental Effects – Subclass 6.8

16.1. General considerations

16.1.1. Reproductive and developmental effects overview

See section 9.6 in chapter 9 for definitions of the key terms used in this chapter.

This classification subclass considers:

- adverse effects on sexual function and fertility (that is, reproductive effects); and
- adverse effects on the development of offspring (that is, developmental effects).

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, chemicals with these effects would be classified as reproductive/developmental toxicants.

For classification purposes, the known induction of genetically based inheritable effects in the offspring is addressed in mutagenicity (subclass 6.6 – see chapter 14), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of mutagenicity.

16.1.2. Weight-of-evidence approach

The best quality data should be used as the fundamental basis for classification. Classification should preferably be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development (OECD) Test Guidelines or equivalent according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

See Appendix 16A [below](#) for a detailed list of acceptable test methods for reproductive and developmental toxicity.

16.2. Reproductive or developmental effects hazard and classification criteria

16.2.1. Reproductive or developmental effects threshold criteria

Schedule 4 of the threshold criteria defined in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 state:

2 Minimum degrees of hazard

(1) A substance with toxic properties is not hazardous for the purposes of the Act unless—

...

- (q) *reliable information for the substance indicates to an expert that exposure to the substance causes an adverse reproductive effect; or*
- (r) *reliable information for the substance indicates to an expert that exposure to the substance causes an adverse developmental effect.*

16.2.2. Reproductive or developmental effects classification criteria for substances

Schedule 4 to the Hazardous Substances Classification Regulations 2001 identifies two classification categories for substances that are reproductive or development toxicants and one classification category for substances that product effects on or via lactation (subclass 6.8).

- Category 6.8A – substances that are known or presumed human reproductive or developmental toxicants
 - a. A substance for which data indicate evidence of a causal relationship in humans between exposure to the substance and adverse effects on reproductive ability, reproductive capacity, or development.
 - b. A substance for which data indicate evidence of adverse reproductive or adverse developmental effect in animals as a result of exposure to the substance, where that adverse effect occurs:
 - i. in the absence of other adverse effects from exposure to the substance; or
 - ii. in the presence of other adverse effects that occur as a result of exposure to the substance, where the adverse reproductive or adverse developmental effect is considered by an expert not to be a secondary non-specific consequence of those other adverse effects.
- Category 6.8B – substances that are suspected human reproductive or developmental toxicants
A substance for which data indicates evidence from human epidemiological or animal studies of an adverse reproductive or developmental effect as a result of exposure to the substance, where:
 - a. that effect is considered by an expert not to be a secondary non-specific consequence of any other adverse effect; and
 - b. the strength and weight of the evidence indicate to an expert that the evidence is not sufficient to classify the substance in hazard classification 6.8A.
- Category 6.8C – substances that produce toxic human reproductive or developmental effects on or via lactation
 - A substance for which data from studies of absorption, metabolism, distribution, and excretion of the substance indicate evidence that the substance would be present in potentially toxic levels in human breast milk.
 - A substance for which data indicate evidence in humans of toxicity to babies during the lactation period as a result of exposure.
 - A substance for which data from one- or two-generation studies indicate evidence of any adverse effect in the offspring of animals due to the transfer of the substance in the milk as a result of exposure

- A substance for which data from one- or two-generation studies indicate evidence of any adverse effect in the offspring of animals due to any adverse effect on the quality of milk as a result of exposure.

The classification criteria are based on the Globally Harmonised System of Classification and Labelling (GHS) (United Nations, 2007) criteria. See Appendix 16B for a comparison for the HSNO Act criteria with the GHS and Appendix 16C for comparisons with EU risk phrases for reproductive and developmental toxicity.

The placing of a substance in category 6.8A for effects on fertility and/or developmental toxicity is done on the basis of epidemiological data. Placement into category 6.8A or 6.8B is done primarily on the basis of animal data. Data from *in vitro* studies or studies on avian eggs are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

For classification into category 6.8A, for developmental toxicity, there should be clear evidence of adverse effects in well-conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of events such as:

- maternal toxicity;
- reduced food or water intake;
- maternal stress;
- lack of maternal care;
- specific dietary deficiencies;
- poor animal husbandry; and
- intercurrent infections.

It is important that the effects observed should occur in well-conducted studies and at dose levels that are not associated with marked maternal toxicity.

The route of exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents. The results of such studies should be interpreted with caution, and on their own would not normally lead to classification.

Classify as category 6.8A, (known human reproductive or developmental toxicant) if evidence from human data shows a causal relationship between exposure to the substance and the development of reproductive and/or developmental effects and in which chance, bias, and confounding could be ruled out with reasonable confidence.

Classify as category 6.8A (presumed human reproductive or developmental toxicant), if one of the following is the case.

- Evidence from animal data establishes a causal relationship between the substance and the development of reproductive and/or developmental effects. Data should provide clear evidence of specific reproductive toxicity in the absence of toxic effects or, if occurring together with other toxic effects, the adverse effect is considered not to be a secondary non-specific consequence of other toxic effects. Mechanistic information should also support the relevance of the effect in humans.

- Evidence from human data suggests a reproductive and/or developmental effect, but the evidence is not sufficiently convincing to classify the substance as a known human reproductive or developmental toxicant, and evidence from animal data establishes a causal relationship between the substance and the development of reproductive and/or developmental effects.
- There is clear evidence in one animal species of impaired fertility, with supporting evidence on the mechanism of action or site of action and information that would lead to the conclusion that the effect would likely to be seen in humans.
- There is a chemical relationship to other known anti-fertility agents.

When studies are in only one species without other relevant supporting evidence then classification in category 6.8B may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification in this category should be made only when there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it is demonstrated that impaired fertility in animals studies was due to a failure to mate, then for classification it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Classify as category 6.8B (suspected human reproductive or developmental toxicant), if:

- evidence from human data shows a positive association between exposure to the substance and the development of reproductive and/or developmental effects, but chance, bias, or confounding could not be ruled out with reasonable confidence; or
- evidence from animal data suggests a reproductive and/or developmental effect, but the evidence is not sufficiently convincing to place the substance in category 6.8A; for example:
 - a. the evidence of adverse effects is restricted to a single experiment; or
 - b. there are unresolved questions regarding the adequacy of the design, conduct, or interpretation of the study.

Classify as category 6.8C (causing effects on or via lactation), if one of the following is the case.

- Evidence from human data establishes a causal relationship between exposure to the substance and evidence of toxicity to babies, where that substance interferes with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child.
- Evidence from absorption, metabolism, distribution, and excretion studies indicates the likelihood the substance would be present at potentially toxic levels in breast milk.
- Results of one- or two-generation studies in animals provide clear evidence of an adverse effect in offspring due to the transfer of the substance in the milk or an adverse effect on the quality of the milk. Special studies, such as cross-fostering studies, may also demonstrate an adverse effect on or via lactation.
- Substances are known to accumulate in the body and subsequently may be released into milk at potentially toxic levels during lactation.

A classification is not assigned, if it can be shown:

- the clearly identified mechanism or mode of action has no relevance for humans;
- the toxicokinetic differences are so marked it is certain that the hazardous property will not be expressed in humans; or
- that the route of administration (for example intravenous or intraperitoneal injection) results in exposure of the reproductive organs to unrealistically high levels of test substance or elicits local damage to the reproductive organs (such as irritation). This effect in isolation is not considered to be above the reproductive or development effect threshold.

16.2.3. Considerations for reproductive and developmental toxicity classification

Classification as a reproductive or developmental toxicant is made on the basis of an assessment of the total weight of evidence. This means that all available information that bears on the determination of reproductive or developmental toxicity is considered together. This includes epidemiological studies and case reports in humans and specific reproduction studies along with subchronic, chronic, and special study results in animals that provide relevant information about toxicity to reproductive and related endocrine organs.

An evaluation of substances chemically related to the material under study may also be undertaken, particularly when information on the material is scarce. The weight given to the available evidence is influenced by factors such as the quality of the study, the consistency of results, the nature and severity of effects, the level of statistical significance for intergroup differences, the number of endpoints affected, the relevance of the route of administration to humans, and freedom from bias. Both positive and negative results are assembled together into a weight-of-evidence determination. However, a single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.

Toxicokinetic studies in animals and humans, and results from site of action and mechanism or mode of action studies may provide relevant information that could reduce or increase concerns about the hazard to human health. If it can be conclusively demonstrated that the clearly identified mechanism or mode of action in the animal model has no relevance for humans or the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans, then a substance that produces an adverse effect on reproduction in experimental animals should not be classified.

In some reproductive toxicity studies in experimental animals, the only effects recorded may be considered of low or minimal toxicological significance, and classification may not necessarily be the outcome. These include, for example, small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations or in foetal weights, or small differences in postnatal developmental assessments.

Data from animal studies ideally should provide clear evidence of specific reproductive or developmental toxicity in the absence of other, systemic, toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dams in a study, the potential influence of the generalised adverse effects should be assessed to the extent possible. The preferred approach is to consider adverse effects in the

embryo or foetus first, and then evaluate maternal toxicity, along with any other factors, that are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses should not be automatically discounted. Discounting developmental effects that are observed at maternally toxic doses can be done only on a case-by-case basis when a causal relationship is established or refuted.

If appropriate information is available, it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity should not be used to negate findings of embryo or foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, for example, irreversible effects such as structural malformations. In some situations, it is reasonable to assume that reproductive or developmental toxicity is due to a secondary consequence of maternal toxicity and discount the effects; for example, if the chemical is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups, or they are prostrate or dying.

16.2.4. Maternal toxicity

Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally mediated mechanisms. This can occur in the context of a developmental or a reproductive toxicity study (one- or two-generation study). Therefore, in the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight-of-evidence approach, using all available studies, should be used to determine the degree of influence that should be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo or foetus should be first considered, and then maternal toxicity, along with any other factors that are likely to have influenced these effects, using a weight-of-evidence approach to reach a conclusion about classification.

Based on pragmatic observation, it is believed that maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited numbers of studies that have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects, which occur even in the presence of maternal toxicity, are considered evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification should be considered when there is significant toxic effect in the offspring, for example, irreversible effects such as structural malformations, embryo or foetal lethality, and significant post-natal functional deficiencies.

Classification should not be automatically discounted for chemicals that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally mediated mechanism has been demonstrated. In such a case, a 6.8B classification may be considered more appropriate than a 6.8A classification. However, when a chemical is so toxic that maternal death or severe inanition results, or the dams in animal studies are prostrate and incapable of nursing, it may be reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification may not necessarily be the outcome in the case of minor developmental changes, for example, a small reduction in foetal or pup bodyweight, or the retardation of ossification when seen in association with maternal toxicity.

Some of the endpoints used to assess maternal toxicity are listed below. Data on these endpoints, if available, needs to be evaluated in light of their statistical or biological significance and dose–response relationship.

- Maternal mortality

An increased incidence of mortality among treated dams over the controls should be considered evidence of maternal toxicity, if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10% is considered excessive and the data for that dose level should not normally be considered for further evaluation.

- Mating index

$(\text{number of animals with seminal plugs or sperm} \div \text{number of animals mated}) \times 100^5$

- Fertility index

$(\text{number of animals with implants} \div \text{number of matings}) \times 100^6$

- Gestation length (if allowed to deliver)

- Bodyweight and bodyweight change

Consideration of the maternal bodyweight change and/or adjusted (corrected) maternal bodyweight should be included in the evaluation of maternal toxicity whenever such data are available. The calculation of an adjusted (corrected) mean maternal bodyweight change, which is the difference between the initial and terminal bodyweight minus the gravid uterine weight (or the sum of the weights of the fetuses) may indicate whether the effect is maternal or intrauterine. In rabbits, the bodyweight gain may not be a useful indicator of maternal toxicity, because of normal fluctuations in bodyweight during pregnancy.

- Food and water consumption (if relevant)

The observation of a significant decrease in the average food or water consumption in treated dams compared with the control group may be useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption should be

⁵ These indices can also be affected by the male.

⁶ These indices can also be affected by the male.

evaluated in conjunction with maternal bodyweights when determining whether the effects noted are reflective of maternal toxicity or, more simply, the unpalatability of the test material in feed or water.

- Clinical evaluations (including clinical signs, markers, and haematology and clinical chemistry studies)
The observation of an increased incidence of significant clinical signs of toxicity in treated dams relative to the control group may be useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree, and duration of clinical signs should be reported in the study. Examples of frank clinical signs of maternal intoxication include coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.
- Post-mortem data
Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data; for example, absolute organ weight, organ-to-bodyweight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared with those in the control group, may be considered evidence of maternal toxicity.

16.2.5. Animal and experimental data

Internationally accepted test methods are available, including methods for developmental toxicity testing (for example, OECD Test Guideline 414, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Test Guideline S5A, 1993), methods for peri- and post-natal toxicity testing (for example ICH Test Guideline S5B, 1995) and methods for one- or two-generation toxicity testing (for example, OECD Test Guidelines 415 and 416).

Results obtained from screening tests (for example, OECD Test Guidelines 421 (reproduction/developmental toxicity screening test) and 422 (combined repeated dose toxicity study with reproduction/development toxicity screening test)) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and occur in the absence of significant generalised toxicity, may be used as a basis for classification (for example, histopathological changes in the gonads).

Evidence from *in vitro* assays or non-mammalian tests and from analogous substances using structure activity relationships, can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data should not be used as a primary support for classification.

It is preferable that animal studies are conducted using routes of administration that relate to the potential route of human exposure. However, in practice, reproductive or developmental toxicity studies are commonly conducted using the oral route, and such studies are usually suitable for evaluating the hazardous properties of the substance with respect to reproductive or developmental toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or the

toxicokinetic differences are so marked that it is certain the hazardous property will not be expressed in humans, then a substance that produces an adverse effect on reproduction in experimental animals should not be classified.

Studies involving routes of administration such as intravenous or intraperitoneal injection, which may result in exposure of the reproductive organs to unrealistically high levels of the test substance or elicit local damage to the reproductive organs (for example, by irritation), must be interpreted with extreme caution and on their own would not normally be the basis for classification.

There is general agreement about the concept of a limit dose, above which the production of an adverse effect may be considered to be outside the criteria that lead to classification. Some Test Guidelines specify a limit dose; other Test Guidelines qualify the limit dose with a statement that higher doses may be necessary if expected human exposure is sufficiently high that an adequate margin of exposure would not be achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example, doses that induce prostration, severe inappetence, or excessive mortality) would not normally lead to classification, unless other information were available (for example, toxicokinetic information, indicating that humans may be more susceptible than animals) to suggest that classification is appropriate. See also section 16.2.4.

However, specification of the actual 'limit dose' will depend on the test method that has been used to provide the test results (for example, in OECD Test Guideline 408 for repeated dose toxicity studies by the oral route, an upper dose of 1,000 milligrams per kilogram unless the expected human response indicates the need for a higher dose level, has been recommended as a limit dose).

16.3. Classification of mixtures

16.3.1. Classification of mixtures when data are available for the complete mixture

Classification of mixtures is based on the available test data of the individual constituents of the mixture using cut-off values or concentration limits for the components of the mixture. The classification may be modified on a case-by case basis based on the available test data for the mixture as a whole. In such cases, the test results for the mixture as a whole must be shown to be conclusive, taking into account dose and other factors such as duration, observations, and analysis (for example, statistical analysis and test sensitivity) of reproduction test systems.

16.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

Where the mixture itself has not been tested to determine its reproductive or developmental toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data are used in accordance with the following agreed bridging rules. This

ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that is not expected to affect the reproductive or developmental toxicity of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

b. Batching

The reproductive or developmental toxicity potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product produced by and under the control of the same manufacturer, unless there is reason to believe there is significant variation in composition such that the reproductive or developmental toxicity potential of the batch has changed. If the latter occurs, a new classification is necessary.

c. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of ingredient B, toxic to reproduction, is the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
- v. data on toxicity for ingredients A and C are available and substantially equivalent; that is, they are in the same hazard category and are not expected to affect the reproductive or developmental toxicity of ingredient B; then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

d. Aerosols

A hazard classification may be assigned for reproductive or developmental toxicity for aerosol products. The classification should also take into account the propellant in the aerosol.

16.3.3. Classification of mixtures when data are available for all or some ingredients of the mixture

The mixture will be classified as a reproductive or developmental toxicant when at least one ingredient has been classified as a 6.8A or 6.8B reproductive or developmental toxicant and is present at or above the appropriate cut-off value or concentration limit as shown in Table 16.1 for 6.8A and 6.8B respectively.

The mixture will be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate cut-off value or concentration limit as shown in Table 16.1 for the 6.8C classification for effects on or via lactation.

Table 16.1: Cut-off values or concentration limits of ingredients of a mixture classified as a reproductive or developmental toxicant or for reproductive or developmental effects on or via lactation that trigger classification of the mixtures

| Ingredient classified as category | Cut-off values or concentration limits triggering classification of a mixture as category | | |
|---|---|--------|--------|
| | 6.8A | 6.8B | 6.8C |
| 6.8A Reproductive or developmental toxicant | ≥ 0.1% | – | – |
| 6.8B Reproductive or developmental toxicant | – | ≥ 0.1% | – |
| 6.8C Reproductive or developmental toxicant | – | – | ≥ 0.1% |

Note: The cut-off values or concentration limits in the above table apply to solids and liquids (by weight) as well as gases (by volume).

The generic hazard cut-off level or concentration limits do not apply, if it can be shown that the substance causes a reproductive or developmental hazard that will be evident below the generic hazard cut-off levels or concentration limits.

Appendix 16A: Acceptable test methods for reproductive or developmental toxicity

16A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.

16A.2 Reproductive or developmental toxicity test guidelines

The guidelines in Table 16A.1 are primarily relevant to substances which are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.

See also Table 16A.1.

Table 16A.1: Reproductive or developmental toxicity test guidelines for chemicals

| Test protocols | Test guideline | | |
|--|----------------|----------------|---|
| | OECD | USEPA OPPTS | EC |
| Reproduction or developmental toxicity screening test | 421 | 870.3550 | |
| Combined repeated dose toxicity with the reproduction or developmental toxicity screening test | 422 | 870.3650 | |
| Prenatal developmental toxicity study | 414 | 870.3700 | EC B.31 Teratogenicity test – rodent and non-rodent |
| Reproduction and fertility studies | 415 | 870.3800 | EC B.34 One-generation reproduction toxicity test |
| | 416 | | EC B 35 Two-generation reproduction toxicity test |

Appendix 16B: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act reproductive or developmental toxicity hazard classification

Table 16B.1 displays the reproductive or developmental categories from the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2007) and the Hazardous Substances and New Organisms Act 1996 (HSNO Act) equivalent.

Table 16B.1: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act reproductive or developmental toxicity hazard classification

| GHS reproductive or development toxicity classification | HSNO Act equivalent category* |
|---|-------------------------------|
| <p>Category 1: Known or presumed human reproductive toxicant</p> <p>This category includes substances that are known to have produced an adverse effect on sexual function and fertility or on development in humans, or for which there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. For regulatory purposes, a substance can be further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B).</p> <p>Category 1A: Known human reproductive toxicant</p> <p>The placing of the substance in this category is largely based on evidence from humans.</p> <p>Category 1B: Presumed human reproductive toxicant</p> <p>The placing of the substance in this category is largely based on evidence from experimental animals. Data from animal studies should provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate.</p> | 6.8A |
| <p>Category 2: Suspected human reproductive toxicant</p> <p>This category includes substances for which there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, in the absence of other toxic effects, or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects, and where the evidence is not sufficiently convincing to place the substance in category 1. For instance, deficiencies in the study may make the quality of evidence less convincing, so category 2 could be the more appropriate classification.</p> | 6.8B |

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 16C: Comparison of European Union reproductive or developmental toxicity risk phrases with HSNO Act reproductive or developmental classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 16C.1. Note that some cut-off levels are not totally aligned with HSNO Act classification categories. This is noted in the table, and for classification purposes a precautionary approach is advocated such that the higher hazard category is assigned.

Table 16C.1: Comparison of European Union reproductive or developmental toxicity risk phrases with HSNO Act classifications

| European Union risk phrases | HSNO Act equivalent category |
|---|---|
| <p>Reproductive toxicants</p> <p>Substances are determined to be hazardous due to reproductive effects if they fall into one of two categories.</p> <p>a. Effects on fertility</p> <p>Category 1: Substances known to impair fertility in humans.</p> <p>Category 2: Substances that should be regarded as if they impair fertility in humans.</p> <p>Category 3: Substances that cause concern for human fertility.</p> <p>b. Developmental toxicity</p> <p>Category 1: Substances known to cause developmental toxicity in humans.</p> <p>Category 2: Substances that should be regarded as if they cause developmental toxicity to humans.</p> <p>Category 3: Substances that cause concern for humans owing to possible developmental toxic effects.</p> | <p>6.8A</p> <p>6.8A</p> <p>6.8B</p> <p>6.8A</p> <p>6.8A</p> <p>6.8B</p> |
| <p>Effects on fertility</p> <p>Category 1</p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R60 in accordance with the criterion given below.</p> <p>R60 May impair fertility</p> <p>A substance is included in category 1, if there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.</p> | <p>6.8A</p> |
| <p>Category 2</p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R60 in accordance with the criterion given below.</p> <p>R60 May impair fertility</p> <p>A substance is included in category 2, if there is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:</p> <p>c. clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects; and</p> | <p>6.8A</p> |

| European Union risk phrases | HSNO Act equivalent category |
|--|------------------------------|
| <p>d. other relevant information.</p> <hr/> <p>Category 3</p> <p>Substances are determined to be hazardous and classified as Harmful (Xn) and assigned risk phrase R62 in accordance with the criterion given below.</p> <p>R62 Possible risk of impaired fertility</p> <p>A substance is included in category 3 generally on the basis of:</p> <p>e. results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in category 2; and</p> <p>f. other relevant information.</p> | 6.8B |
| <p>Developmental effects</p> <p>Developmental toxicity, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embryotoxic/foetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion (including resorptions), structural defects (reproductive effects), functional defects, peripostnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.</p> | |
| <p>Category 1</p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R61 in accordance with the criterion given below.</p> <p>R61 May cause harm to the unborn child</p> <p>A substance is included in category 1, if there is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.</p> | 6.8A |
| <p>Category 2</p> <p>Substances are determined to be hazardous and classified as Toxic (T) and assigned risk phrase R61 in accordance with the criterion given below.</p> <p>R61 May cause harm to the unborn child</p> <p>A substance is included in category 2, if there is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:</p> <p>g. clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but that are not a secondary non-specific consequence of the other toxic effects; and</p> <p>h. other relevant information.</p> | 6.8A |
| <p>Category 3</p> | 6.8B |

| European Union risk phrases | HSNO Act equivalent category |
|---|------------------------------|
| <p>Substances are determined to be hazardous and classified as Harmful (Xn) and assigned risk phrase R63 in accordance with the criteria given below.</p> <p>R63 possible risk of harm to the unborn child</p> <p>A substance is included in category 3 generally on the basis of:</p> <ul style="list-style-type: none"> i. results in appropriate animal studies that provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but that are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in category 2; and j. other relevant information. | |
| <p>Lactation (Xn)</p> <p>R64 may cause harm to breast fed babies</p> <p>Substances that are determined to cause effects on reproduction and cause concern due to their effects on lactation should also be assigned R64.</p> <p>Substances that are absorbed by women and may interfere with lactation or may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breast-fed child.</p> <ul style="list-style-type: none"> k. For the purpose of classification, toxic effects on offspring resulting only from exposure via the breast milk, or toxic effects resulting from direct exposure of children are not regarded as toxic to reproduction, unless such effects result in impaired development of the offspring. l. Substances that are not classified as toxic to reproduction but that cause concern due to toxicity when transferred to the baby during the period of lactation should be classified as Harmful (Xn) and assigned R64. This risk phrase may also be appropriate for substances that affect the quantity or quality of the milk. | 6.8C |

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances.* European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

17. Specific Target Organ Toxicity (Single or Repeated Exposure) – Subclass 6.9

17.1. General considerations

17.1.1. Specific target organ toxicity overview

See section 9.6 in chapter 9 for definitions of the key terms used in this chapter.

This subclass provides a means of classifying substances that produce specific target organ or systemic toxicity arising from single or repeated exposure. All significant health effects that can impair function, reversible and irreversible, immediate and/or delayed are included (other than those used to derive another classification).

Classification identifies the chemical substance as being a specific target organ or systemic toxicant, so it potentially presents adverse health effects in people who are exposed to it.

Classification depends on the availability of reliable evidence that single or repeated exposure to the substance has produced a consistent and identifiable toxic effect in humans or in experimental animals. This includes toxicologically significant changes that have affected the function or morphology of a tissue or organ, or have produced serious changes to the biochemistry or haematology of the organism. To be eligible for classification, these changes need to be relevant for human health.

Assessment should take into consideration not only significant changes in a single organ or biological system, but also generalised changes of a less severe nature involving several organs. Specific target organ or systemic toxicity can occur by any route that is relevant for humans; that is, principally oral, dermal, or inhalation.

Non-lethal toxic effects observed after a single-event exposure are also classified under subclass 6.9.

Specific toxic effects that are eligible for classification under class 6 or class 8 are assessed separately under the appropriate toxic endpoints and are not used to derive a subclass 6.9 classification. These effects are:

- acute lethality or toxicity (subclass 6.1 – see chapter 10);
- skin corrosivity (subclass 8.2) or irritation (subclass 6.3) (see chapter 11);
- eye corrosivity (subclass 8.3) or eye irritation (subclass 6.4) (see chapter 12);
- respiratory or contact sensitisation (subclass 6.5 – see chapter 13);
- carcinogenicity (subclass 6.7 – see chapter 14);
- mutagenicity (subclass 6.6 – see chapter 15); and
- reproductive toxicity (subclass 6.8 – see chapter 16).

17.1.2. Weight of evidence approach

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Animal data from internationally harmonised test methods are preferred for classification under this subclass. Data should preferably be derived using Organisation for Economic Co-operation and Development Test Guidelines or equivalent according to the principles of Good Laboratory Practice. When such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 for information about assessing data quality.

See Appendix 17A for a detailed list of acceptable test methods for specific target organ toxicity (single or repeated exposure).

17.2. Specific target organ toxicity (single or repeated exposure) hazard and classification criteria

17.2.1. Specific target organ toxicity (single or repeated exposure) effects threshold criteria

Schedule 4 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with toxic properties is not hazardous for the purposes of the Act unless—*

...

(s) *data for the substance indicates, in the opinion of an expert, evidence of a significant adverse biological effect or a significant toxic effect (other than an effect referred to in any of paras (a) to (r)) on the function or morphology of an organ, or on the biochemistry or haematology of an organism or human being as a result of exposure to the substance and, in the case of a significant biological effect, the change is relevant to human health.*

17.2.2. Specific target organ toxicity (single or repeated exposure) classification criteria for substances

Schedule 4 to the Hazardous Substances (Classification) Regulations 2001 identifies two classification categories for substances that are target organ toxicants (subclass 6.9).

- Category 6.9A – substances that are toxic to human target organs or systems

A substance for which data indicate to an expert evidence of a causal relationship between exposure of humans to the substance and the development of target organ or systemic toxicity that would not result in the substance being classified in any of subclasses 6.1 and 6.3–6.8.

A substance for which data indicate to an expert evidence of a significant adverse biological effect on the function or morphology of an organ or on the biochemistry or haematology of an organism as a result of

exposure to the substance that would not result in the substance being classified in any of subclasses 6.1 and 6.3–6.8 and that are produced at low exposure concentrations and are of relevance to human health.

- Category 6.9B – substances that are harmful to human target organs or systems

A substance for which data indicate to an expert evidence of a significant adverse biological effect on the function or morphology of an organ or on the biochemistry or haematology of an organism or human being as a result of exposure to the substance that would not result in the substance being classified in any of subclasses 6.1 and 6.3–6.8, and that are produced at moderate exposure concentrations and are of relevance to human health.

The classification criteria above are based on the Globally Harmonised System for Classification and Labelling (GHS) (United Nations, 2007). See Appendix 17C for a comparison of the HSNO Act and GHS criteria. See Appendix 17D for a comparison with the EU risk phrases for target organ toxicity.

17.2.3. Considerations for specific target organ toxicity (single or repeated exposure) classification

The relevant route of exposure by which the classified substance produces damage should be identified. Classification is determined by expert judgement, on the basis of the weight of all evidence available, including the guidance presented below.

A weight-of-evidence approach to all data, including human incidents, epidemiology, and studies conducted in experimental animals, is used to substantiate specific target organ or systemic toxic effects that merit classification.

The information required to evaluate specific target organ toxicity (single exposure) comes from single exposure in humans (for example, exposure at home, in the workplace, or environmentally) or from studies conducted in experimental animals. The standard animal studies in rats or mice that provide this information are acute toxicity studies that can include clinical observations and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues or organs to be identified. Results of acute toxicity studies conducted in other species may also provide relevant information.

The information required to evaluate specific target organ toxicity (repeated exposure) comes from repeated exposure in humans (for example, exposure at home, in the workplace, or environmentally) or from studies conducted in experimental animals. The standard animal studies in rats or mice that provide this information are 28-day, 90-day, or lifetime studies (up to two years) that include haematological, clinicochemical, and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues or organs to be identified. Data from repeat-dose studies performed in other species may also be used. Other long-term exposure studies (for example, for carcinogenicity, neurotoxicity, or reproductive toxicity) may also provide evidence of specific target organ or systemic toxicity that could be used in the assessment of classification.

In exceptional cases, based on expert judgement, it may be appropriate to classify certain substances with human evidence of specific target organ or systemic toxicity as 6.9B when the weight of human evidence is not sufficiently convincing to warrant a 6.9A classification and/or based on the nature and severity of effects.

Dose or concentration levels in humans should not be considered in the classification, and any available evidence from animal studies should be consistent with the 6.9B classification. In other words, if animal data are also available on the chemical that it warrants 6.9A classification, the chemical should be classified as 6.9A.

17.2.4. Effects considered to support classification

Reliable evidence associating single or repeated exposure to the substance with a consistent and identifiable toxic effect demonstrates support for classification.

It is recognised that evidence from human experience and incidents is usually restricted to reports of adverse health consequences, often with uncertainty about exposure conditions (for example, information on dose and exposure to other substances or confounding factors that may have influenced the outcome). This evidence may not provide the scientific detail that can be obtained from well-conducted studies in experimental animals.

Evidence from appropriate studies in experimental animals can furnish much more detail than can be gained from human experience and incidents, in the form of clinical observations, and macroscopic and microscopic pathological examination (haematology and clinical chemistry for repeat dose studies). This can often reveal hazards that may not be life-threatening, but may indicate functional impairment. Consequently, all available evidence and relevance to human health must be taken into consideration in the classification process.

Relevant toxic effects in humans and/or animals are as follows.

- Morbidity resulting from single exposure.
- Morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses or concentrations, due to the bioaccumulation of the substance or its metabolites, or the accumulation of effect as a result of the detoxification process becoming overwhelmed by repeated exposure to the substance or its metabolites.
- Significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (for example, sight, hearing, and smell).
- Any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters.
- Significant organ damage that may be noted at necropsy and/or subsequently seen or confirmed at microscopic examination.
- Multifocal or diffuse necrosis, fibrosis, or granuloma formation in vital organs with regenerative capacity.
- Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (for example, severe fatty change in the liver).
- Evidence of appreciable cell death (including cell degeneration, severe acute tubular nephrosis in the kidney, ulcerative gastritis, and reduced cell numbers) in vital organs incapable of regeneration (for example, fibrosis of the myocardium or dying back of a nerve) or in stem cell populations (for example, aplasia or hypoplasia of the bone marrow).

Effects that are not considered to support classification are:

- clinical observations or small changes in bodyweight gain, food consumption, or water intake that may have some toxicological importance, but that do not, by themselves, indicate 'significant' toxicity;
- small changes in clinical biochemistry, haematology, or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;
- changes in organ weight with no evidence of organ dysfunction;
- adaptive responses that are not considered toxicologically relevant;
- substance-induced species-specific mechanisms of toxicity demonstrated with reasonable certainty to be not relevant for human health; and
- local-only effects, after single-dose exposure, at the site of administration for the routes tested, especially when adequate testing by other principal routes show lack of specific target organ or systemic toxicity.

17.2.5. Guidance value ranges for single and repeat dose exposures

Specific target organ toxicity (single exposure)

To help to decide whether and to what extent (6.9A or 6.9B) a substance should be classified, dose–concentration 'guidance values' are provided in Table 17.1. These are the dose–concentration values that have been shown to produce significant health effects. The principal argument for proposing such guidance values is that all chemicals are potentially toxic and there has to be a reasonable dose–concentration above which a degree of toxic effect is acknowledged.

Thus, in animal studies, when significant toxic effects are observed that indicate classification is necessary, consideration of the dose–concentration at which these effects were seen, in relation to the suggested guidance values, provides useful information to help to assess the need to classify (since the toxic effects are a consequence of the hazardous property or properties and the dose–concentration value).

The range of guidance values for single-dose exposure that has produced a significant non-lethal toxic effect are those applicable to acute toxicity testing, as indicated in Table 17.1.

These single dose–concentration values produce a significant non-lethal toxic effect, that is, they are not median lethal dose (LD₅₀) or median lethal concentration (LC₅₀) values. These values are not strict demarcation values, but should be used within a weight-of-evidence approach for deciding classification.

Table 17.1: Guidance value ranges for single dose exposures

| Route of exposure | Units | Guidance value ranges for category | |
|-------------------------|----------|------------------------------------|-----------------|
| | | 6.9A | 6.9B |
| Oral (rat) | mg/kg bw | ≤ 300 | > 300 – 2,000 |
| Dermal (rat or rabbit) | mg/kg bw | ≤ 1,000 | > 1,000 – 2,000 |
| Inhalation (rat) gas | ppm | ≤ 2,500 | > 2,500 – 5,000 |
| Inhalation (rat) vapour | mg/L | ≤ 10 | > 10 – 20 |

| | | | |
|-----------------------------------|--------------|-------|-------------|
| Inhalation (rat) dust, mist, fume | mg/L/4 hours | ≤ 1.0 | > 1.0 – 5.0 |
|-----------------------------------|--------------|-------|-------------|

Note: L = litre; mg/kg bw = milligrams per kilogram of bodyweight; mg/L = milligrams per litre; ppm = parts per million.

Thus, it is feasible that a specific profile of toxicity is seen to occur at a dose–concentration below the guidance value, for example, < 2,000 milligrams per kilogram of bodyweight (mg/kg bw) by the oral route. However the nature of the effect may result in the decision not to classify. Conversely, a specific profile of toxicity may be seen in animal studies occurring at above a guidance value (for example, at or above 2,000 mg/kg bw by the oral route), but in addition there may be supplementary information from other sources (for example, other single-dose studies or human case experience), which supports a conclusion that, in view of the weight of evidence, classification would be prudent.

Specific target organ toxicity (repeated exposure)

In studies conducted in experimental animals, reliance on observation of effects alone (that is, without reference to the duration of experimental exposure and dose–concentration value), omits a fundamental concept of toxicology; that is, all substances are potentially toxic, and what determines the toxicity is a function of the dose–concentration and the duration of exposure. In most studies conducted in experimental animals the test guidelines use an upper limit dose value.

To help to decide whether and to what degree (6.9A or 6.9B) a substance should be classified, dose–concentration ‘guidance values’ are provided in Table 17.2. These are the dose–concentration values that have been shown to produce significant health effects. The principal argument for proposing such guidance values is that all chemicals are potentially toxic and there has to be a reasonable dose–concentration above which a degree of toxic effect is acknowledged. Also, repeated-dose studies conducted in experimental animals are designed to produce toxicity at the highest dose used in order to optimise the test objective, so most studies will reveal some toxic effect at least at this highest dose. Therefore, what is to be decided is not only what effects have been produced, but also at what dose–concentration level and over what period were they produced and how relevant are they for humans.

Thus, in animal studies, when significant toxic effects are observed that would indicate classification is necessary, consideration of the duration of experimental exposure and the dose–concentration value at which these effects were seen, in relation to the suggested guidance values, provides useful information to help to assess the need to classify (since the toxic effects are a consequence of the hazardous property or properties, the duration of exposure, and the dose–concentration value).

The decision to classify can be influenced by the dose–concentration guidance values at or below which a significant toxic effect has been observed.

The guidance values proposed in Table 17.2 refer to effects seen in a standard 90-day toxicity study conducted in rats. They can be used as a basis from which to extrapolate equivalent guidance values for toxicity studies of longer or shorter duration, using a dose–exposure time extrapolation similar to Haber’s rule

for inhalation. This rule states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure.

The assessment should be done on a case-by-case basis; for example, for a 28-day study the guidance values in Table 17.2 would be increased by a factor of three. Thus, for 6.9A and 6.9B classification, significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals and seen to occur at or below the (suggested) guidance values in the table would justify classification.

Table 17.2: Guidance value ranges for repeated dose exposures

| Route of exposure | Units | Guidance value ranges for category | |
|-----------------------------------|------------------|------------------------------------|-----------|
| | | 6.9A | 6.9B |
| Oral (rat) | mg/kg bw | ≤10 | >10–100 |
| Dermal (rat or rabbit) | mg/kg bw | ≤20 | >20–200 |
| Inhalation (rat) gas | ppm/6 hours/day | ≤50 | >50–250 |
| Inhalation (rat) vapour | mg/L/6 hours/day | ≤0.2 | >0.2–1.0 |
| Inhalation (rat) dust, mist, fume | mg/L/6 hours/day | ≤0.02 | >0.02–0.2 |

Note: L = litre; mg/kg bw = milligrams per kilogram of bodyweight; mg/L = milligrams per litre; ppm = parts per million.

The values and ranges in Table 17.1 and Table 17.2 are intended to be only guides; that is, they are to be used as part of the weight-of-evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values. These values are not no observed effect levels (NOELs), but are lowest observed adverse effect levels (LOAELs).

It is feasible that a specific profile of toxicity is seen to occur in repeat-dose animal studies at a dose–concentration level below the guidance value (for example, < 100 mg/kg bw/day by the oral route). However, the nature of the effect (for example, nephrotoxicity seen only in male rats of a particular strain known to be susceptible to this effect) may result in a decision not to classify. Conversely, a specific profile of toxicity may be seen in animal studies occurring at above a guidance value (for example, at or above 100 mg/kg bw/day by the oral route) and with supplementary information from other sources (for example, other long-term administration studies or human case experience) supports a conclusion that, in view of the weight of evidence, classification would be prudent.

See Appendix 17B for converting a concentration of a substance in the diet (ppm) to a dietary intake (mg/kg bw/day).

17.2.6. Study duration

As stated above, the use of factors based on Haber's rule should take into account rat studies of a duration shorter than 90 days (3 months). A similar approach should be taken in a weight-of-evidence approach when

assessing data from longer-term studies. This is not a strictly arithmetic approach, but a consideration of data close to the guideline values.

Similarly, when considering species other than rats, there is evidence that species generally differ in their response. A consideration in relation to the weight of evidence could be that mice are likely to respond at higher dose levels than are rats, while dogs are likely to respond at lower dose levels than are rats. Thus, a mouse study with an LOAEL in the range 100–200 mg/kg bw/day may be appropriate for classification (as 6.9B), while a dog study with a LOAEL in the range 50–100 mg/kg bw/day may not be appropriate for classification.

It is emphasised that these aspects should be considered in the overall determination of the weight of evidence for the classification, not as 'rules'. Therefore, no guideline values are provided here.

17.2.7. Other considerations

When a substance is characterised only by use of animal data (typical of new chemicals, but also true for many existing chemicals), the classification process would include reference to dose–concentration guidance values as one of the elements that contribute to the weight-of-evidence approach.

When well-substantiated human data are available showing a specific target organ or systemic toxic effect that can be reliably attributed to repeated or prolonged exposure to a chemical substance, the substance may be classified. Positive human data, regardless of probable dose, predominates over animal data. Thus, if a substance is unclassified because no specific target organ or systemic toxicity was seen at or below the proposed dose–concentration guidance value for animal testing, and subsequent human incident data shows a specific target organ or systemic toxic effect, then the substance should be classified.

A substance that has not been tested for specific target organ or systemic toxicity may be classified on the basis of data from a validated structure activity relationship and an expert, judgement-based extrapolation from a structural analogue that has previously been classified, and with substantial support from a consideration of other important factors (such as the formation of common significant metabolites). This could include consideration of data from other routes such as injection.

17.3. Classification of mixtures

Mixtures are classified using the same criteria as for substances or as described below. As with substances, mixtures may be classified for specific target organ or systemic toxicity following a single exposure and/or repeated exposure.

17.3.1. Classification of mixtures when data are available for the complete mixture

When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture can be classified using a weight-of-evidence evaluation of the data. Care should be exercised when evaluating data on mixtures that the dose, duration, observation, or analysis does not render the results inconclusive.

17.3.2. Classification of mixtures when data are not available for the complete mixture: bridging principles

When the mixture itself has not been tested to determine its specific target organ or systemic toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data can be used in accordance with the following bridging principles. This ensures the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without needing additional testing in animals.

a. Dilution

If a mixture is diluted with a diluent that has the same or a lower toxicity classification as the least toxic original ingredient and is not expected to affect the toxicity of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

b. Batching

The toxicity of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product, where produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the toxicity of the batch has changed. If the latter occurs, a new classification is necessary.

c. Concentration of highly toxic mixtures

If, in a mixture classified 6.9A, the concentration of a toxic ingredient is increased, the concentrated mixture should remain classified as 6.9A without additional testing.

d. Interpolation within one toxicity category

For three mixtures with identical ingredients, where mixtures A and B are in the same toxicity category and mixture C has the same toxicologically active ingredients with concentrations intermediate to the concentrations of those ingredients in mixtures A and B, then mixture C is assumed to be in the same toxicity category as mixtures A and B.

e. Substantially similar mixtures

Given:

- i. two mixtures: (A + B) and (C + B);
- ii. the concentration of ingredient B is essentially the same in both mixtures;
- iii. the concentration of ingredient A in mixture (A + B) equals that of ingredient C in mixture (C + B);
and
- iv. data on toxicity for ingredients A and C are available and substantially equivalent; that is they are in the same hazard category and are not expected to affect the toxicity of ingredient B; then

if mixture (A + B) has already been classified by testing, mixture (C + B) can be assigned the same category.

f. Aerosols

A hazard classification may be assigned for specific target organ toxicity (single or repeat exposure) for aerosols. Although in many cases, data are available only from repeat-dose oral studies, classification for

specific target organ toxicity is dependent on the internal dose of a substance. Unless data are available for each component in an aerosol classified as 6.9 to confirm that the dermal and inhalation routes are not relevant, they cannot be excluded. When the propellant is not excluded, the propellant is taken into consideration for classification. If data are available to exclude the inhalation route for an aerosol, then the propellant is not taken into consideration.

17.3.3. Classification of mixtures when data are available for all or some ingredients of the mixture

When there is no reliable evidence or test data for the specific mixture itself, and the bridging principles cannot be used to enable classification, then classification of the mixture is based on the classification of the ingredients. In this case, the mixture will be classified as a specific target organ or systemic toxicant (specific organ specified), following single exposure and/or repeated exposure, when at least one ingredient has been classified as a 6.9A or 6.9B specific target organ or systemic toxicant and is present at or above the appropriate cut-off value or concentration limit as mentioned in Table 17.3 for 6.9A and 6.9B respectively.

Table 17.3: Cut-off values or concentration limits of ingredients of a mixture classified as a specific target organ or systemic toxicant that would trigger classification of the mixture.

| Ingredient classified as category | Cut-off or concentration limits triggering classification of a mixture as category | |
|--|--|---------------|
| | 6.9A | 6.9B |
| 6.9A Target organ or systemic toxicant | ≥ 10% | ≥ 1 but < 10% |
| 6.9B Target organ or systemic toxicant | – | ≥ 1% |

Note: The cut-off values or concentration limits in the table apply to solids and liquids (by weight) as well as gases (by volume).

These cut-off values and consequent classifications should be applied equally and appropriately to both single- and repeated-dose target organ toxicants.

Care should be exercised when toxicants affecting more than one organ system are combined that the potentiation or synergistic interactions are considered, because certain substances can cause specific target organ toxicity at < 1% concentration when other ingredients in the mixture are known to potentiate its toxic effect.

Appendix 17A: Acceptable test methods for specific target organ toxicity (single or repeated exposure)

17A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.

17A.2 Specific target organ toxicity test guidelines

The guidelines in Table 17A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in mammals.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011..

See also Table 17A.1.

Table 17A.1: Specific target organ toxicity (single or repeated exposure) test guidelines for chemicals, including mixtures

| Test protocols | Test guideline | | USEPA OPPTS |
|--|----------------|---|-------------|
| | OECD | EC | |
| Chronic toxicity | 452 | EC B.30 Chronic toxicity test | 870.4100 |
| Combined chronic toxicity and carcinogenicity | 453 | EC B.33 Combined chronic toxicity/carcinogenicity test | 870.4300 |
| 90-day oral toxicity | 408 | EC B.26 Subchronic oral toxicity test: repeated dose 90-day study in rodents | 870.3100 |
| Subchronic non-rodent oral toxicity | 409 | EC B.27 Subchronic oral toxicity test: repeated dose 90-day study in non- rodents | 870.3150 |
| Repeated dose oral toxicity – 28 days | 407 | EC B.7 Repeated dose (28 days) toxicity (oral) | 870.3050 |
| Repeated dose dermal toxicity – 28 days | 410 | EC B.9 | 870.3200 |
| Repeated dose inhalation toxicity – 28 days | 412 | EC B.8 Repeated dose (28 days) toxicity (inhalation) | – |
| Subchronic dermal toxicity | 411 | EC B.28 Subchronic dermal toxicity test: 90-day repeated dermal dose study using rodent species | 870.3250 |
| Subchronic inhalation toxicity | 413 | EC B.29 Subchronic inhalation toxicity test: 90-day repeated dermal dose study using rodent species | 870.3465 |
| Delayed neurotoxicity of organophosphorous substances – acute and 28 day | 418 | EC B.37 Delayed neurotoxicity of organophosphorous substances following acute exposure | 870.6100 |
| | 419 | EC B.38 Delayed neurotoxicity of organophosphorous substances 28-day repeated dose study | |
| Neurotoxicity screening battery | – | – | 870.6200 |
| Developmental neurotoxicity study | – | – | 870.6300 |
| Schedule-controlled operant behaviour | – | – | 870.6500 |
| Peripheral nerve function | – | – | 870.6850 |
| Neurophysiology: sensory evoked potentials | – | – | 870.6855 |
| Companion animal safety | – | – | 870.7200 |

| | | | |
|--------------------|-----|-------------------------|----------|
| Toxicokinetics | 417 | EC B.36: Toxicokinetics | 870.7485 |
| Dermal penetration | – | – | 870.7600 |
| Immunotoxicity | – | – | 870.7800 |

Note: Data from reproductive or developmental toxicity studies may be used when specific target organ effects are found in parental animals, particularly when data are sparse from other studies.

Appendix 17B: Conversion of parts per million in the diet per day to milligrams of the substance per kilogram of bodyweight per day

Use Table 17B.1 to convert data from repeat-dose studies with the substance in the diet per day to milligrams of the substance per kilogram of bodyweight per day.

Table 17B.1: Approximate relation of concentration of the substance in the diet (ppm) to dietary intake (mg/kg bw/day)

| Animal | Weight (kg) | Food consumed per day (g) | Type of diet | One ppm in food = mg/kg bw/day | One mg/kg bw/day = ppm of diet |
|-------------------|-------------|---------------------------|--------------------------------------|--------------------------------|--------------------------------|
| Mouse | 0.02 | 3 | Dry laboratory chow diets | 0.150 | 7 |
| Chick | 0.4 | 50 | | 0.125 | 8 |
| Rat (young) | 0.1 | 10 | | 0.100 | 10 |
| Rat (old) | 0.4 | 20 | | 0.050 | 20 |
| Guinea pig | 0.75 | 30 | | 0.040 | 25 |
| Rabbit | 2.0 | 60 | | 0.030 | 33 |
| Dog | 10.0 | 250 | | 0.025 | 40 |
| Cat | 2 | 100 | Moist, semi-solid diets | 0.050 | 20 |
| Monkey | 5 | 250 | | 0.050 | 20 |
| Dog | 10 | 750 | | 0.075 | 13 |
| Man | 60 | 1,500 | | 0.025 | 40 |
| Pig or sheep | 60 | 2,400 | Relatively dry grain forage mixtures | 0.040 | 25 |
| Cow (maintenance) | 500 | 750 | | 0.015 | 65 |
| Cow (fattening) | 500 | 15,000 | | 0.030 | 33 |
| Horse | 500 | 10,000 | | 0.200 | 50 |

Notes

- g = gram; kg = kilogram; mg/kg bw/day = milligrams of the substance per kilogram of bodyweight per day; ppm = parts per million.
- The values in this table are average figures derived from numerous sources.

Source: Adapted from IPCS (1990), originally from Lehman (1954).

17B.1 Example

Question: What is the value in parts per million (ppm) and mg/kg bw/day of 0.5% substance X mixed in the diet of an adult rat?

Answer: 0.5% corresponds to 5,000 ppm and from Table 17B.1 1 ppm in the diet of a rat is equivalent to 0.05 mg/kg bw/day. Consequently, 5,000 ppm is equivalent to 250 mg/kg bw/day (that is, $5,000 \times 0.05$).

References

IPCS 1990. *Principles for the Toxicological Assessment of Pesticides Residues in Food*, Environmental Health Criteria 104. International Programme for Chemical Safety.

Lehman, AJ 1954. *Association of Food and Drug Officials Quarterly Bulletin* 18: 66.

Appendix 17C: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals and HSNO Act specific target organ toxicity (single or repeated exposure)

Table 17C.1 and Table 17C.2 compare the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) classifications for specific target organ toxicity (single or repeated exposure) and Hazardous Substances and New Organisms Act 1996 (HSNO Act) subclass 6.9 classification.

Note that the GHS assigns separate classifications for substances causing specific target organ toxicity, depending on whether this occurred from single exposure or repeat exposure. The HSNO Act classifications for this subclass can be assigned from either single or repeat exposure.

Table 17C.1: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act specific target organ toxicity (single exposure)

| GHS specific target organ toxicity single exposure classification | HSNO Act equivalent category |
|---|------------------------------|
| <p>Category 1: Substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following a single exposure</p> <p>Placing a substance in category 1 is done on the basis of:</p> <ul style="list-style-type: none"> reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations (guidance dose–concentration values are used as part of the weight-of-evidence evaluation). | 6.9A |
| <p>Category 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following a single exposure</p> <p>Placing a substance in category 2 is done on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose–concentration values are used to help in classification.</p> <p>In exceptional cases, human evidence can also be used to place a substance in category 2.</p> | 6.9B |
| <p>Category 3: Transient target organ effects</p> <p>There are target organ effects for which a substance or mixture may not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects that adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. This category includes only narcotic effects and respiratory tract irritation. Substances or mixtures may be classified specifically for these effects.</p> | No equivalent |

Note: For these categories, the specific target organ or system that has been primarily affected by the classified substance may be identified, or the substance may be identified as a general systemic toxicant. Attempts should be made to determine the primary target organ of toxicity and classify for that purpose (for example, hepatocarcinogens)

and neurotoxicants). One should carefully evaluate the data and, where possible, not include secondary effects (for example, a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal system).

Table 17C.2: Comparison of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and HSNO Act specific target organ toxicity (repeat exposure)

| GHS specific target organ toxicity repeat exposure classification | HSNO Act equivalent category |
|---|------------------------------|
| <p>Category 1: Substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following repeated exposure</p> <p>Placing a substance in category 1 is done on the basis of:</p> <ul style="list-style-type: none"> reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose–concentration values are used as part of the weight-of-evidence evaluation. | 6.9A |
| <p>Category 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure</p> <p>Placing a substance in category 2 is done on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose–concentration values are provided to help in classification. In exceptional cases, human evidence can also be used to place a substance in category 2.</p> | 6.9B |

Note: For both categories, the specific target organ or system that has been primarily affected by the classified substance may be identified, or the substance may be identified as a general systemic toxicant. Attempts should be made to determine the primary target organ of toxicity and classify for that purpose (for example, hepatotoxicants and neurotoxicants). One should carefully evaluate the data and, where possible, not include secondary effects (for example, a hepatotoxicant can produce secondary effects in the nervous or gastrointestinal system).

References

United Nations 2007. *The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

Appendix 17D: Comparison of European Union specific target organ toxicity risk phrases with HSNO Act specific target organ toxicity

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 17D.1.

Table 17D.1: Comparison of European Union specific target organ toxicity risk phrases with HSNO Act equivalent classification specific target organ toxicity

| European Union risk phrases | HSNO Act equivalent category |
|--|--|
| <p>Very Toxic (T+)</p> <p>A substance is determined to be hazardous and classified as Very Toxic (T+) and assigned one of the following risk phrases in accordance with the criteria given below.</p> | |
| <p>R39 Danger of very serious irreversible effects</p> <p>Strong evidence that irreversible damage, other than the carcinogenicity, mutagenicity and reproductive effects referred to below, is likely to be caused by a single exposure in the dose ranges used for classification as being Very Toxic (T+) by different routes; that is:</p> <ul style="list-style-type: none"> • inhalation LC₅₀ rat, for aerosols or particulates: ≤ 0.25 mg/L over 4 hours; • inhalation LC₅₀ rat, for gases and vapours: ≤ 0.5 mg/L over 4 hours; • dermal LD₅₀ rat or rabbit; ≤ 50 mg/kg; • oral LD₅₀ rat ≤ 25 mg/kg. | 6.9A |
| <p>Toxic (T)</p> <p>A substance is determined to be hazardous and classified as Toxic (T) and assigned one or more of the following risk phrases in accordance with the criteria given below.</p> | |
| <p>R39 Danger of very serious irreversible effects</p> <p>Strong evidence that irreversible damage other than carcinogenicity, mutagenicity and reproductive effects, is likely to be caused by a single exposure by an appropriate route. Substances are classified at least as Toxic (T) when these effects are observed at acutely toxic dose levels, that is:</p> <ul style="list-style-type: none"> • inhalation LC₅₀ rat, for aerosols or particulates: 0.25 < LC₅₀ ≤ 1 mg/L over 4 hours; • inhalation LC₅₀ rat, for gases and vapours: 0.5 < LC₅₀ ≤ 2 mg/L over 4 hours; • dermal LD₅₀ rat or rabbit: 50 < LD₅₀ ≤ 400 mg/kg; • oral LD₅₀ rat: 25 < LD₅₀ ≤ 200 mg/kg. | 6.9A |
| <p>R48 Danger of serious damage to health by prolonged exposure (not acute)</p> <p>Serious damage (clear functional disturbance or morphological change that has toxicological significance) is likely to be caused by repeated or prolonged exposure by an appropriate route. Substances are classified at least as Toxic (T) when these effects are observed at the following dose ranges:</p> <ul style="list-style-type: none"> • inhalation, rat ≤ 0.025 mg/L, 6 hours/day; • oral, rat ≤ 5 mg/kg bw/day; • dermal, rat or rabbit ≤ 10 mg/kg bw/day. | 6.9A Note the inhalation cut-off also |

| | |
|---|----------------------|
| | crosses into 6.9B |
| <p>Harmful (Xn)</p> <p>A substance is determined to be hazardous and classified as Harmful (Xn) and assigned one or more of the following risk phrases in accordance with the criteria given below.</p> | |
| <p>R48 Danger of serious damage to health by prolonged exposure</p> <p>Serious damage (clear functional disturbance or morphological changes that have toxicological significance) is likely to be caused by repeated or prolonged exposure by an appropriate route. Substances are classified at least as Harmful (Xn) when these effects are observed at the following dose ranges:</p> <ul style="list-style-type: none"> • inhalation, rat ≤ 0.25 mg/L, 6 hours/day; • oral, rat ≤ 50 mg/kg bw/day; and • dermal, rat or rabbit ≤ 100 mg/kg bw/day. <p>These guide values can apply directly when severe lesions have been observed in a subchronic (90 days) toxicity test. As a guideline, when interpreting the results of a sub-acute (28 days) toxicity test these figures should be increased at least threefold. If a chronic (two years) toxicity test is available, it should be evaluated on a case-by-case basis. If results of studies of more than one duration are available, then those from the study of the longest duration should normally be used.</p> | 6.9A |

Notes: bw = bodyweight; LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; mg/kg = milligrams per kilogram; mg/L = milligrams per litre.

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. *Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances*. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

18. Ecotoxicity – General Information

18.1. Introduction

The four subclasses under the ecotoxicity property in the Hazardous Substances and New Organisms Act 1996 (HSNO Act) are:

- subclass 9.1 – aquatic ecotoxicity (see chapter 19 [below](#)).
- subclass 9.2 – ecotoxicity to the soil environment (see chapter 20 [below](#)).
- subclass 9.3 – ecotoxicity to terrestrial vertebrates (chapter 21 [below](#)).
- subclass 9.4 – ecotoxicity to terrestrial invertebrates (see chapter 22 [below](#)).

A threshold is also set for a substance that is used as a biocide. If a substance is used as a biocide and does not trigger one of the above thresholds, then it is classified as 9.1D (biocide). See chapter 23 for more information on the biocidal classification.

This introductory chapter covers matters that are common across the four subclasses.

The key terms used in this chapter are defined in section 18.6

18.2. Classification of substances

18.2.1. Content of following sections

Each of the following sections explains how to classify a substance for each of the four subclasses. Each section outlines the key considerations required to assign a classification to a substance and acceptable test methods for deriving data for classification purposes. Additional guidance is provided where it may be difficult to interpret the regulations or for more complex types of data.

18.2.2. Consideration of metabolites

When you are evaluating the ecotoxicity hazards of a substance, the metabolites of the substance may also be relevant for classifying the parent substance.

Data on metabolites in aquatic and terrestrial systems come from the relevant degradation studies, including information on the time course of appearance and concentration. These metabolites are relevant for organisms that may be exposed through the environmental medium (soil or water) or food.

Supporting evidence is needed to evaluate the hazards of major metabolites, but a qualitative approach can be used for minor metabolites. Valuable sources of information include:

- the molecular structure of the metabolite (that is, is the active part intact?);
- the occurrence of metabolites in the medium in existing tests with the substance or major metabolites;
- for mammals and birds, the appearance of the metabolite in rats and poultry;
- general knowledge about the relationship between the toxicity of the metabolite and its parent substance (for example, from the aquatic data set (fish, *Daphnia*, algae));
- information about pesticidal activity from biological screening data; and

- available knowledge on related compounds.

No further studies are required and the metabolite is not considered ecotoxicologically relevant if the metabolite is:

- carbon dioxide (CO₂) or an inorganic compound, not being or containing a heavy metal; or
- an organic compound of aliphatic structure, with a chain length of four or less, which consists only of carbon (C), hydrogen (H), nitrogen (N) or oxygen (O) atoms and has no 'structures' or functional groups that are known to be of ecotoxicological concern.

Test data on metabolites may not be required when they are formed relatively rapidly and are short-lived, as their toxicity may be exerted in the tests on the parent substance. Such conclusions should be supported by analytical measurements or other justifiable arguments (for example, data from laboratory or field studies).

If there is more than one metabolite, it may be sufficient to conduct tests only with the most important metabolite (that is, the one with the highest concentration or the most comparable structure with the parent).

Where the parent substance degrades to a more hazardous metabolite, consider the rate at which it is formed when assigning a classification to the parent substance.

Metabolites in or on potential feed items have to be considered. However, apart from the general considerations explained above, experimental toxicity testing is not necessary in the following cases.

- If the metabolite in question also appears in birds and mammals, it may be assumed that any toxic effects would be expressed in the toxicity test with the parent compound, and that the risk from the metabolite is covered. Note that the toxicology section of the dossier or monograph always provides information on metabolism in rats, but not necessarily on metabolism in birds (poultry), and it cannot be assumed that the metabolic pathway in birds is identical to that in mammals.
- The toxicology data package may already contain mammalian toxicity tests with the metabolite. The absolute toxicity of the metabolite cannot be directly extrapolated from mammals to birds, but the relation can be used as an indication that such information might be sufficient for an assessment. For example, consider the following information.
 - LD₅₀ rat (parent) = 238 mg/kg,
 - LD₅₀ rat (metabolite) = 680 mg/kg,
 - LD₅₀ quail (parent) = 42 mg/kg.

So, in rats the metabolite is 2.9 times less toxic than the parent. It is not appropriate to multiply the quail LD₅₀ (parent) by 2.9 because that would imply an undue level of accuracy. However, it would be reasonable in most cases to assume that also in birds the metabolite is not more toxic than the parent compound.

Should testing become necessary an acute oral study would be the first choice to serve as a bridging study, that is, to compare the inherent toxicity of the metabolite with that of the parent compound.

18.3. Classification of mixtures: generic guidance

Once a substance triggers a threshold, it is then classified. While this is relatively straightforward for single substances, substances as mixtures are more complex. Mixtures can not be tested for degradability or bioaccumulation as these properties apply only to components of the mixture.

Note that classification issues associated with degradability and bioaccumulation are addressed in more detail in the chapters on aquatic ecotoxicity and ecotoxicity to the soil environment.

18.3.1. General process for classifying ecotoxicity hazards

The general process for classifying ecotoxicity hazards is as follows.

- a. Where ecotoxicity test data are available for the complete substance (mixture), then classification is based on the test results.
- b. Where test data are not available for the mixture itself, then bridging principles should be considered to see whether they permit classification of the mixture.
- c. Where test data are not available for the mixture (that is, formulation test data), and the available information is not sufficient to allow application of the bridging principles, the agreed method for estimating the hazards of the mixture is based on information on the components. This is used to derive the classification of the mixture, which is known as the summation of classified components approach.

The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) does not include classifications for ecotoxicity to the soil environment, terrestrial vertebrates, or terrestrial invertebrates. However, the same principles used to classify substances for aquatic ecotoxicity can be applied to these other subclasses under the HSNO Act.

18.3.2. Synergistic and antagonistic effects

Consider any information about possible synergistic effects that may enhance the ecotoxicity of the substance as a mixture when classifying the substance.

Note if there is information that antagonistic effects may occur such that the mixture classification is lower than that indicated from the calculated value.

18.3.3. Test data available on the mixture

See the specific ecotoxicity chapters for details on using test data available on the mixture to classify.

- Aquatic ecotoxicity – chapter 19.
- Ecotoxicity to the soil environment – chapter 20.
- Ecotoxicity to terrestrial vertebrates – chapter 21.
- Ecotoxicity to terrestrial invertebrates – chapter 22.

18.3.4. Bridging principles: test data not available for the mixture

If the substance as a mixture has not been tested to determine its ecotoxicity, but sufficient data exist about the individual components and similar tested mixtures to adequately characterise the hazards of the mixture, these data should be used in accordance with the following five bridging principles.

a. Dilution

- i. If a substance as a mixture is diluted with a diluent that has an equivalent or lower hazard classification than the least ecotoxic original component and is not expected to affect the hazards of other components, then assign the new mixture the same classification as the original mixture or substance.
- ii. If the mixture is diluted with water or other non-ecotoxic material, calculate the ecotoxicity of the mixture from the original mixture or substance.

b. Batching

Assume the ecotoxicity hazard classification of one batch of substance as a mixture is substantially equivalent to that of another batch of the same commercial product produced by or under the control of the same manufacturer. If there is reason to believe significant variation exists, such that the ecotoxicity hazard classification of the batch has changed, consider conducting testing or assessing the hazard using the mixture rules.

c. Concentration of highly ecotoxic mixtures

If a mixture is classified as very ecotoxic (that is, 9.1A, 9.2A, 9.3A, or 9.4A), and components of the mixture that are classified as very ecotoxic in their own right are further concentrated, classify the more concentrated mixture as 9.nA without additional testing.

d. Interpolation within one ecotoxicity class

If mixtures X and Y are in the same classification category and mixture Z is made in which the ecotoxic components have concentrations intermediate to those in mixtures X and Y, then assume mixture Z is in the same classification category as mixtures X and Y. Note that this assumes the identity of the components is the same in all three mixtures.

Substantially similar mixtures

For example, assume:

- i. mixture one comprises components A and B; and
- ii. mixture two comprises components C and B.

The concentration of component B is the same for both mixtures and the concentration of component A equals that of component C. If the data on the ecotoxicity of A and C are available and substantially equivalent (that is, A and C are from the same hazard class and are not expected to affect the ecotoxicity of B), and mixture one has already been tested, mixture two does not need to be tested. That is, mixture one and mixture two are classified in the same category.

18.3.5. Classification of mixture based on classifications of components: the summation approach

See the specific ecotoxicity chapters for details about classifying a mixture based on classifications of components.

- Aquatic ecotoxicity – chapter 19.
- Ecotoxicity to the soil environment – chapter 20.

- Ecotoxicity to terrestrial vertebrates – chapter 21.
- Ecotoxicity to terrestrial invertebrates – chapter 22.

18.4. Data requirements and data quality

18.4.1. Minimum data sets

The HSNO Act covers many types of substances with varying degrees of hazardous properties. These substances also have different uses and circumstances of use. The risk associated with a hazardous substance is a function of the degree of hazard of the substance and the level and duration of exposure to the hazard.

Different types of hazardous substances present different levels of risk, so require different types and levels of information to be considered in an application for approval. Different levels of information could relate to the quantity, extent, or degree of detail of the information, as applicable to the substance and type of approval involved.

Further guidance on the likely information requirements (that is, the minimum data sets) for applications for approval of hazardous substances can be found in the user guides to the HSNO Act application forms.

18.4.2. Data quality

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential test conditions are clearly and completely articulated.

See section 1.3 in chapter 1 for information about assessing data quality.

18.4.3. Weight of evidence

Where multiple studies for a taxonomic group are available, a decision on which studies are the most sensitive and of the highest quality must be made. A judgement has to be made on a case-by-case basis whether to use a study that is not based on Good Laboratory Practice (GLP) that has a more sensitive observation or a study based on GLP that has a less sensitive observation.

Substances that are difficult to test may yield apparent results that are more or less severe than the true toxicity of the substance. Expert judgement is needed for classification in these cases.

When more than one acceptable test is available for the same taxonomic group, generally use the most sensitive (that is, the one with the lowest median effect concentration or median lethal concentration (L(E)C₅₀) or no observable effect concentration (NOEC) – see the definitions in section 18.6) for classification. However, decide this on a case-by-case basis.

When larger data sets (that is, data sets with four or more values) are available for the same species, use the geometric mean of toxicity values as the representative toxicity value for that species. In estimating a

mean value, it is not advisable to combine tests of different species within a taxonomic group or in different life stages or tested under different conditions or durations.

18.4.4. Absence of measured data

The EPA recognises that measured data may not be available for all hazard effect endpoints for all substances. The Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 also acknowledge that:

data includes values that are directly measured, calculated, or estimated for any of the measures given.

Therefore, although no measured data may be available, the classification of a substance into a HSNO Act hazard classification category may still occur, using a weight of evidence approach that acknowledges all other data that is available on the substance or closely related substances. If this approach is used, any assumptions made and the weight of evidence approach for hazard classification should be clearly documented.

If no measured (direct) data or indirect data are available on the substance, the substance cannot be assigned a definitive hazard classification.

18.5. Data sources

The possible data sources listed in Table 18.1 below and Table 18.2 below are provided as a starting point; they are not exhaustive.

As noted in section 1.3 in chapter 1, the quality of data is highly variable within and between various sources. It is your responsibility to ensure the data used for classification meets the criteria of reliability, relevance, and adequacy.

Some sources listed in Table 18.1 and Table 18.2 require a subscription, but most are free. See also chapter 9 for a more extensive listing of data sources.

Table 18.1: Ecotoxicity and environmental fate – information sources

| Information source | URL |
|---|---|
| Chemical Evaluation Search and Retrieval System (CESARS) | http://www.ccohs.ca/products/databases/cesars.html |
| ECETOC Aquatic toxicity database (EAT III) | http://www.ecetoc.org/Content/Default.asp? |
| ECOTOX (US EPA integration of AQUIRE, PHYTOTOX and TERRETOX) | http://www.epa.gov/ecotox |
| OECD SIDS | http://cs3-hq.oecd.org/scripts/hpv |
| Biodegradation and Bioaccumulation Database on Existing Chemicals, Japan (MITI) | http://www.safe.nite.go.jp/english/db.html |

| | |
|--|---|
| SRC Environmental Fate data base (BIOLOG, BIODEG, CHEMFATE, DATALOG) | http://www.syrres.com/esc/efdb.htm |
| TRACE | http://www.bibra-information.co.uk |
| TOXNET (includes TOXLINE SPECIAL, TOXLINE CORE/MEDLINE and HSDB) | http://toxnet.nlm.nih.gov |
| OECD E-Chem Portal | http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en |
| USEPA Pesticide Fate Database | http://cfpub.epa.gov/pfate/Home.cfm |
| EU Pesticides Database FOOTPRINT | http://www.eu-footprint.org/ppdb.html |
| EFSA Pesticide Risk Assessment Reports | http://dar.efsa.europa.eu/dar-web/provision |

Note: These URLs may not be the only routes to the information.

Table 18.2: Physicochemical properties – information sources

| Information source | URL |
|---|---|
| HSDB | http://toxnet.nlm.nih.gov |
| Chemical Evaluation Search and Retrieval System (CESARS) | http://www.ccohs.ca/products/databases/cesars.html |
| Biodegradation and Bioaccumulation Database on Existing Chemicals, Japan (MITI) | http://www.safe.nite.go.jp/english/db.html |
| OECD SIDS | http://cs3-hq.oecd.org/scripts/hpv |
| ChemFinder | http://chemfinder.cambridgesoft.com |
| SRC PhysProp | http://www.srcinc.com/what-we-do/product.aspx?id=133 |
| SRC Environmental Fate data base (BIOLOG, BIODEG, CHEMFATE, DATALOG) | http://www.syrres.com/esc/efdb.htm |
| Merck Index | http://library.dialog.com/bluesheets/html/bl0304.html |
| IUPAC solubility data series | http://www.iupac.org/publications/sds |
| Beilstein | http://www.beilstein-online.de/frameset.htm |

Note: These URLs may not be the only routes to the information.

18.6. Definitions

The following definitions are particularly relevant to chapters 18–23.

| Term | Definition |
|-----------------------------------|--|
| acute aquatic ecotoxicity value | <p>The lowest value expressed in milligrams of a substance per litre of water from:</p> <ul style="list-style-type: none"> a. fish LC₅₀ data after a 96-hour exposure period; or b. crustacean EC₅₀ data after a 48-hour exposure period; or c. algal, or other aquatic plant, EC₅₀ data after a 72-hour or 96-hour exposure period. <p>See Schedule 6 to the Hazardous Substances (Classification) Regulations 2001. See also LC₅₀, EC₅₀.</p> |
| BCF | See bioconcentration factor (BCF). |
| bioaccumulative | <p>Any substance that has a bioconcentration factor (BCF) greater than or equal to 500 or, if BCF data are not available, a log K_{OW} equal to or less than 4; and, for the purposes of this definition, measured log K_{OW} values take precedence over estimated values.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. See also bioconcentration factor (BCF), K_{OW}.</p> |
| biocidal action | <p>In relation to a substance, means the substance causes mortality, inhibited growth, or inhibited reproduction in an organism.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| bioconcentration factor (BCF) | <p>The steady state concentration of a substance in an aquatic organism divided by the concentration of the substance in the surrounding water.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| BOD ₅ | <p>The five-day biochemical oxygen demand, being the mass of oxygen consumed by micro-organisms during oxidation of the substance in water over five days, expressed in milligrams of oxygen consumed per milligrams of the substance.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| chemical oxygen demand | See COD. |
| chronic aquatic ecotoxicity value | <p>The lowest value expressed in milligrams of a substance per litre of water from chronic fish, crustacean, algal, or other aquatic plant NOEC data.</p> <p>See Schedule 6 to the Hazardous Substances (Classification) Regulations 2001. See also NOEC.</p> |
| COD | <p>The chemical oxygen demand, being the equivalent mass of oxygen from an oxidising agent, of a strength at least equal to the oxidising strength of potassium permanganate or potassium dichromate, that is consumed during oxidation of the substance in water, expressed in milligrams of oxygen consumed per milligram of the substance.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |

| Term | Definition |
|--|--|
| data | Includes values that are directly measured, calculated, or estimated for any of the measures given. See Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| DT ₅₀ | The time required to reduce the concentration of the original substance in an environmental medium by 50% as a result of biotic or abiotic processes. |
| EC ₅₀ | The median effect concentration, being a statistically derived concentration of a substance that can be expected to cause: a. an adverse reaction in 50% of organisms; or b. a 50% reduction in growth or in the growth rate of organisms. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| ecotoxicologically relevant metabolite | A metabolite that poses a higher or comparable risk to terrestrial or aquatic organisms as the parent substance. Such a metabolite is relevant for the overall decision on classification of the parent substance. |
| five-day biochemical oxygen demand | See BOD ₅ . |
| K _{OW} | The steady state ratio of the solubility of a substance in n-octanol to the solubility of that substance in water. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| LC ₅₀ | The median lethal concentration, being a statistically derived concentration of a substance that can be expected to cause death in 50% of organisms exposed for a specified time. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| LD ₅₀ | A median lethal dose, being a statistically derived single dose of a substance that can be expected to cause death in 50% of organisms. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| L(E)C ₅₀ | Either LC ₅₀ or EC ₅₀ data. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. See also LC ₅₀ , EC ₅₀ . |
| LOEC | The lowest observed effect concentration, being the lowest concentration of a substance that produces a significant ecotoxic effect in an organism or organism population. See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| lowest observed effect concentration | See LOEC. |

| Term | Definition |
|---|---|
| major metabolite | All metabolites that are formed in amounts of equal to or more than 10% of the applied amount of substance at any time-point evaluated during the degradation studies in the appropriate compartment under consideration (soil or water). |
| MATC | <p>The maximum acceptable toxicant concentration, being the geometric mean of the NOEC and LOEC where the NOEC and LOEC are derived from the same study.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. See also LOEC, NOEC.</p> |
| maximum acceptable toxicant concentration | See MATC. |
| median effect concentration | See EC ₅₀ . |
| median lethal concentration | See LC ₅₀ . |
| median lethal dose | See LD ₅₀ . |
| metabolite | All breakdown products of a substance that are formed in the environment by biotic or abiotic processes. |
| minor metabolite | All metabolites, degradation and reaction products that are formed in amounts of less than 10% of the parent substance at any time during the degradation studies under consideration. |
| no observed effect concentration | See NOEC. |
| NOEC | <p>The no observed effect concentration, being the highest concentration of a substance that does not produce a significant ecotoxic effect in an organism or organism population.</p> <p>See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001.</p> |
| rapidly degradable | <p>In relation to a substance in water, means:</p> <ol style="list-style-type: none"> a. 28 days after a solution containing the substance is inoculated with micro-organisms, there is at least a: <ol style="list-style-type: none"> i. 70% reduction in dissolved organic carbon in the solution; or ii. 60% depletion of oxygen in the solution, when compared with the maximum depletion of oxygen that would occur if the substance were completely degraded; or iii. 60% generation of carbon dioxide in the solution, when compared with the maximum generation of carbon dioxide that would occur if the substance were completely degraded; or b. if only COD and BOD₅ data are available, the ratio of BOD₅ to COD is greater than or equal to 0.5:1; or c. at least 70% of the substance can be degraded biotically or abiotically, in the aquatic environment within 28 days. |

| Term | Definition |
|-----------------------------|--|
| | See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. See also BOD ₅ , COD. |
| significant ecotoxic effect | An ecotoxicologically significant change in an organism or organism population observed during the study where the probability that the change is different from any recognised background history of change or from the value in a recognised unexposed control organism or organism population is greater than 0.95 (equivalent to a probability of 0.05 or less). See Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001. |
| soil DT ₅₀ | The half-life of a substance in soil, which is the time required to reduce the original concentration of the substance in the soil by 50%. See Schedule 6 to the Hazardous Substances (Classification) Regulations 2001. See also DT ₅₀ . |
| soil ecotoxicity value | The lower value in milligrams of a substance per kilogram (dry weight) of soil from: a. plant or soil invertebrate EC ₅₀ data after 14 days' exposure to the substance; or b. data that demonstrate a 25% reduction in soil micro-organism respiration or nitrification after 28 days' exposure to the substance. See Schedule 6 to the Hazardous Substances (Classification) Regulations 2001. See also EC ₅₀ . |

References

United Nations 2007. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2nd revised edition. United Nations, Geneva.

19. Aquatic Ecotoxicity – Subclass 9.1

19.1. Basic elements and general considerations

The basic elements to consider in determining aquatic hazard classification under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) are:

- acute aquatic ecotoxicity;
- potential for or actual bioaccumulation;
- degradation (biotic or abiotic) for organic chemicals; and
- chronic aquatic ecotoxicity.

While data from internationally harmonised test methods are preferred, data from national methods may also be used where they are considered as equivalent. In general, freshwater and marine species toxicity data can be considered as equivalent data, preferably derived using test guidelines from the Organisation for Economic Co-operation and Development (OECD) or according to the principles of Good Laboratory Practice (GLP). Where such data are not available, classification should be based on the best available data using a weight of evidence approach.

See section 18.6 in chapter 18 for definitions of the key terms used in this chapter.

See section 1.3 in chapter 1 for information about assessing data quality.

See Appendix 19A for a detailed list of acceptable test methods for aquatic toxicity, aquatic degradation, and bioaccumulation.

See Appendix 19B and Appendix 19C for comparisons of the HSNO aquatic hazard classifications with those of the GHS and EU.

19.1.1. Acute aquatic toxicity

Aquatic toxicity testing involves dissolving the substance under test in the water used and maintaining a stable bioavailable exposure concentration over the course of the test. Some substances are difficult to test under standard procedures, so special guidance has been developed on interpreting and using the data when applying the classification criteria.

Acute aquatic toxicity is normally determined using:

- a fish 96-hour LC50 (OECD Test Guideline 203 or equivalent);
- a crustacean 48-hour EC50 (OECD Test Guideline 202 or equivalent); and/or
- an algal 72- or 96-hour EC50 (OECD Test Guideline 201 or equivalent).

These species are considered surrogates for all aquatic organisms. Data on other species such as the floating aquatic macrophyte *Lemna* spp. may also be considered if the test methodology is suitable. For *Lemna*, a standard 7- or 14-day EC50 test is considered appropriate (OECD Test Guideline 221).

Although the algal growth inhibition test is a chronic test, the EC50 is treated as an acute value for classification purposes. The algal EC50 should normally be based on growth rate inhibition. If only the EC50

based on reduction in biomass is available, or it is not indicated which EC50 is reported, these values may be used.

Ideally, data on all three standard taxa will be available for classification purposes, with classification based on the most sensitive test result.

19.1.2. Chronic aquatic toxicity

Chronic effects usually include a range of sublethal endpoints and are generally expressed in terms of a no observable effect concentration (NOEC) or an equivalent ECx. Endpoints typically include survival, growth, and/or reproduction. Exposure durations vary widely, depending on the endpoint being assessed and the test species being used.

Chronic toxicity data are generally less available than are acute data, so for classification purposes the potential for chronic or long-term toxicity is often assessed through a combination of acute toxicity, lack of rapid degradability, and potential or actual bioaccumulation. Where chronic data do exist, these are taken into account in assigning a classification to a substance.

Use of chronic data to reduce a classification

Where a substance is acutely toxic, not rapidly degradable, and/or has potential to bioconcentrate, chronic test data > 1 mg/L can be used to 'de-classify' or reduce the classification of a substance from 9.1B or 9.1C to the less restrictive classification of 9.1D. Several aspects of the chronic data must be considered before a classification can be reduced.

The general approach is to demonstrate the chronic NOEC > 1 mg/L for the most sensitive species identified by the acute toxicity data. For example, if the classification has been applied on the basis of acute toxicity to fish, it would generally not be possible to reduce the classification based on a NOEC for an aquatic invertebrate. If the classification has resulted from acute toxicity to more than one taxa, a NOEC > 1 mg/L for each would be needed to reduce the classification.

Tests with algae and Lemna cannot be used to reduce the classification of a substance because the:

- duration of the studies is not long term;
- acute to chronic ratio is generally narrow; and
- endpoints are more consistent with those for other organisms.

Combined acute and chronic classifications

While the current HSNO Act classification system will continue to rely on the use of acute toxicity data in combination with a lack of rapid degradation and/or a potential to bioaccumulate as the basis for aquatic hazard classification, actual chronic toxicity data form a better basis for classification where these data are available. The OECD is developing a chronic aquatic hazard classification system based on chronic aquatic test data. It is anticipated that under the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2007) the available chronic toxicity data would be used to assign a chronic hazard classification in preference to that derived from acute toxicity in combination with a

lack of rapid degradation and/or a potential to bioaccumulate. Changes to the HSNO Act regulations would be required in order to adopt any changes to the GHS system.

See Appendix 19D for more detailed guidance on interpreting aquatic toxicity data and test methods.

19.1.3. Bioaccumulation potential

The bioaccumulation of substances within aquatic organisms can give rise to toxic effects over longer time scales even when actual water concentrations are low. The potential to bioaccumulate is determined in the laboratory by the partitioning between n-octanol and water. The relationship between the partition coefficient of an organic substance and its bioconcentration as measured by the bioconcentration factor (BCF) in fish has considerable scientific literature support. Using a cut-off value of $\log KOW \geq 4$ is intended to identify only those substances with a real potential to bioconcentrate (see the definition of KOW in section 18.6 in chapter 18). In recognition that the log Kow is only an imperfect surrogate for a measured BCF, such a measured value would always take precedence. A BCF in fish of < 500 is considered indicative of a low level of bioconcentration.

Under the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001, the criterion for determining whether a substance has the potential to bioaccumulate is:

- a BCF greater than or equal to 500; or
- if BCF data are not available, a log KOW equal to or greater than 4.

The order of preference (from most preferable to least preferable) in terms of acceptability of data for assessing bioaccumulation potential is:

- measured BCF (generally in whole fish);
- measured log KOW;
- estimated KOW; and
- estimated BCF.

See Appendix 19F for detailed guidance on the principles and interpretation of bioaccumulation studies.

19.1.4. Rapid degradability

Substances that rapidly degrade can be quickly removed from the environment. While effects can occur, particularly in a spillage or an accident, they can be localised and of short duration. The absence of rapid degradation in the environment can mean a substance in the water has the potential to exert toxicity over a wide temporal and spatial scale.

One way to demonstrate rapid degradation uses the biodegradation screening tests designed to determine whether a substance is ‘readily biodegradable’. Thus, a substance that passes this screening test is one that is likely to biodegrade ‘rapidly’ in the aquatic environment, so is unlikely to be persistent. However, a fail in the screening test does not necessarily mean the substance will not degrade rapidly in the environment. A further criterion allows the use of data to show that the substance did actually degrade biotically or abiotically in the aquatic environment by $> 70\%$ in 28 days. Thus, if degradation is demonstrated under environmentally

realistic conditions, then the HSNO Act definition of 'rapid degradability' would be met (see the definition in section 18.6 in chapter 18).

Many degradation data are available in the form of degradation half-lives (DT50), and they can also be used to define rapid degradation. In general, a DT50 of 16 days is considered equivalent to > 70% degradation in 28 days.

(A flowchart summarising the evaluation of rapid degradation is in Figure 19.1.)

Environmental degradation may be biotic or abiotic (for example, hydrolysis) and the HSNO Act criteria reflect this. Ready biodegradation can most easily be defined using the OECD biodegradability tests (OECD Test Guideline 301 (A–F)). A pass level in these tests can be considered indicative of rapid degradation in most environments. These are freshwater tests, so the use of the results from OECD Test Guideline 306, which is more suitable for marine environments, has also been included. Where such data are not available, a BOD₅ to COD ratio > 0.5 is considered indicative of rapid degradation.

Some tests measure the ultimate biodegradation of the substance, that is, when full mineralisation is achieved. Primary biodegradation would not normally qualify in the assessment of rapid degradability unless it can be demonstrated that the metabolites or degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

See Appendix 19E for detailed guidance on the principles of degradation testing and the interpretation of degradation data.

Default classification in the absence of data on degradation and bioconcentration

Where there are no data on the degradation or bioconcentration potential of a substance, the default position is that the substance attracts the same classification as if those data were available and indicated that the substance was not rapidly degradable and/or likely to bioconcentrate, unless there are chronic data to 'de-classify' to a lesser classification.

Inorganic compounds and metals

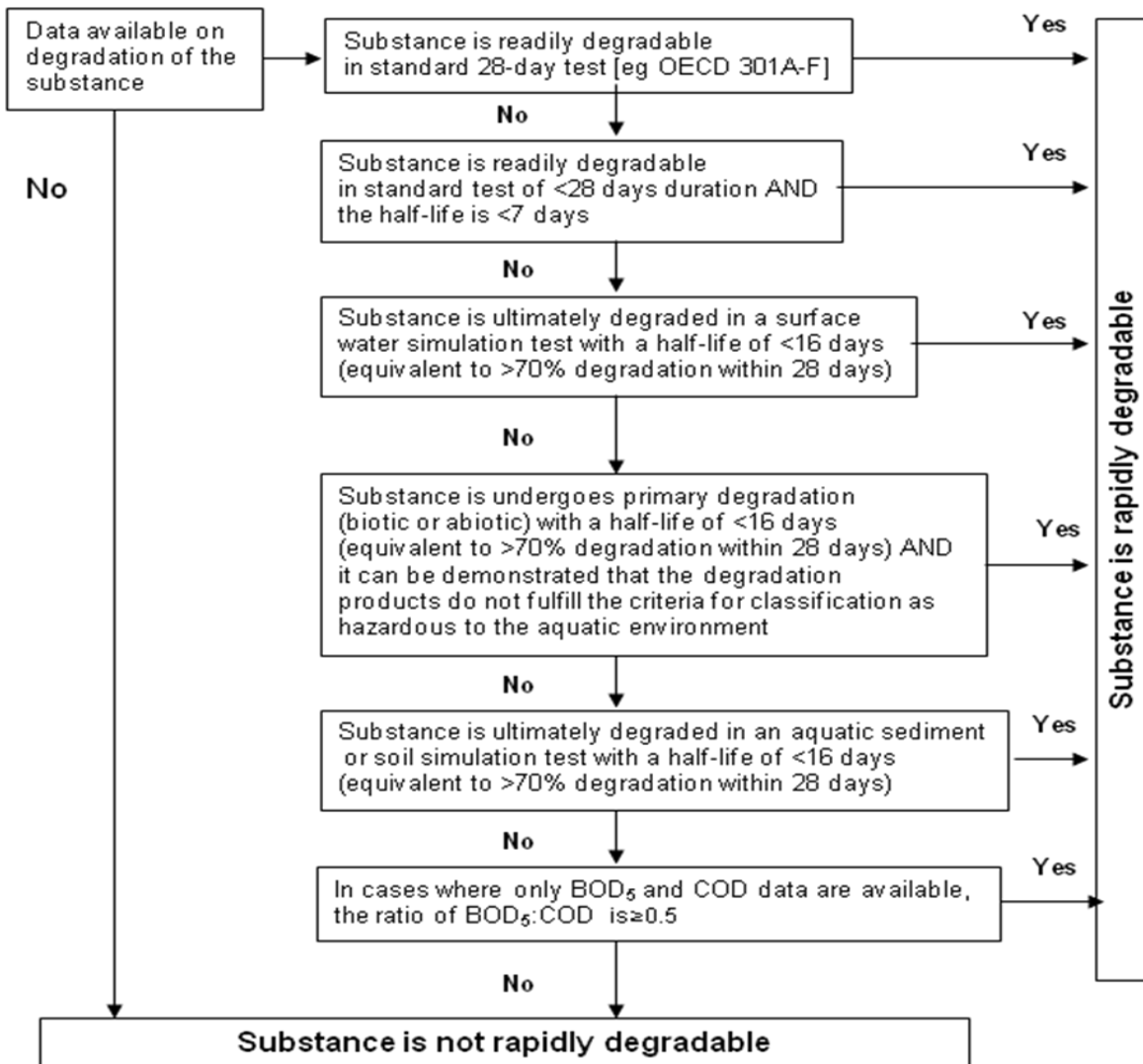
For inorganic compounds and metals, the concept of degradability as applied to organic compounds has limited or no meaning. Rather the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally, the use of bioaccumulation data should be treated with care. Specific guidance is provided on how these data for such materials may be used in meeting the requirements of the classification criteria.

Poorly soluble inorganic compounds and metals may be acutely or chronically toxic in the aquatic environment, depending on the intrinsic toxicity of the bioavailable inorganic species and the rate and amount of this species that may enter solution.

A protocol for testing these poorly soluble materials is in Appendix 19G. This protocol is undergoing inter-laboratory validation testing under the auspices of the OECD.

See Appendix 19D for detailed guidance on the classification of metals and inorganic metal compounds, and Appendix 19G for the details of the metal transformation and dissolution protocol.

Figure 19.1: Determining rapid degradability of an organic substance



Note: BOD₅ = 5-day biochemical oxygen demand; COD = chemical oxygen demand; OECD = Organisation for Economic Co-operation and Development

19.1.5. Metabolites

Data on metabolites in aquatic systems come from the aquatic degradation studies, including information on the time course of appearance and concentration. These metabolites are relevant for aquatic organisms.

Toxicity data or other supporting information may be needed to evaluate the hazards of the major metabolites.

Where the parent substance degrades to a more hazardous metabolite, the rate at which it is formed should be taken into consideration when assigning a classification to the parent substance.

See section 18.2.2 in chapter 18 for further information on metabolites.

19.1.6. Use of Quantitative Structure Activity Relationships

While experimentally derived test data are preferred, where no experimental data are available, validated Quantitative Structure Activity Relationships (QSARs) for aquatic toxicity and log KOW may be used in the classification process. Such validated QSARs may be used without modification to the agreed criteria, if restricted to chemicals for which their mode of action and applicability are well characterised.

QSARs for predicting ready biodegradation are not yet sufficiently accurate to predict rapid degradation.

See Appendix 19D for further information on the use of QSARs.

19.1.7. Weight of evidence

See section 1.3 in chapter 1 for information about assessing data quality.

The best quality data should be used as the fundamental basis for classification. It is preferable that classification is based on primary data sources, and it is essential that test conditions are clearly and completely articulated.

Where multiple studies for a taxonomic group are available, a decision on which studies are the most sensitive and of the highest quality must be made. A judgement has to be made on a case-by-case basis whether to use a study that is not based on Good Laboratory Practice (GLP) that has a more sensitive observation, or a study based on GLP that has a less sensitive observation. It appears that results that indicate high toxicity from tests performed according to non-standard or non-GLP guidelines should be able to be used for classification, whereas studies that demonstrate negligible toxicity require more careful consideration.

Substances that are difficult to test may yield apparent results that are more or less severe than the true toxicity of the substance. Expert judgement is needed for classification in these cases.

When more than one acceptable test is available for the same taxonomic group, the most sensitive (the one with the lowest L(E)C50 (that is, LC50 or EC50 data) or NOEC) is generally used for classification. However, this must be dealt with on a case-by-case basis. When larger data sets (that is, with four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. In estimating a mean value, it is not advisable to combine tests of different species within a taxonomic group or in different life stages or tested under different conditions or duration.

19.2. Aquatic hazard threshold and classification criteria

19.2.1. Aquatic hazard threshold criteria

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 Minimum degrees of hazard

- (1) *A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—*
- (a) *the substance is ecotoxic to aquatic organisms because—*
- i. *data for the substance indicates that the fish LC50 is 100 milligrams or less of the substance per litre of water over a 96-hour exposure period, as a result of exposure to the substance; or*
 - ii. *data for the substance indicates that the crustacean EC50 is 100 milligrams or less of the substance per litre of water over a 48-hour exposure period, as a result of exposure to the substance; or*
 - iii. *data for the substance indicates that the algal or other aquatic plant EC50 is 100 milligrams or less of the substance per litre of water over a 72-hour or 96-hour exposure period, as a result of exposure to the substance; or*
 - iv. *data for the substance indicates that the chronic fish NOEC, or chronic crustacean NOEC, or algal or other aquatic plant chronic NOEC, is 1 milligram or less of the substance per litre of water, as a result of exposure to the substance; or*
 - v. *in the absence of the NOEC data prescribed in subpara (iv) data for the substance indicates that it is not rapidly degradable and is bioaccumulative.*

If data for the substance meet one or more of the above criteria, then the substance needs to be assigned an aquatic hazard classification.

19.2.2. Aquatic hazard classification criteria for substances

Schedule 6 to the Hazardous Substances (Classification) Regulations 2001 specifies four classification categories for substances that are ecotoxic to the aquatic environment (subclass 9.1).

A subclass 9.1 classification and the subsequent category apply to any substance that meets the following criteria.

- Category 9.1A – substances that are very ecotoxic in the aquatic environment
A substance for which data indicate an acute aquatic ecotoxicity value \leq 1 milligram of the substance per litre of water.
- Category 9.1B – substances that are ecotoxic in the aquatic environment
Unless the chronic aquatic ecotoxicity value is $>$ 1 mg of the substance per litre of water, a substance—
 - a. for which data indicate an acute aquatic ecotoxicity value $>$ 1 mg but \leq 10 mg, of the substance per litre of water; and
 - b. that is not readily degradable or is bioaccumulative, or is not readily degradable and is bioaccumulative.
- Category 9.1C – substances that are harmful in the aquatic environment
Unless the chronic aquatic ecotoxicity value is $>$ 1 milligram of the substance per litre of water, a substance—

- a. for which data indicate an acute aquatic ecotoxicity > 10 mg, but ≤ 100 mg, of the substance per litre of water; and
 - b. that is not readily degradable or is bioaccumulative, or is not readily degradable and is bioaccumulative.
- Category 9.1D – substances that are slightly harmful to the aquatic environment or are otherwise designed for biocidal action
 - A substance for which data indicate that:
 - i. the acute aquatic ecotoxicity value is > 1 mg per litre of water but ≤ 100 mg of the substance per litre of water, but which does not meet the criteria for hazard classification 9.1B or 9.1C; or
 - ii. the chronic aquatic ecotoxicity value is ≤ 1 mg of the substance per litre of water, but which does not meet the criteria for hazard classification 9.1A, 9.1B, or 9.1C; or
 - a substance that is designed for biocidal action, other than a substance that is designed for biocidal action against a virus, protozoan, bacterium, or an internal organism in humans or in other vertebrates, but does not meet the criteria for any hazard classification in class 9 other than 9.1D; or
 - a substance that is not rapidly degradable and that is bioaccumulative unless the chronic aquatic ecotoxicity value is > 1 mg of the substance per litre of water, but does not meet the criteria for hazard classification 9.1A, 9.1B, or 9.1C.

Note that assignment to category 9.1D due solely to biocidal action is discussed chapter 23.

The aquatic classification criteria for single component substances are summarised in Table 19.1 and Figure 19.2. The application of the criteria to mixtures is set out in more detail in section 19.3.

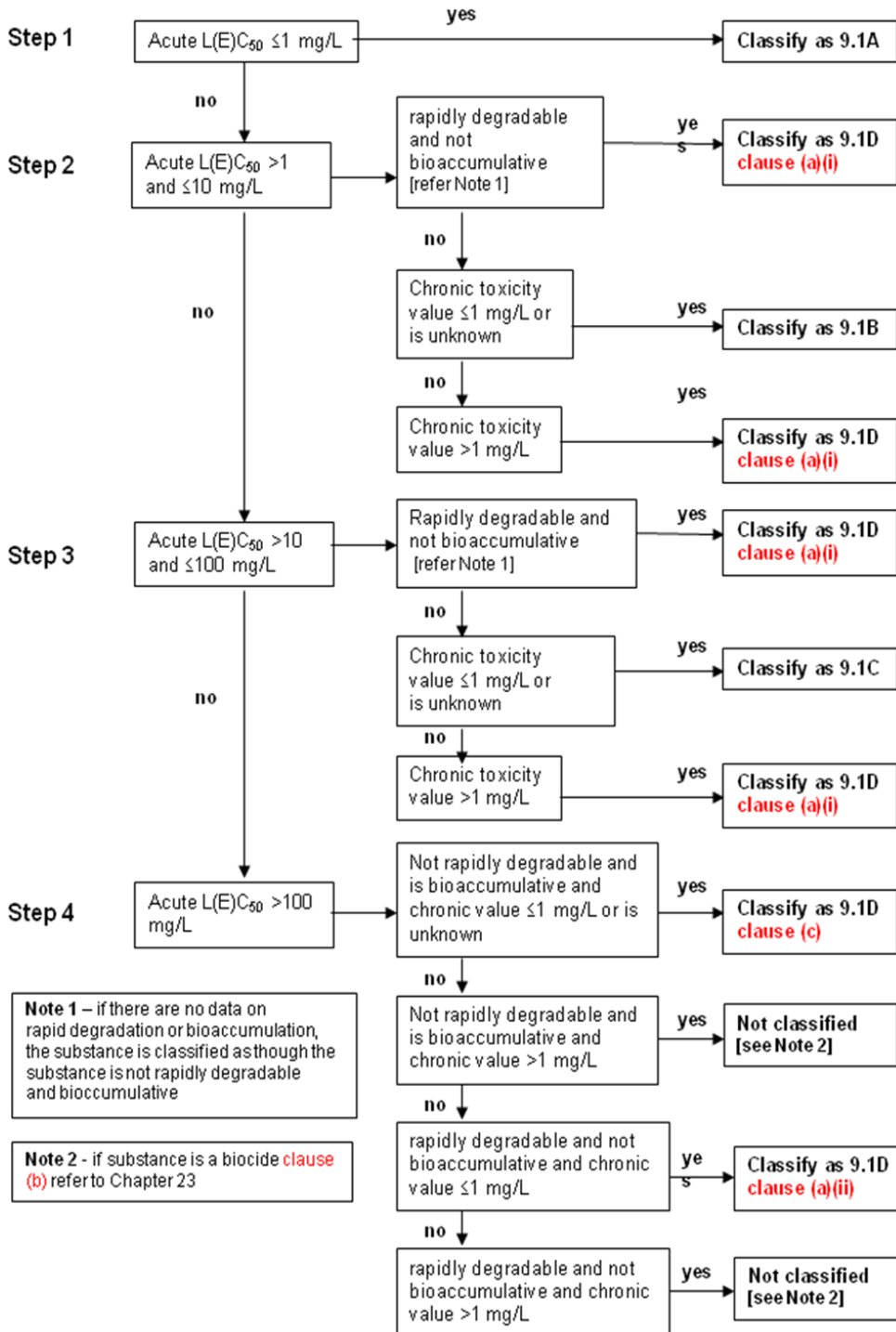
Table 19.1: Aquatic classification of a single component substance

| Acute L(E)C ₅₀ of the substance | Chronic NOEC of the substance | Substance is not rapidly degradable or is bioaccumulative | Classification category for the substance |
|--|-------------------------------|---|--|
| ≤ 1 mg/L | ≤ 1 mg/L or unknown: | Not considered for 9.1A classification | 9.1A |
| ≤ 1 mg/L | > 1 mg/L | | |
| > 1 and ≤ 10 mg/L | ≤ 1 mg/L or unknown | Yes or unknown | 9.1B |
| > 10 and ≤ 100 mg/L | ≤ 1 mg/L or unknown | | 9.1C |
| > 1 and ≤ 10 mg/L | > 1 mg/L | Yes or unknown; the chronic test data 'de-classify' substance | 9.1D |
| > 10 and ≤ 100 mg/L | > 1 mg/L | | |
| > 10 and ≤ 100 mg/L | < 1 mg/L | No | |
| > 100 mg/L | ≤ 1 mg/L or unknown | Yes or unknown | |
| > 100 mg/L | > 1 mg/L | No for either property; the chronic test data 'de- | Substance not classified, not hazardous unless |

| | | |
|--|---------------------|---|
| | classify' substance | intended for biocidal use in which case 9.1D applies (see chapter 23) |
|--|---------------------|---|

Note: L(E)C₅₀ = median lethal concentration or median effect concentration; NOEC = no observable effect concentration.

Figure 19.2: Aquatic hazard classification of a single substance



Note: L(E)C₅₀ = median lethal concentration or median effective dose

19.3. Classification of mixtures

To make use of all available data for classifying the aquatic environmental hazards of the mixture, the following assumption has been made and should be applied where appropriate.

The 'relevant components' of a mixture are those that are present in a concentration of 1% (weight/weight) or greater, unless there is a presumption (for example, in the case of highly toxic components) that a component present at less than 1% can still be relevant for classifying the mixture for aquatic environmental hazards.

The approach for classifying aquatic hazards is tiered, and depends on the type of information available for the mixture itself and for its components. Elements of the tiered approach include classification based on:

- tested mixtures (see section 19.3.1);
- bridging principles (see section 19.3.2); and
- the summation approach, using the classifications of components (see section 19.3.3).

19.3.1. Tested mixtures

For aquatic hazard classification, the test data on the mixture can be used directly to assign a classification to a substance on the basis of acute toxicity, as indicated in the examples in Table 19.2, with additional consideration given to whether the components of the mixture are not rapidly degradable and/or are potentially bioaccumulative.

Where components of the mixture are acutely toxic and either are not rapidly degradable or are bioaccumulative, or in the absence of data on these properties, the concentrations of components with these properties are weighted and summed to determine the classification of the mixture. Where the weighted sum of these components is $\geq 25\%$ the more conservative classification applies.

To calculate the weighted sum of the components which are not rapidly degradable and/or are bioaccumulative, use the summation approach set out in section 19.3.3 and see the worked example in Table 19.5. and accompanying text.

Table 19.2: Aquatic classification of a tested mixture

| Acute L(E)C ₅₀ of the tested mixture | Chronic NOEC of tested mixture | Components in mixture are not rapidly degradable and/or are bioaccumulative | Classification category |
|---|--------------------------------|---|-------------------------|
| ≤ 1 mg/L | ≤ 1 mg/L or unknown | Not considered for 9.1A classification | 9.1A |
| ≤ 1 mg/L | > 1 mg/L | | |
| > 1 and ≤ 10 mg/L | ≤ 1 mg/L or unknown | Yes or unknown | 9.1B* |

| | | | |
|--------------------------------------|---------------------|---|--|
| > 10 and ≤100 mg/L | ≤ 1 mg/L or unknown | | 9.1C* |
| > 1 and ≤10 mg/L | > 1 mg/L | Yes or unknown; the chronic test data 'de-classify' substance | 9.1D |
| > 10 and ≤100 mg/L | > 1 mg/L | | |
| > 10 and ≤100 mg/L | < 1 mg/L | No | |
| > 100 mg/L or above water solubility | ≤ 1 mg/L or unknown | Yes or unknown | |
| > 100 mg/L or above water solubility | > 1 mg/L | No for either property; the chronic test data 'de-classify' substance | Substance not classified If intended for biocidal use see Biocidal Classification |

Notes: L(E)C₅₀ = median lethal concentration or median effect concentration; NOEC = no observable effect concentration.

* If a mixture is classified as 9.1B or 9.1C on the basis of acute toxicity data, and the weighted sum of components, which are not rapidly degradable or are bioaccumulative, is < 25% then the mixture is assigned a 9.1C or D classification ie, 9.1B drops to 9.1C and 9.1 C to 9.1D.

19.3.2. Bridging principles

Guidance on the bridging principles for classifying mixtures without test data is in chapter 18.

19.3.3. Classification of a mixture based on the classifications of components: summation approach

When test data on the mixture are not available and the bridging principles are not applicable, the summation approach is used to derive an aquatic hazard classification for the mixture.

Rationale

The toxicity criteria for the aquatic classification categories differ by a factor of 10 in moving from one category to another. Substances with a classification in a high toxicity band may, therefore, contribute to the classification of a mixture in a lower band. The calculation of these classification categories, therefore, needs to consider the contribution of all substances that are classified for aquatic toxicity.

When components are classified as 9.1A and their acute toxicity is well below the cut-off value (that is, 1 mg/L) they contribute to the toxicity of the mixture even at a low concentration. Active ingredients in pesticides often possess such high aquatic toxicity but so do some other substances such as organometallic compounds. Under these circumstances the application of the normal cut-off values or concentration limits may lead to an 'under-classification' of the mixture. Therefore, multiplying factors are applied to account for highly toxic components, as described in 'Mixtures with highly toxic components' under 'Classification procedure' below.

Classification procedure

Rapid degradability and potential for bioaccumulation

When classifying a mixture for aquatic hazards, separate consideration must be given to the rapid degradability and potential bioaccumulation of the components of the mixture. In general, a mixture cannot be directly tested for these properties. The classification criteria for 9.1B and 9.1C require that the mixture includes a weighted sum of the components with one or both of these properties to be $\geq 25\%$.

If the weighted sum of these components is $<25\%$ the aquatic hazard classification is reduced to the next classification.

The steps to follow in applying the summation approach to aquatic hazard classification are set out below and summarised in Table 19.3 and accompanying text and Figure 19.3.

Mixtures with no highly toxic components

The steps to follow in applying the summation approach to aquatic hazard classification for mixtures with no highly toxic components are set out below.

- Step 1: Consider all components classified as 9.1A.
If:
 $\sum(9.1A)\% \geq 25\%$
then the mixture is classified as 9.1A, and the classification process is complete.
- Step 2a: Consider all components classified as 9.1A and 9.1B.
If:
 $(\sum(9.1A)\% \times 10) + \sum(9.1B)\% \geq 25\%$
then the mixture is classified as 9.1B, unless
Step 2b: consider components that are not rapidly degradable or are bioaccumulative.
If the weighted sum of components which are not rapidly degradable or are bioaccumulative is $<25\%$,
then the mixture is classified as 9.1C.
- Step 3a: Consider all components classified as 9.1A, 9.1B, and 9.1C.
If:
 $(\sum(9.1A)\% \times 100) + (\sum(9.1B)\% \times 10) + \sum(9.1C)\% \geq 25\%$
then the mixture is classified as 9.1C unless
Step 3b: consider components that are not rapidly degradable or are bioaccumulative.
If the weighted sum of components which are not rapidly degradable or are bioaccumulative is $<25\%$,
then the mixture is classified as 9.1D.
- Step 4: Consider all components classified as 9.1A, 9.1B, 9.1C, and 9.1D.
If:
 $\sum(9.1A)\% + \sum(9.1B)\% + \sum(9.1C)\% + \sum(9.1D)\% \geq 25\%$
then the classification process is complete.

If the sum is < 25% then the substance is not classified for hazards to the aquatic environment. The exception to this is where the substance is used as a biocide (see chapter 23 for further guidance).

Table 19.3: Classification of a mixture for aquatic ecotoxicity based on summation of classified components

| Process | Sum of % of components classified as | Cut-off | Mixture classified as |
|---------|---------------------------------------|---------|-----------------------|
| Step 1 | 9.1A × M | ≥ 25% | 9.1A |
| Step 2 | (M × 10 × 9.1A) + 9.1B | ≥ 25% | 9.1B* |
| Step 3 | (M × 100 × 9.1A) + (10 × 9.1B) + 9.1C | ≥ 25% | 9.1C* |
| Step 4 | 9.1A + 9.1B + 9.1C + 9.1D | ≥ 25% | 9.1D |

Notes: M = multiplying factor.

* If a mixture is classified as 9.1B or 9.1C and the weighted sum of components that are not rapidly degradable or are bioaccumulative is < 25% then the mixture is assigned the classification at the step below, ie 9.1B reduces to 9.1C, and 9.1C to 9.1D.

Mixtures with highly toxic components

Components with toxicities well below the cut-off for 9.1A classification (that is, << 1 mg/L) may influence the toxicity of the mixture, so are given increased weight in applying the summation of classification approach.

The multiplying factors to be applied to these components are defined using the toxicity value, as summarised in Table 19.4. Therefore, to classify a mixture containing highly toxic components, the classifier needs to apply the multiplying factor M when assigning an aquatic hazard classification to the mixture.

See Table 19.5 and the worked example below.

Table 19.4: Aquatic ecotoxicity: multiplying factors

| Acute L(E)C ₅₀ value (mg/L) | Multiplying factor (M) |
|--|------------------------|
| 0.1 < L(E)C ₅₀ ≤ 1 | 1 |
| 0.01 < L(E)C ₅₀ ≤ 0.1 | 10 |
| 0.001 < L(E)C ₅₀ ≤ 0.01 | 100 |
| 0.0001 < L(E)C ₅₀ ≤ 0.001 | 1,000 |
| 0.00001 < L(E)C ₅₀ ≤ 0.0001 | 10,000 |
| (continue in factor 10 intervals) | |

Note: L(E)C₅₀ = median lethal concentration or median effect concentration.

Table 19.5: Example calculation for aquatic classification of mixture Z containing one highly toxic component

| Component | L(E)C ₅₀ (mg/L) | Aquatic classification of individual component | Component not rapidly degradable or bioaccumulates | Concentration of component in mixture (%) | Multiplying factor M (from table 19.4.) | Adjusted concentration of component in mixture (M x %) |
|-----------|-------------------------------|--|--|---|---|--|
| B | 5 | 9.1B | No | 5 | 1 | 5 |
| P | 0.002 | 9.1A | Yes | 0.05 | 100 | 5 |
| Q | 0.9 | 9.1A | No | 1 | 1 | 1 |
| T | 50 | 9.1C | No | 40 | 1 | 40 |
| U | Not classified | Not classified | No | 53.95 | 1 | 53.95 |

Note: L(E)C₅₀ = median lethal concentration or median effect concentration.

The steps to follow in applying the summation approach to aquatic hazard classification for mixtures with highly toxic components are set out below, using the information in Table 19.5.

- Step 1

Component P is highly ecotoxic and attracts a multiplier of 100, resulting in a weighted concentration of that component of 5%.

Component Q, although classified as 9.1A, is not given addition weighting, that is:

$$\Rightarrow (100 \times P) + Q$$

$$\Rightarrow (100 \times 0.05\%) + 1\% = 6\%$$

which is < 25% therefore mixture Z is not classified as 9.1A.

- Step 2a: Consider components classified as 9.1A and 9.1B

$$\Rightarrow 10((100 \times P) + Q) + B$$

$$\Rightarrow 10((100 \times 0.05\%) + 1\%) + 5\% = 60\% + 5\% = 65\%$$

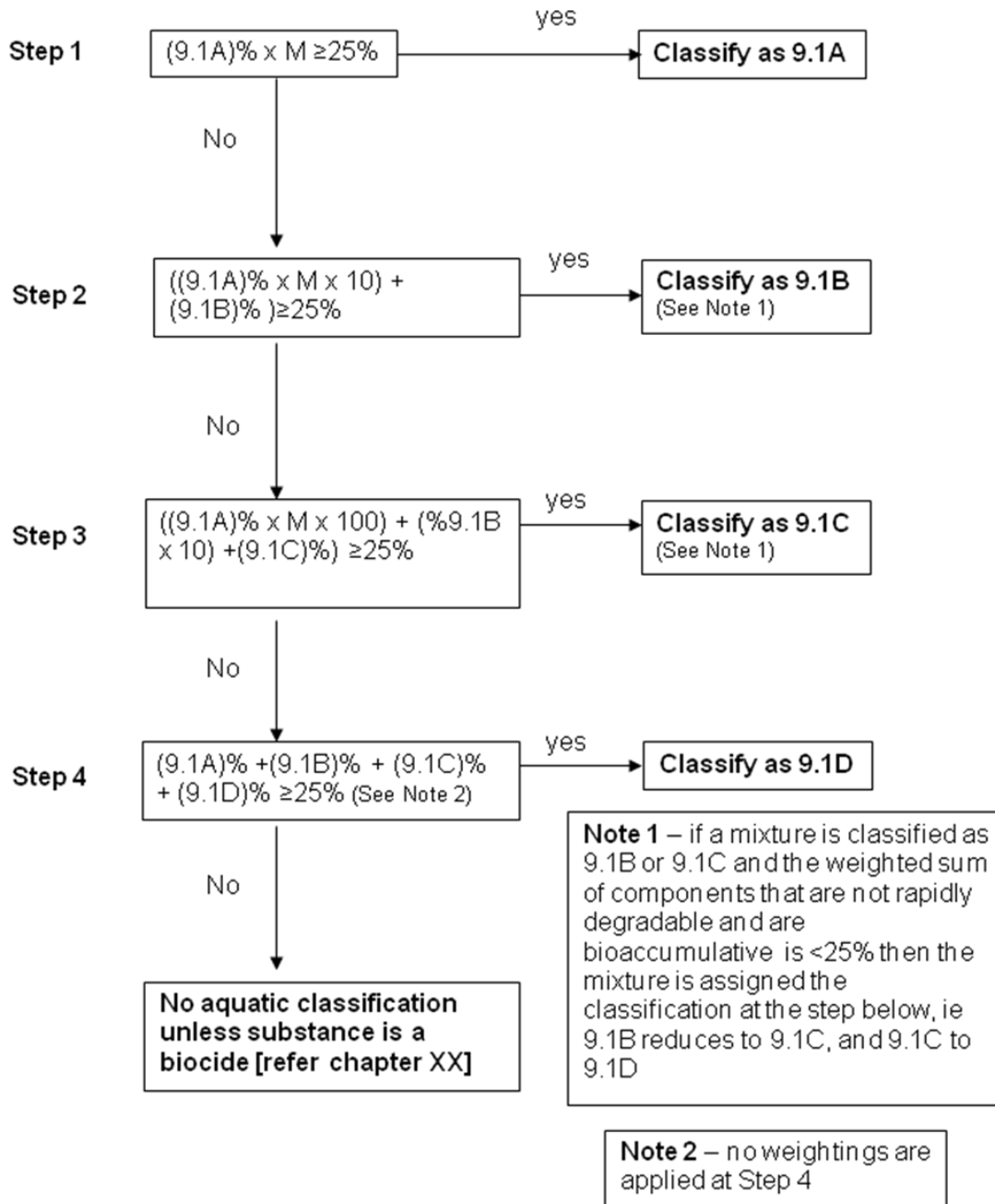
which is \geq 25% therefore mixture Z is classified as 9.1B unless

Step 2b: Consider components that are not rapidly degradable or are bioaccumulative

Component P is not rapidly degradable in the aquatic environment and attracts a multiplier of 100 due to its high toxicity, resulting in an adjusted concentration for that component of 5%. As mixture Z is not classified as 9.1A, an additional weighting is given to component P at Step 2, i.e. $10((100 \times 0.05\%) = 50\%$.

Mixture Z retains the 9.1B classification based on the weighted presence of $\geq 25\%$ of components in the mixture that are not rapidly degradable.

Figure 19.3: Aquatic hazard classification of mixtures



Appendix 19A: Acceptable test methods for aquatic toxicity, biodegradation, and bioconcentration, and relevant physico-chemical tests

19A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The guidelines listed below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable.

The main references to international guidelines referred to in the tables in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1996. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- International Organization for Standardization (ISO) guidelines:
Guidelines are available from the national standardisation organisations or the ISO website (<http://www.iso.ch> Retrieved 14 August 2007).
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011.
- ASTM International (ASTM) guidelines are available from the ASTM homepage (<http://www.astm.org>, search on 'standards').

19A.2 Aquatic toxicity test guidelines

The guidelines in Table 19A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides, which include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in the aquatic environment.

For tests specific to the testing of microbial biopesticides, see:

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011..

See also Table 19A.2.

Table 19A.1: Aquatic toxicity test guidelines for chemicals, including mixtures

| Species | Test guideline number | | |
|----------------------------|--|---|---|
| | OECD | EC | USEPA OPPTS |
| Algae/aquatic plant | | | |
| | OECD 201 (2006) Alga, Growth Inhibition Test | EC C.3: Algal Inhibition Test (1992) | 850.5400 Algal toxicity, Tiers I and II |
| | OECD 221 (2006) Lemna sp. Growth inhibition test | | 850.4400 Aquatic plant toxicity test using Lemna spp. Tiers I and II 850.4450 Aquatic plants field study, Tier III |
| Crustacea | | | |
| Acute | OECD 202 (2004) Daphnia sp. Acute Immobilisation Test | EC C.2: Acute Toxicity for Daphnia (1992) | 850.1010 Aquatic invertebrate acute toxicity, test, freshwater daphnids |
| | | | 850.1020 Gammarid acute toxicity test 850.1035 Mysid acute toxicity test 850.1045 Penaeid acute toxicity test |
| Chronic | OECD 211 (1998) Daphnia magna Reproduction Test | EC C.20: Daphnia magna Reproduction Test (2001) | 850.1300 Daphnid chronic toxicity test |
| | | | 850.1350 Mysid chronic toxicity test |
| Fish | | | |
| Acute | OECD 203 (1992) Fish, Acute Toxicity Test | EC C.1: Acute Toxicity for Fish (1992) | 850.1075 Fish acute toxicity test, freshwater and marine |
| Chronic | OECD 204 (1984) Fish, Prolonged Toxicity Test: 14-Day Study | | |
| | OECD 210 (1992) Fish, Early-Life Stage Toxicity Test | | 850.1400 Fish early-life stage toxicity test |
| | OECD Test Guideline 212 (1998) Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages | EC C.15: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages (2001) | |
| | OECD Test Guideline 215 (2000) Fish, Juvenile Growth Test | EC C.14: Fish Juvenile Growth Test (2001) | |
| | | | 850.1500 Fish life cycle toxicity |

Table 19A.2: Aquatic toxicity test guidelines for microbial biopesticides

| USEPA OPPTS guidelines |
|--|
| 885.4000 Background for non-target organism testing of microbial pest control agents |
| 885.4200 Freshwater fish testing, Tier I |
| 885.4240 Freshwater aquatic invertebrate testing, Tier I |
| 885.4280 Estuarine and marine animal testing, Tier I |
| 885.4650 Aquatic invertebrate range testing, Tier III |
| 885.4700 Fish life cycle studies, Tier III |
| 885.4750 Aquatic ecosystem test |
| 885.5000 Background for microbial pesticides testing |
| 885.5300 Expression in a freshwater environment |
| 885.5400 Expression in a marine or estuarine environment |

19A.3 Abiotic and biotic degradation

A large number of test methods are available for evaluating the degradability of a substance in the aquatic environment (see Table 19A.3).

A pass in one of the ready biodegradability tests will meet the HSNO Act criteria for a substance to be considered rapidly degradable. Results from other test methods will usually require further interpretation to assess whether the HSNO Act criteria are met or not.

See Appendix 19E for detailed guidance on the interpretation of test results and further information on degradation testing.

Table 19A.3: Test methods for degradability of organic substances in aquatic systems

| Test type | Test guidelines | | | |
|-------------------------------------|-------------------------------------|---|---------------------------------|----------------------------------|
| | OECD | EC | USEPA OPPTS | Other |
| Ready biodegradability tests | | | | |
| | 301A DOC die-away test | EC C.4. A to F: Determination of ready biodegradability. Directive 67/548/EEC, Annex V. (1992) (are the same as the OECD 301 tests) | 835.3110 Ready biodegradability | |
| | 301B CO ₂ evolution test | | | ISO 9439 (1990). Water quality – |

| Test type | Test guidelines | | | |
|---------------------------------|--|----|------------------------------------|--|
| | OECD | EC | USEPA OPPTS | Other |
| | | | | Evaluation in an aqueous medium of the 'ultimate' biodegradability of organic compounds – Method by analysis of released CO ₂ |
| | 301C Modified MITI (I) | | | |
| | 301D Closed bottle test | | | ISO 10707 (1994). Water quality – Evaluation in an aqueous medium of the 'ultimate' biodegradability of organic compounds – Method by analysis of biochemical oxygen demand (closed bottle test) |
| | 301E Modified OECD screening test | | | |
| | 301F Manometric respirometry test | | | ISO 9408 (1991). Water quality – Evaluation in an aqueous medium of the 'ultimate' biodegradability of organic compounds – Method by determining the oxygen demand in a closed respirometer |
| | OECD 306 (1992). Biodegradability in seawater. | | | |
| | OECD. 310 (2006) Ready Biodegradability – CO ₂ in sealed vessels (Headspace Test) | | | |
| Aquatic simulation tests | | | | |
| | | | 835.3170 Shake flask die-away test | ASTM E 1279-89(95) Standard test method |

| Test type | Test guidelines | | | |
|----------------------------------|--|--|---|---|
| | OECD | EC | USEPA OPPTS | Other |
| | | | | for biodegradation by a shake-flask die-away method |
| | | | | ISO/DIS 14592 (1999) Water quality – Evaluation of the aerobic biodegradability of organic compounds at low concentrations in water. Part 1: Shake flask batch test with surface water or surface water/sediment suspensions (22.11.1999) |
| | OECD 308 (2002). Aerobic and anaerobic transformation in aquatic sediment systems | EC C24 Aerobic and anaerobic transformation in aquatic sediment systems | 835.3180 Sediment/water microcosm biodegradability test | |
| | OECD 309 (2004) Aerobic mineralisation in surface water – simulation biodegradation test | | | |
| Inherent biodegradability | | | | |
| | OECD 302A (1981). Inherent biodegradability: Modified SCAS test. | EC C.12. Biodegradation: Modified SCAS test. Directive 67/548/EEC, Annex V. (1998) | OPPTS 835.3210 Modified SCAS test | ASTM E 1625-94 Standard test method for determining biodegradability of organic chemicals in semicontinuous activated sludge (SCAS) |
| | OECD 302B (1992). Zahn-Wellens/EMPA test | EC C.9. Biodegradation: Zahn-Wellens test. Directive 67/548/EEC, Annex V. (1988) | 835.3200 Zahn-Wellens/EMPA test | ISO 9888 (1991). Water quality – Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium – Static test (Zahn- |

| Test type | Test guidelines | | | |
|---------------------------------|--|---|---|-----------------|
| | OECD | EC | USEPA OPPTS | Other |
| | | | | Wellens method) |
| | OECD 302C (1981). Inherent biodegradability: Modified MITI test (II) | | | |
| Abiotic degradation | | | | |
| Hydrolysis | OECD Test Guideline 111 (2004). Hydrolysis as a function of pH | EC C.7. Degradation: abiotic degradation: hydrolysis as a function of pH. Directive 67/548/EEC, Annex V. (1992) | OPPTS 835.2110 Hydrolysis as a function of pH | |
| | | | OPPTS 835.2130 Hydrolysis as a function of pH and temperature | |
| Photolysis in water | | | OPPTS 835.2210 Direct photolysis rate in water by sunlight | |
| | | | OPPTS 835.5270 Indirect photolysis screening test: Sunlight photolysis in waters containing dissolved humic substances | |
| Other methods | | | | |
| Soil degradation | OECD 307 (2002). Aerobic and anaerobic transformation in soil | | 835.3300 Soil biodegradation | |
| | OECD Test Guideline 304A (1981). Inherent biodegradability in soil. OECD guidelines for testing of chemicals | | | |
| BOD (biochemical oxygen demand) | | EC C.5. Degradation: biochemical oxygen demand. Directive 67/548/EEC, Annex V. (1992) | | |

| Test type | Test guidelines | | | |
|---------------------------------|-----------------|---|-------------|-------|
| | OECD | EC | USEPA OPPTS | Other |
| COD (chemical oxygen demand) | | EC C6 Degradation: chemical oxygen demand | | |

19A.4 Bioconcentration test guidelines

Table 19A.4: Bioconcentration test guidelines

| OECD | European Commission | USEPA | Other |
|--|---|--|--|
| OECD 305, 1996. Bioconcentration: Flow-through Fish Test | EC.C.13 Bioconcentration: Flow-through Fish Test | USEPA OPPTS 850.1730 Fish BCF | ASTM E 1022-94. 1997. Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs |
| OECD 107 (1995) Partition Coefficient (n-octanol/ water): Shake Flask Method | EC A.8 Partition coefficient | 830.7550 | |
| OECD 117, (1989). OECD Guideline for testing of chemicals. Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method | | 830.7570 | |
| OECD 123 (2006). Partition Coefficient n-Octanol/Water Pow. Slow-stirring method for highly hydrophobic chemicals | | | |
| | | 830.7560 Estimation by generator column method | |

19A.5 Test guidelines for relevant physico-chemical properties for interpretation of toxicity and degradation data

Table 19A.5: Test guidelines for relevant physico-chemical properties for interpretation of toxicity and degradation data

| Test type | OECD | EC | USEPA OPPTS |
|---------------------------|--|-------|----------------------|
| Vapour pressure | OECD 104 (2006) Vapour pressure | EC AA | 830.7950 |
| Water solubility | OECD 105 (1995) Water solubility | EC A6 | 830.7840 830.7869 |
| pH | – | – | 830.7000 |
| Dissociation constant pKa | OECD 112 (1981) Dissociation constants in water | | 830.7370 |

Appendix 19B: Comparison of Hazardous Substances and New Organisms Act 1996 and Globally Harmonized System of Classification and Labelling of Chemicals aquatic hazard classifications

The GHS system of aquatic hazard classifications comprises three acute classes and four chronic classes. The Hazardous Substances and New Organisms Act 1996 (HSNO Act) combines these seven classes into four categories in recognition of the overlap between the GHS classes. The GHS classes and its equivalent HSNO Act category are in Table 19B.1.

Table 19B.1: Comparison of HSNO Act and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) aquatic hazard classifications

| GHS aquatic classification | HSNO Act equivalent |
|--|----------------------------|
| Acute I | 9.1A |
| Acute toxicity – all values ≤ 1 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour E _r C ₅₀ (for algae or other aquatic plants) This class may be subdivided for some regulatory systems to include a lower band at LEC ₅₀ ≤ 0.1 mg/L | |
| Acute II | 9.1D clause (a) |
| Acute toxicity – all values > 1 to ≤ 10 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour E _r C ₅₀ (for algae or other aquatic plants) | |
| Acute III | 9.1D clause (a) |
| Acute toxicity all values > 10 to ≤ 100 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour E _r C ₅₀ (for algae or other aquatic plants) This class may be extended beyond an LEC ₅₀ of 100 mg/L through the introduction of another class. | |
| Chronic I | 9.1A |
| Acute toxicity all values ≤ 1 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour E _r C ₅₀ (for algae or other aquatic plants) | |

| | |
|--|------------------------|
| and the substance is not rapidly degradable and/or the $\log K_{OW} \geq 4$ (unless the experimentally determined BCF < 500) | |
| Chronic II | 9.1B |
| Acute toxicity – all values > 1 to ≤ 10 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour ErC ₅₀ (for algae or other aquatic plants) and the substance is not rapidly degradable and/or the $\log K_{OW} \geq 4$ (unless the experimentally determined BCF < 500), unless the chronic toxicity NOECs are > 1 mg/L | |
| Chronic III | 9.1C |
| Acute toxicity > 10 to ≤ 100 mg/L 96-hour LC ₅₀ (for fish) 48-hour EC ₅₀ (for crustacea) 72- or 96-hour ErC ₅₀ (for algae or other aquatic plants) and the substance is not rapidly degradable and/or the $\log K_{OW} \geq 4$ (unless the experimentally determined BCF < 500), unless the chronic toxicity NOECs are > 1 mg/L | |
| Chronic IV | 9.1D clause (c) |
| Poorly soluble substance for which no acute toxicity is recorded at levels up to the water solubility, and which are not rapidly degradable and have a $\log K_{OW} > 4$, indicating a potential to bioaccumulate, will be classified in this class unless other scientific evidence exists showing classification to be unnecessary. Such evidence would include an experimentally determined BCF < 500, or a chronic toxicity NOEC > 1 mg/L, or evidence of rapid degradation in the environment. | |

Notes: BCF = bioconcentration factor; EC₅₀ = median effect concentration; ErC₅₀ = median effect concentration based on growth rate; K_{OW} = steady state ratio of the solubility of a substance in n-octanol to the solubility of that substance in water; LC₅₀ = median lethal concentration; L(E)C₅₀ = median lethal concentration or median effective concentration; NOEC = no observable effect concentration.

- * OECD Acute II and Acute III are included under HSNO Act equivalent 9.1D. Substances assigned to 9.1D based on acute toxicity alone may require different controls than those substances classified due to chronic effects (Chronic IV substances). These acutely toxic substances may also have degradation and bioaccumulation properties that would classify the substance as OECD Chronic II or OECD Chronic III. In this instance, the overall classification would be to the higher category, that is, 9.1B or 9.1C respectively.

Appendix 19C: Comparison of European Union aquatic risk phrases with Hazardous Substances and New Organisms Act 1996 aquatic classifications

The European Union (EC, 1967) risk phrases are converted into the equivalent Hazardous Substances and New Organisms Act 1996 (HSNO Act) classification in Table 19C.1.

Table 19C.1: Comparison of European Union (EU) aquatic risk phrases with HSNO Act aquatic classifications

| EU risk phrases | HSNO Act equivalent |
|---|---|
| R50 Very toxic to aquatic organisms For substances with acute toxicity ≤ 1 mg/L | 9.1A |
| R50/53 | |
| R51 Toxic to aquatic organisms For substances with acute toxicity $1 \text{ mg/L} < \text{LC}_{50} \leq 10 \text{ mg/L}$ | R51 alone: 9.1D unless there is no data to indicate the substance is rapidly degradable or not bioaccumulative in which case 9.1B applies |
| R51/R53 | 9.1B |
| R52 Harmful to aquatic organisms For substances with acute toxicity $10 \text{ mg/L} < \text{LC}_{50} \leq 100 \text{ mg/L}$ | 9.1D unless there is no data to indicate the substance is rapidly degradable or not bioaccumulative in which case 9.1C applies |
| R52/53 | 9.1C |
| R53 May cause long-term adverse effects in aquatic environment Substances not falling under the criteria listed above, but which, on the basis of the available evidence concerning their persistence, potential to accumulate, and predicted or observed environmental fate and behaviour may nevertheless present a long-term and/or delayed danger to the structure and/or functioning of aquatic ecosystems. For example, poor water-soluble substances (solubility of less than 1 mg/L) if: they are not readily degradable; and the $\log P_{\text{OW}} \geq 3.0$ (unless the experimentally determined BCF is ≤ 100). | 9.1D clause c |

Notes

- BCF = bioconcentration factor; LC_{50} = median lethal dose; P_{OW} = Kow the octanol/water partition co-efficient.
- The EU classification criteria for bioconcentration potential and biodegradation are more conservative than the HSNO Act criteria for these properties, so may result in a more precautionary HSNO Act aquatic hazard classification than might otherwise be the case under the EU system.

Source: EC (1967).

References

EC 1967. General classification and labelling requirements for dangerous substances and preparations. Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances. European Commission, Annex VI. http://ec.europa.eu/environment/dansub/consolidated_en.htm.

Appendix 19D: Globally Harmonized System of Classification and Labelling – additional guidance on aquatic hazard data interpretation

19D.1 Introduction

This appendix is largely the same as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Annex 9 guidance on aquatic hazard classification (United Nations, 2007). Changes have been made where necessary to refer to the Hazardous Substances and New Organisms Act 1996 (HSNO Act) aquatic classification criteria as these sometimes differ from the GHS.

19D.2 Aquatic toxicity

Introduction

The basis for the identification of hazard to the aquatic environment for a substance is the aquatic toxicity of that substance. Classification is predicated on having toxicity data for fish, crustacea, and algae/aquatic plant available. These taxa are generally accepted as representative of aquatic fauna and flora for hazard identification. Data on these particular taxa are more likely to be found because of this general acceptance by regulatory authorities and the chemical industry. Other information on the degradation and bioaccumulation behaviour is used to better delineate the aquatic hazard. This section describes the appropriate tests for ecotoxicity, provides some basic concepts in evaluating the data and using combinations of testing results for classification, summarises approaches for dealing with difficult substances, and includes a brief discussion on interpretation of data quality.

Description of tests

For classifying substances in the harmonised system, freshwater and marine species toxicity data can be considered as equivalent data. It should be noted that some types of substances, for example, ionisable organic chemicals or organometallic substances may express different toxicities in freshwater and marine environments. Since the purpose of classification is to characterise hazard in the aquatic environment, the result showing the highest toxicity should be chosen.

The GHS criteria for determining health and environmental hazards should be test method neutral, allowing different approaches as long as they are scientifically sound and validated according to international procedures and criteria already referred to in existing systems for the endpoints of concern and produce mutually acceptable data. According to the GHS system:

*Acute toxicity would normally be determined using a fish 96-hour LC50 (OECD Test Guideline 203 or equivalent), a crustacea species 48-hour EC50 (OECD Test Guideline 202 or equivalent) and/or an algal species 72- or 96-hour EC50 (OECD Test Guideline 201 or equivalent). These species are considered as surrogate for all aquatic organisms and data on other species such as the duckweed *Lemna* may also be considered if the test methodology is suitable.*

Chronic testing involves an exposure that is lingering or continues for a longer time; the term can signify periods from days to a year, or more depending on the reproductive cycle of the aquatic organism. Chronic tests can be done to assess certain endpoints relating to growth, survival, reproduction, and development.

Chronic toxicity data are less available than acute data and the range of testing procedures less standardised. Data generated according to the OECD Test Guidelines 210 (Fish Early Life Stage), 202 or 211 (Daphnia Reproduction) and 201 (Algal Growth Inhibition) can be accepted. Other validated and internationally accepted tests could also be used. The NOECs or other equivalent L(E)Cx should be used.

It should be noted that several of the OECD guidelines cited as examples for classification are being revised or are being planned for updating. Such revisions may lead to minor modifications of test conditions. Therefore, the expert group that developed the harmonised criteria for classification intended some flexibility in test duration or even species used.

Guidelines for conducting acceptable tests with fish, crustacea, and algae can be found in Appendix 19A. The OECD's (1998) Detailed Review Paper on Aquatic Toxicity Testing for Industrial Chemicals and Pesticides is a good compilation of pelagic test methods and sources of testing guidance. This document is also a source of appropriate test methodologies.

Fish tests

Acute testing

Acute tests are generally performed with young juveniles 0.1–5 g in size for a period of 96 hours. The observational endpoint in these tests is mortality. Fish larger than this range and/or durations shorter than 96 hours are generally less sensitive. However, for classification, they could be used if no acceptable data with the smaller fish for 96 hours are available or the results of these tests with different size fish or test durations would influence classification in a more hazardous category. Tests consistent with OECD Test Guideline 203 (Fish 96-hour LC50) or equivalent should be used for classification.

Chronic testing

Chronic or long-term tests with fish can be initiated with fertilised eggs, embryos, juveniles, or reproductively active adults. Tests consistent with OECD Test Guideline 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used in the classification scheme. Durations can vary widely depending on the test purpose (anywhere from 7 days to over 200 days). Observational endpoints can include hatching success, growth (length and weight changes), spawning success, and survival. Technically, the OECD 210 Guideline (Fish Early Life Stage) is not a 'chronic' test, but a sub-chronic test on sensitive life stages. It is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system. Fish early life stage toxicity data are much more available than fish life cycle or reproduction studies.

Crustacea tests

Acute testing

Acute tests with crustacea generally begin with first instar juveniles. For daphnids, a test duration of 48 hours is used. For other crustacea, such as mysids or others, a duration of 96 hours is typical. The observational endpoint is mortality or immobilisation as a surrogate to mortality. Immobilisation is defined as unresponsive to gentle prodding. Tests consistent with OECD Test Guideline 202 Part 1 (Daphnia acute) or US-EPA OPPTS 850.1035 (Mysid acute toxicity) or their equivalents should be used for classification.

Chronic testing

Chronic tests with crustacea also generally begin with first instar juveniles and continue through maturation and reproduction. For daphnids, 21 days is sufficient for maturation and the production of 3 broods. For mysids, 28 days is necessary. Observational endpoints include time to first brood, number of offspring produced per female, growth, and survival. It is recommended that tests consistent with OECD Test Guideline 202 Part 2 (Daphnia reproduction) or US-EPA 850.1350 (Mysid chronic) or their equivalents be used in the classification scheme.

Algae/plant tests

Tests in algae

Algae are cultured and exposed to the test substance in a nutrient-enriched medium. Tests consistent with OECD Test Guideline 201 (Algal growth inhibition) should be used. Standard test methods employ a cell density in the inoculum in order to ensure exponential growth through the test, usually 3 to 4 days' duration. The algal test is a short-term test and, although it provides both acute and chronic endpoints, only the acute EC_{50} is used for classification in the harmonised system. The preferred observational endpoint in this study is algal growth rate inhibition because it is not dependent on the test design, whereas biomass depends both on growth rate of the test species as well as test duration and other elements of test design. If the endpoint is reported only as reduction in biomass or is not specified, then this value may be interpreted as an equivalent endpoint.

Tests in aquatic macrophytes

The most commonly used vascular plants for aquatic toxicity tests are duckweeds (*Lemna gibba* and *Lemna minor*). The *Lemna* test is a short-term test and, although it provides both acute and sub-chronic endpoints, only the acute EC_{50} is used for classification in the harmonised system. The tests last for up to 14 days and are performed in nutrient enriched media similar to that used for algae, but may be increased in strength. The observational endpoint is based on change in the number of fronds produced. Tests consistent with OECD Test Guideline 221 on *Lemna* and US-EPA 850.4400 (aquatic plant toxicity, *Lemna*) should be used.

Aquatic toxicity concepts

This section addresses the use of acute and chronic toxicity data in classification, and special considerations for exposure regimes, algal toxicity testing, and use of Quantitative Structure Activity Relationships (QSARs). For a more detailed discussion of aquatic toxicity concepts, see Rand (1995).

Acute toxicity

Acute toxicity for purposes of classification refers to the intrinsic property of a substance to be injurious to an organism in a short-term exposure to that substance. Acute toxicity is generally expressed in terms of a concentration which is lethal to 50% of the test organisms (LC₅₀), causes a measurable adverse effect to 50% of the test organisms (EC₅₀, for example, immobilisation of daphnids), or leads to a 50% reduction in test (treated) organism responses from control (untreated) organism responses (EC₅₀, for example, growth rate in algae).

Chronic toxicity

Chronic toxicity, for purposes of classification, refers to the potential or actual properties of a substance to cause adverse effects to aquatic organisms during exposures that are determined in relation to the life-cycle of the organism. Such chronic effects usually include a range of sublethal endpoints and are generally expressed in terms of a no observable effect concentration (NOEC), or an equivalent EC_x. Observable endpoints typically include survival, growth and/or reproduction. Chronic toxicity exposure durations can vary widely depending on test endpoint measured and test species used.

Since chronic toxicity data are less common in certain sectors than acute data, for classification schemes, the potential for chronic toxicity is identified by appropriate combinations of acute toxicity, lack of degradability, and/or the potential or actual bioaccumulation. Where such data exist and show long-term NOEC > 1 mg/L, this can be taken into account when deciding whether the classification based on the acute data should be applied. In this context, the following general approach should be used. In order to remove a 9.1B or 9.1C classification, it must be demonstrated that the NOEC used would be suitable in removing the concern for all taxa that resulted in classification. This can often be achieved by showing a long-term NOEC > 1 mg/L for the most sensitive species identified by the acute toxicity. Thus, if a classification has been applied based on a fish acute LC₅₀, it would generally not be possible to remove this classification using a long-term NOEC from an invertebrate toxicity test. In this case, the NOEC would normally need to be derived from a long-term fish test of the same species or one of equivalent or greater sensitivity. Equally, if classification has resulted from the acute toxicity to more than one taxa, it is likely that NOECs > 1 mg/L from each taxa will need to be demonstrated. In case of classification of a poorly soluble substance as 9.1D, it is sufficient to demonstrate that NOECs are greater than the water solubility of the substances under consideration.

Testing with algae/Lemna cannot be used for de-classifying chemicals because (1) the algae and Lemna tests are not long-term studies, (2) the acute to chronic ratio is generally narrow and (3) the endpoints are more consistent with the endpoints for other organisms. However, where classification is applied solely due to the acute toxicity (L(E)C₅₀) observed in single algae/aquatic plant tests, but there is evidence from a range

of other algae tests that the chronic toxicity (NOECs) for this taxonomic group is above 1 mg/L, this evidence could be used to consider declassification. At present, this approach cannot be applied to aquatic plants since no standardised chronic toxicity tests have been developed.

The GHS is intended to contain a specific value of chronic toxicity below which substances would be classified as chronically toxic, but the criteria are not yet set.

Exposure regimes

Four types of exposure conditions are employed in both acute and chronic tests and in both freshwater and saltwater media: static, static-renewal (semi-static), recirculation, and flow-through. The choice for which test type to use usually depends on test substance characteristics, test duration, test species, and regulatory requirements.

Test media for algae

Algal tests are performed in nutrient-enriched media and use of one common constituent, EDTA, or other chelators, should be considered carefully. When testing the toxicity of organic chemicals, trace amounts of a chelator like EDTA are needed to complex micronutrients in the culture medium; if omitted, algal growth can be significantly reduced and compromise test utility. However, chelators can reduce the observed toxicity of metal test substances. Therefore, for metal compounds, it is desirable that data from tests with high concentration of chelators and/or tests with stoichiometrical excess of chelator relative to iron be critically evaluated. Free chelator may mask heavy metal toxicity considerably, in particular with strong chelators like EDTA. However, in the absence of available iron in the medium, the growth of algae can become iron limited, and consequently data from tests with no or with reduced iron and EDTA should be treated with caution.

Use of Quantitative Structure Activity Relationships

For purpose of classification, and in the absence of experimental data, QSARs can be relied upon to provide predictions of acute toxicity for fish, Daphnia, and algae for non-electrolyte, non-electrophilic, and otherwise non-reactive substances (See section 19D.5 on the use of QSARs.) Problems remain for substances such as organophosphates that operate by means of special mechanisms such as functional groups which interact with biological receptors, or which can form sulfhydryl bonds with cellular proteins. Reliable QSARs have been derived for chemicals acting by a basic narcosis mechanism. These chemicals are nonelectrolytes of low reactivity such as hydrocarbons, alcohols, ketones and certain aliphatic chlorinated hydrocarbons that produce their biological effects as a function of their partition coefficients. Every organic chemical can produce narcosis. However, if the chemical is an electrolyte or contains specific functional groups leading to non-narcotic mechanisms as well, any calculations of toxicity based on partition coefficient alone would severely underestimate the toxicity. QSARs for acute aquatic toxicity of parent compounds cannot be used to predict the effects of toxic metabolites or degradates, when these arise after a longer period than the duration of acute tests.

Weight of evidence

The best quality data should be used as the fundamental basis for classification. Classification should preferably be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Where multiple studies for a taxonomic group are available, a decision on what is the most sensitive and highest quality must be made. A judgement has to be made on a case-by-case basis whether a non-Good Laboratory Practice (GLP) study with a more sensitive observation is used in lieu of a GLP study. It would appear that results that indicate high toxicity from tests performed according to non-standard or non-GLP guidelines should be able to be used for classification, whereas studies, which demonstrate negligible toxicity, would require more careful consideration. Substances, which are difficult to test, may yield apparent results that are more or less severe than the true toxicity. Expert judgement would also be needed for classification in these cases.

Where more than one acceptable test is available for the same taxonomic group, the most sensitive (the one with the lowest L(E)C₅₀ or NOEC) is generally used for classification. However, this must be dealt with on a case-by-case basis. When larger data sets (four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. In estimating a mean value, it is not advisable to combine tests of different species within a taxa group or in different life stages or tested under different conditions or duration.

Difficult to test substances

Introduction

Valid aquatic toxicity tests require the dissolution of the test substance in the water media under the test conditions recommended by the guideline. In addition, a bioavailable exposure concentration should be maintained for the duration of the test. Some chemical substances are difficult to test in aquatic systems and guidance has been developed to assist in testing these materials. The OECD (2000) guidance document Aquatic Toxicity Testing of Difficult Substances and Mixtures is a good source of information on the types of substances that are difficult to test and the steps needed to ensure valid conclusions from tests with these materials.

Nevertheless, much test data exist that may have used testing methodologies which, while not in conformity with what might be considered best practice today, can still yield information suitable for application of the classification criteria. Such data require special guidance on interpretation, although ultimately, expert judgement must be used in determining data validity. Such difficult to test substances may be poorly soluble, volatile, or subject to rapid degradation due to such processes as phototransformation, hydrolysis, oxidation, or biotic degradation. When testing algae, coloured materials may interfere with the test endpoint by attenuating the light needed for cell growth. In a similar manner, substances tested as cloudy dispersions above solubility may give rise to false toxicity measurements. Loading of the water column with test material can be an issue for particulates or solids such as metals. Petroleum distillate fractions can also pose loading

problems, as well as difficult interpretational problems when deciding on the appropriate concentrations for determining L(E)C₅₀ values. *Aquatic Toxicity Testing of Difficult Substances and Mixtures* (OECD, 2000) describes the more common properties of many types of substances that are likely to pose testing difficulties.

- **Stability**

If test chemical concentrations are expected to fall below 80% of nominal, testing, in order to be valid, may require exposure regimes that provide for renewal of the test material. Semi-static or flow-through conditions are preferred. Special problems arise, therefore, with respect to testing on algae, where the standard guidelines generally include static tests to be conducted. While alternative exposure regimes are possible for crustacea and fish, these tests are frequently conducted on static conditions as included in the internationally agreed guidelines. In these tests, a certain level of degradation as well as other relevant factors has to be tolerated and appropriate account must be taken in calculations of toxic concentrations. Some approaches on how this can be dealt with are covered in 'Unstable substances' under 'Difficult to test substances' later in this section.. Where degradation occurs, it is also important to consider the influence of the toxicity of the degradation products on the recorded toxicity in the test. Expert judgement will need to be exercised when deciding if the data can be used for classification.

- **Degradation**

When a compound breaks down or degrades under test condition, expert judgement should be used in calculating toxicity for classification, including consideration of known or likely breakdown products. Concentrations of the parent material and all significant toxic degradates are desirable. If degradates are expected to be relatively non-toxic, renewable exposure regimes are desirable in order to ensure that levels of the parent compounds are maintained.

- **Saturation**

For single component substances, classification should be based only on toxic responses observed in the soluble range, and not on total chemical loading above solubility. Frequently, data are available which indicate toxicity at levels in excess of water solubility and, while these data will often be regarded as not valid, some interpretation may be possible. These problems generally apply when testing poorly soluble substances, and guidance on how to interpret such data is included in 'Poorly soluble substances' under 'Difficult to test substances' later in this section (see also *Aquatic Toxicity Testing of Difficult Substances and Mixtures* (OECD, 2000)).

- **Perturbation of test media**

Special provisions may be needed to ensure dissolution of difficult to test substances. Such measures should not lead to significant changes in the test media when such changes are likely to lead to an increase or decrease in the apparent toxicity and hence the classification level of the test substance.

- **Complex substances**

Many substances covered by the classification scheme are in fact mixtures, for which measurement of exposure concentrations is difficult, and in some cases impossible. Substances such as petroleum distillate fractions, polymers, substances with significant levels of impurities, etc can pose special problems since the toxic concentration is difficult to define and impossible to verify. Typical testing procedures often rely on the formation of a Water Soluble Fraction (WSF) or Water Accommodated

Fraction (WAF) and data are reported in terms of loading rates. These data may be used in applying the classification criteria.

For classification of organic compounds, it is desirable to have stabilised and analytically measured test concentrations. Although measured concentrations are preferred, classification may be based on nominal concentration studies when these are the only valid data available under certain circumstances. If the material is likely to substantially degrade or otherwise be lost from the water column, care must be taken in data interpretation and classification should be done taking the loss of the toxicant during the test into account, if relevant and possible. Additionally, metals present their own set of difficulties and are discussed separately.

Table 19D.1 lists several properties of difficult to test substances and their relevance for classification.

In most difficult to test conditions, the actual test concentration is likely to be less than the nominal or expected test concentration. Where toxicities (L(E)C50s) are estimated to be less than 1 mg/L for a difficult to test substance, one can be fairly confident the classification in the 9.1A is warranted. However, if the estimated toxicity is greater than 1 mg/L, the estimated toxicity is likely to under-represent the toxicity. In these circumstances, expert judgement is needed to determine the acceptability of a test with a difficult to test substance for use in classification. Where the nature of the testing difficulty is believed to have a significant influence on the actual test concentration when toxicity is estimated to be greater than 1 mg/L and the test concentration is not measured, then the test should be used with due caution in classification.

The following paragraphs provide some detailed guidance on some of these interpretational problems. In doing so, it should be remembered that this is guidance and hard and fast rules cannot be applied. The nature of many of the difficulties mean that expert judgement must always be applied both in determining whether there is sufficient information in a test for a judgement to be made on its validity, and also whether a toxicity level can be determined suitable for use in applying the classification criteria.

Unstable substances

While testing procedures should ideally have been adopted which minimised the impacts of instability in the test media, in practice, in certain tests, it can be almost impossible to maintain a concentration throughout the test. Common causes of such instability are oxidation, hydrolysis, photodegradation, and biodegradation. While the latter forms of degradation can more readily be controlled, such controls are frequently absent in much existing testing. Nevertheless, for some testing, particularly acute and chronic fish toxicity testing, a choice of exposure regimes is available to help minimise losses due to instability, and this should be taken into account in deciding on the test data validity.

Where instability is a factor in determining the level of exposure during the test, an essential prerequisite for data interpretation is the existence of measured exposure concentrations at suitable time points throughout the test. In the absence of analytically measured concentrations at least at the start and end of test, no valid interpretation can be made and the test should be considered as invalid for classification purposes. Where measured data are available, a number of practical rules can be considered by way of guidance in interpretation.

- Where measured data are available for the start and end of test (as is normal for the acute Daphnia and algal tests), the L(E)C₅₀, for classification purposes, may be calculated based on the geometric mean of the start and end of test concentrations. Where the end of test concentrations are below the analytical detection limit, such concentrations shall be considered to be half that detection limit.
- where measured data are available at the start and end of media renewal periods (as may be available for the semi-static tests), the geometric mean for each renewal period should be calculated, and the mean exposure over the whole exposure period calculated from these data.
- Where the toxicity can be attributed to a degradation breakdown product, and the concentrations of this are known, the L(E)C₅₀ for classification purposes, may be calculated based on the geometric mean of the degradation product concentration, back calculated to the parent substance.
- Similar principles may be applied to measured data in chronic toxicity testing.

Poorly soluble substances

These substances, usually taken to be those with a solubility in water of < 1 mg/L, are frequently difficult to dissolve in the test media, and the dissolved concentrations will often prove difficult to measure at the low concentrations anticipated. For many substances, the true solubility in the test media will be unknown, and will often be recorded as less than the detection limit in purified water. Nevertheless, such substances can show toxicity, and where no toxicity is found, judgement must be applied to whether the result can be considered valid for classification. Judgement should err on the side of caution and should not underestimate the hazard.

Ideally, tests using appropriate dissolution techniques and with accurately measured concentrations within the range of water solubility should be used. Where such test data are available, they should be used in preference to other data. It is normal, however, particularly when considering older data, to find such substances with toxicity levels recorded in excess of the water solubility, or where the dissolved levels are below the detection limit of the analytical method. Thus, in both circumstances, it is not possible to verify the actual exposure concentrations using measured data. Where these are the only data available on which to classify, some practical rules can be considered by way of general guidance.

- Where the acute toxicity is recorded at levels in excess of the water solubility, the L(E)C₅₀ for classification purposes, may be considered to be equal to or below the measured water solubility. In such circumstances it is likely that a 9.1A classification should be applied. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. Where this is considered the likely cause of the effects observed, the test should be considered as invalid for classification purposes.
- Where no acute toxicity is recorded at levels in excess of the water solubility, the L(E)C₅₀ for classification purposes may be considered to be greater than the measured water solubility. In such circumstances, consideration should be given to whether the 9.1D classification should apply. In making a decision that the substance shows no acute toxicity, due account should be taken of the techniques used to achieve the maximum dissolved concentrations. Where these are not considered as adequate, the test should be considered as invalid for classification purposes.

- Where the water solubility is below the detection limit of the analytical method for a substance, and acute toxicity is recorded, the $L(E)C_{50}$ for classification purposes, may be considered to be less than the analytical detection limit. Where no toxicity is observed, the $L(E)C_{50}$ for classification purposes, may be considered to be greater than the water solubility. Due consideration should also be given to the quality criteria mentioned above.
- Where chronic toxicity data are available, the same general rules should apply. In principle, only data showing no effects at the water solubility limit, or greater than 1 mg/L need be considered. Again, where these data cannot be validated by consideration of measured concentrations, the techniques used to achieve the maximum dissolved concentrations must be considered as appropriate.

Other factors contributing to concentration loss

A number of other factors can also contribute to losses of concentration and, while some can be avoided by correct study design, interpretation of data where these factors have contributed may, from time to time, be necessary.

- **Sedimentation**
This can occur during a test for a number of reasons. A common explanation is that the substance has not truly dissolved despite the apparent absence of particulates, and agglomeration occurs during the test leading to precipitation. In these circumstances, the $L(E)C_{50}$ for classification purposes, may be considered to be based on the end of test concentrations. Equally, precipitation can occur through reaction with the media. This is considered under instability above.
- **Adsorption**
This can occur for substances of high adsorption characteristics such as high $\log K_{OW}$ substances. Where this occurs, the loss of concentration is usually rapid and exposure may best be characterised by the end of test concentrations.
- **Bioaccumulation**
Losses may occur through the bioaccumulation of a substance into the test organisms. This may be particularly important where the water solubility is low and $\log K_{OW}$ correspondingly high. The $L(E)C_{50}$ for classification purposes, may be calculated based on the geometric mean of the start and end of test concentrations.

Perturbation of the test media

Strong acids and bases may appear toxic because they may alter pH. Generally however changes of the pH in aquatic systems are normally prevented by buffer systems in the test medium. If no data are available on a salt, the salt should generally be classified in the same way as the anion or cation, that is, as the ion that receives the most stringent classification. If the effect concentration is related to only one of the ions, the classification of the salt should take the molecular weight difference into consideration by correcting the effect concentration by multiplying with the ratio: MW_{salt}/MW_{ion} .

Polymers are typically not available in aquatic systems. Dispersible polymers and other high molecular mass materials can perturb the test system and interfere with uptake of oxygen, and give rise to mechanical or

secondary effects. These factors need to be taken into account when considering data from these substances. Many polymers behave like complex substances, however, having a significant low molecular mass fraction that can leach from the bulk polymer. This is considered further below.

Complex substances

Complex substances are characterised by a range of chemical structures, frequently in a homologous series, but covering a wide range of water solubilities and other physico-chemical characteristics. On addition to water, equilibrium will be reached between the dissolved and undissolved fractions that will be characteristic of the loading of the substance. For this reason, such complex substances are usually tested as a WSF or WAF, and the L(E)C₅₀ recorded based on the loading or nominal concentrations. Analytical support data are not normally available since the dissolved fraction will itself be a complex mixture of components. The toxicity parameter is sometimes referred to as LL₅₀, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria.

Polymers represent a special kind of complex substance, requiring consideration of the polymer type and their dissolution/dispersal behaviour. Polymers may dissolve as such without change, (true solubility related to particle size), be dispersible, or portions consisting of low molecular weight fractions may go into solution. In the latter case, in effect, the testing of a polymer is a test of the ability of low molecular mass material to leach from the bulk polymer, and whether this leachate is toxic. It can thus be considered in the same way as a complex mixture in that a loading of polymer can best characterise the resultant leachate, and hence the toxicity can be related to this loading.

Table 19D.1: Classification of difficult to test substances

| Property | Nature of difficulty | Relevance for classification |
|-----------------------------|--|---|
| Poorly water soluble | Achieving/maintaining required exposure concentration. Analysing exposure. | When toxic responses are observed above apparent solubility, expert judgement is required to confirm whether effects are due to chemical toxicity or a physical effect. If no effects are observed, it should be demonstrated that full, saturated dissolution has been achieved. |
| Toxic at low concentrations | Achieving/maintaining required exposure concentration. Analysing exposure. | Classified based on toxicity < 1 mg/L |
| Volatile | Maintaining and measuring exposure concentration. | Classification should be based on reliable measurement of concentrations. |
| Photo-degradable | Maintaining exposure concentrations. Toxicity of breakdown products. | Classification requires expert judgement and should be based on measured concentrations. Toxicity of significant breakdown products should be characterised. |
| Hydrolytically unstable | Maintaining exposure concentrations. Toxicity of | Classification requires expert judgement, should be based on measured concentrations, |

| Property | Nature of difficulty | Relevance for classification |
|--|--|--|
| | breakdown products. Comparison of degradation half-lives to the exposure regimen used in testing. | and needs to address the toxicity of significant breakdown products. |
| Oxidisable | Achieving, maintaining and measuring exposure concentration. Toxicity of modified chemical structures or breakdown products. Comparison of degradation half-lives to the exposure regimen used in testing. | Classification requires expert judgement, should be based on measured concentrations, and needs to address the toxicity of significant breakdown products. |
| Subject to corrosion or transformation (this refers to metals and metal compounds) | Achieving, maintaining and measuring exposure concentration. Comparison of partitioning from the water column half-lives to the exposure regimen used in testing. | Classification requires expert judgement, should be based on measured concentrations, and needs to address the toxicity of significant breakdown products. |
| Biodegradable | Maintaining exposure concentrations. Toxicity of breakdown products. Comparison of degradation half-lives to the exposure regimen used in testing. | Classification requires expert judgement, should be based on measured concentrations, and needs to address the toxicity of significant breakdown products. |
| Adsorbing | Maintaining exposure concentrations. Analysing exposure. Toxicity mitigation due to reduced availability of test substance. | Classification should use measured concentration of available material |
| Chelating | Distinguishing chelated and non-chelated fractions in media. | Classification should use measurement of concentration of bioavailable material. |
| Coloured. | Light attenuation (an algal problem). | Classification must distinguish toxic effects from reduced growth due to light attenuation |
| Hydrophobic | Maintaining constant exposure concentrations. | Classification should use measured concentration. |
| Ionised | Maintaining exposure concentrations. Toxicity of breakdown products. Comparison of degradation half-lives to the exposure regime used in testing. | Classification requires expert judgement, should be based on measured concentrations, and needs to address the toxicity of significant breakdown products. |
| Multi-component | Preparing representative test batches. | Considered same as complex mixture. |

Interpreting data quality

Standardisation

Many factors can influence the results of toxicity tests with aquatic organisms. These factors include characteristics of the test water, experimental design, chemical characteristics of the test material, and biological characteristics of the test organisms. Therefore, it is important in conducting aquatic toxicity tests to use standardised test procedures to reduce the influence of these sources of extraneous variability. The goal of test standardisation and international harmonisation of these standards is to reduce test variability and improve precision, reproducibility, and consistency of test results.

Data hierarchies

See section 1.3 in chapter 1 for information about assessing data quality.

19D.3 Degradation

Introduction

Degradability is one of the important intrinsic properties of chemical substances that determine their potential environmental hazard. Non-degradable substances will persist in the environment and may consequently have a potential for causing long-term adverse effects on biota. In contrast, degradable substances may be removed in the sewers, in sewage treatment plants or in the environment. Classification of chemical substances is primarily based on their intrinsic properties. However, the degree of degradation depends not only on the intrinsic recalcitrance of the molecule, but also on the actual conditions in the receiving environmental compartment, for example, redox potential, pH, presence of suitable micro-organisms, concentration of the substances and occurrence and concentration of other substrates. The interpretation of the degradation properties in an aquatic hazard classification context therefore requires detailed criteria that balance the intrinsic properties of the substance and the prevailing environmental conditions into a concluding statement on the potential for long-term adverse effects. The purpose of the present section is to present guidance for interpretation of data on degradability of organic substances. The guidance is based on an analysis of the above-mentioned aspects regarding degradation in the aquatic environment. Based on the guidance a detailed decision scheme for use of existing degradation data for classification purposes is proposed. The types of degradation data included in this guidance document are ready biodegradability data, simulation data for transformation in water, aquatic sediment and soil, BOD₅/COD-data and techniques for estimation of rapid degradability in the aquatic environment. Also considered are anaerobic degradability, inherent biodegradability, sewage treatment plant simulation test data, abiotic transformation data such as hydrolysis and photolysis, removal process such as volatilisation and finally, data obtained from field investigations and monitoring studies.

The term degradation is defined as the decomposition of organic molecules to smaller molecules and eventually to carbon dioxide, water, and salts. For inorganic compounds and metals, the concept of degradability as applied to organic compounds has limited or no meaning. Rather the substance may be

transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Therefore, the present section deals only with organic substances and organo-metals. Section 19D.6 provides detailed guidance on assessing the transformation of metals in the aquatic environment.

Data on degradation properties of a substance may be available from standardised tests or from other types of investigations, or they may be estimated from the structure of the molecules. The interpretation of such degradation data for classification purposes often requires detailed evaluation of the test data. Guidance is given in the present section and more details can be found in Appendix 19E.

Interpretation of degradability data

Rapid degradability

Aquatic hazard classification of chemical substances is normally based on existing data on their environmental properties. Only seldom will test data be produced with the main purpose of facilitating a classification. Often a diverse range of test data is available that does not necessarily fit directly with the classification criteria. Consequently, guidance is needed on interpretation of existing test data in the context of the aquatic hazard classification. Guidance for interpretation of degradation data is set out below for the three types of data indicated in the HSNO Act definition of 'rapid degradation' in the aquatic environment.

Ready biodegradability

Ready biodegradability is defined in the OECD Test Guideline 301). All organic substances that degrade to a level higher than the pass level in a standard OECD ready biodegradability test or in a similar test should be considered readily biodegradable and consequently also rapidly degradable. Many literature test data, however, do not specify all of the conditions that should be evaluated to demonstrate whether or not the test fulfils the requirements of a ready biodegradability test. Expert judgement is therefore needed as regards the validity of the data before use for classification purposes. Before concluding on the ready biodegradability of a test substance, however, at least the following parameters should be considered.

Concentration of test substance

Relatively high concentrations of test substance are used in the OECD ready biodegradability tests (2–100 mg/L). Many substances may, however, be toxic to the inocula at such high concentrations causing a low degradation in the tests although the substances might be rapidly degradable at lower non-toxic concentrations. A toxicity test with micro-organisms (as, for example, the OECD Test Guideline 209 'Activated Sludge, Respiration Inhibition Test', the International Organization for Standardization (ISO) 9509 nitrification inhibition test, or the ISO 11348 luminescent bacteria inhibition test) may demonstrate the toxicity of the test substance. When it is likely that inhibition is the reason for a substance being not readily degradable, results from a test employing lower non-toxic concentrations of the test substance should be used when available. Such test results could on a case by case basis be considered in relation to the classification criteria for rapid degradation, even though surface water degradation test data with environmentally realistic microbial biomass and non toxic realistic low concentration of the test substance in general are preferred, if available.

Time window

The harmonised criteria include a general requirement for all of the ready biodegradability tests on achievement of the pass level within 10 days. This is not in line with the OECD Test Guideline 301 in which the 10-days time window applies to the OECD ready biodegradability tests except to the MITI I test (OECD Test Guideline 301C). In the Closed Bottle test (OECD Test Guideline 301D), a 14-days window may be used instead when measurements have not been made after 10 days. Moreover, often only limited information is available in references of biodegradation tests. Thus, as a pragmatic approach the percentage of degradation reached after 28 days may be used directly for assessment of ready biodegradability when no information on the 10-days time window is available. This should, however, only be accepted for existing test data and data from tests where the 10-days window does not apply.

(Note that the HSNO Act regulations have no requirement for a pass within a specified window.)

BOD₅/COD

Information on the five-day biochemical oxygen demand (BOD₅) will be used for classification purposes only when no other measured degradability data are available. Thus, priority is given to data from ready biodegradability tests and from simulation studies regarding degradability in the aquatic environment. The BOD₅ test is a traditional biodegradation test that is now replaced by the ready biodegradability tests. Therefore, this test should not be performed today for assessment of the ready biodegradability of substances. Older test data may, however, be used when no other degradability data are available. For substances where the chemical structure is known, the theoretical oxygen demand (ThOD) can be calculated and this value should be used instead of the chemical oxygen demand (COD).

Other convincing scientific evidence

Rapid degradation in the aquatic environment may be demonstrated by other data than referred to in HSNO Act criteria (a) and (b). These may be data on biotic and/or abiotic degradation. Data on primary degradation can only be used where it is demonstrated that the degradation products shall not be classified as hazardous to the aquatic environment, that is, that they do not fulfil the classification criteria.

The fulfilment of HSNO Act criterion (c), requires that the substance is degraded in the aquatic environment to a level of > 70% within a 28-day period. If first-order kinetics are assumed, which is reasonable at the low substance concentrations prevailing in most aquatic environments, the degradation rate will be relatively constant for the 28-day period. Thus, the degradation requirement will be fulfilled with an average degradation rate constant, $k > -(\ln 0.3 - \ln 1)/28 = 0.043 \text{ day}^{-1}$. This corresponds to a degradation half-life, $t_{1/2} < \ln 2/0.043 = 16 \text{ days}$.

Moreover, as degradation processes are temperature dependent, this parameter should also be taken into account when assessing degradation in the environment. Data from studies employing environmentally realistic temperatures should be used for the evaluation. When data from studies performed at different temperatures need to be compared, the traditional Q10 approach could be used, that is, that the degradation rate is halved when the temperature decreases by 10°C.

The evaluation of data on fulfilment of this criterion should be conducted on a case-by-case basis by expert judgement. However, guidance on the interpretation of various types of data that may be used for demonstrating a rapid degradation in the aquatic environment is given below. In general, only data from aquatic biodegradation simulation tests are considered directly applicable. However, simulation test data from other environmental compartments could be considered as well, but such data require in general more scientific judgement before use.

Aquatic simulation tests

Aquatic simulation tests are tests conducted in the laboratory, but simulating environmental conditions and employing natural samples as inoculum. Results of aquatic simulation tests may be used directly for classification purposes, when realistic environmental conditions in surface waters are simulated, that is:

- substance concentration that is realistic for the general aquatic environment (often in the low µg/L range);
- inoculum from a relevant aquatic environment;
- realistic concentration of inoculum (10³–10⁶ cells/mL);
- realistic temperature (for example, 5°C to 25°C); and
- ultimate degradation is determined (that is, determination of the mineralisation rate or the individual degradation rates of the total biodegradation pathway).

Substances that under these conditions are degraded at least 70% within 28 days, that is, with a half-life < 16 days, are considered rapidly degradable.

Field investigations

Parallels to laboratory simulation tests are field investigations or mesocosm experiments. In such studies, fate and/or effects of chemicals in environments or environmental enclosures may be investigated. Fate data from such experiments might be used for assessing the potential for a rapid degradation. This may, however, often be difficult, as it requires that an ultimate degradation can be demonstrated. This may be documented by preparing mass balances showing that no non-degradable intermediates are formed, and which take the fractions into account that are removed from the aqueous system due to other processes such as sorption to sediment or volatilisation from the aquatic environment.

Monitoring data

Monitoring data may demonstrate the removal of contaminants from the aquatic environment. Such data are, however, very difficult to use for classification purposes. The following aspects should be considered before use.

- Is the removal a result of degradation, or is it a result of other processes such as dilution or distribution between compartments (sorption, volatilisation)?
- Is formation of non-degradable intermediates excluded?

Only when it can be demonstrated that removal as a result of ultimate degradation fulfils the criteria for rapid degradability, can such data be considered for use for classification purposes. In general, monitoring data

should only be used as supporting evidence for demonstration of either persistence in the aquatic environment or a rapid degradation.

Inherent biodegradability tests

Substances that are degraded more than 70% in tests for inherent biodegradability (OECD Test Guidelines 302) have the potential for ultimate biodegradation. However, because of the optimum conditions in these tests, the rapid biodegradability of inherently biodegradable substances in the environment cannot be assumed. The optimum conditions in inherent biodegradability tests stimulate adaptation of the micro-organisms, thus increasing the biodegradation potential, compared to natural environments. Therefore, positive results in general should not be interpreted as evidence for rapid degradation in the environment.

The inherent biodegradability tests concerned are the Zahn Wellens test (OECD TG 302 B) and the MITI II test (OECD TG 302 C). The conditions for use in this regard are:

- the methods must not employ pre-exposed (pre-adapted) micro-organisms;
- the time for adaptation within each test should be limited, the test endpoint should refer to the mineralisation only and the pass level and time for reaching these should be, respectively:
 - MITI II pass level > 60% within 14 days; and
 - Zahn Wellens Test > 70% within 7 days.

Sewage treatment plant simulation tests

Results from tests simulating the conditions in a sewage treatment plant (STP) (for example, the OECD Test Guideline 303) cannot be used for assessing the degradation in the aquatic environment. The main reasons for this are that the microbial biomass in a STP is significantly different from the biomass in the environment, that there is a considerably different composition of substrates, and that the presence of rapidly mineralised organic matter in waste water facilitates degradation of the test substance by cometabolism.

Soil and sediment degradation data

It has been argued that for many non-sorptive (non-lipophilic) substances, more or less the same degradation rates are found in soil and in surface water. For lipophilic substances, a lower degradation rate may generally be expected in soil than in water due to partial immobilisation caused by sorption. Thus, when a substance has been shown to be degraded rapidly in a soil simulation study, it is most likely also rapidly degradable in the aquatic environment. It is therefore proposed that an experimentally determined rapid degradation in soil is sufficient documentation for a rapid degradation in surface waters when:

- no pre-exposure (pre-adaptation) of the soil micro-organisms has taken place; and
- an environmentally realistic concentration of substance is tested; and
- the substance is ultimately degraded within 28 days with a half-life < 16 days corresponding to a degradation rate > 0.043 day⁻¹.

The same argument is considered valid for data on degradation in sediment under aerobic conditions.

Anaerobic degradation data

Data regarding anaerobic degradation cannot be used in relation to deciding whether a substance should be regarded as rapidly degradable, because the aquatic environment is generally regarded as the aerobic compartment where the aquatic organisms, such as those employed for aquatic hazard classification, live.

Hydrolysis

Data on hydrolysis (for example, OECD Test Guideline 111) might be considered for classification purposes only when the longest half-life $t_{1/2}$ determined within the pH range 4–9 is shorter than 16 days. However, hydrolysis is not an ultimate degradation and various intermediate degradation products may be formed, some of which may be only slowly degradable. Only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment, can data from hydrolysis studies be considered. When a substance is quickly hydrolysed (for example, with $t_{1/2} < a$ few days), this process is a part of the degradation determined in biodegradation tests. Hydrolysis may be the initial transformation process in biodegradation.

Photochemical degradation

Information on photochemical degradation is difficult to use for classification purposes. The actual degree of photochemical degradation in the aquatic environment depends on local conditions (for example, water depth, suspended solids, turbidity) and the hazard of the degradation products is usually not known. Probably only seldom will enough information be available for a thorough evaluation based on photochemical degradation.

Estimation of degradation

Certain QSARs have been developed for prediction of an approximate hydrolysis half-life, which should only be considered when no experimental data are available. However, a hydrolysis half-life can only be used in relation to classification with great care, because hydrolysis does not concern ultimate degradability (see 'Hydrolysis' in section 19D.3). Furthermore the QSARs developed until now have a rather limited applicability and are only able to predict the potential for hydrolysis on a limited number of chemical classes. The QSAR program HYDROWIN (version 1.67, Syracuse Research Corporation) is, for example, only able to predict the potential for hydrolysis on less than 1/5th of the existing EU substances that have a defined (precise) molecular structure.

In general, no quantitative estimation method (QSAR) for estimating the degree of biodegradability of organic substances is yet sufficiently accurate to predict rapid degradation. However, results from such methods may be used to predict that a substance is not rapidly degradable. For example, when in the Biodegradation Probability Program (for example, BIOWIN version 3.67, Syracuse Research Corporation, Howard and Meylan, 1992) the probability is < 0.5 , estimated by the linear or non-linear methods, the substances should be regarded as not rapidly degradable (Pedersen et al, 1995; Langenberg et al, 1996). Also other (Q)SAR methods may be used as well as expert judgement, for example, when degradation data for structurally analogue compounds are available, but such judgement should be conducted with great care. In general, a

QSAR prediction that a substance is not rapidly degradable is considered a better justification for a classification than application of a default classification, when no useful degradation data are available.

Volatilisation

Chemicals may be removed from some aquatic environments by volatilisation. The intrinsic potential for volatilisation is determined by the Henry's Law constant (H) of the substance. Volatilisation from the aquatic environment is highly dependent on the environmental conditions of the specific water body in question, such as the water depth, the gas exchange coefficients (depending on wind speed and water flow) and stratification of the water body. Because volatilisation only represents removal of a chemical from water phase, the Henry's Law constant cannot be used for assessment of degradation in relation to aquatic hazard classification of substances. Substances that are gases at ambient temperature may however for example be considered further in this regard (see also Pedersen et al, 1995).

No degradation data available

When no useful data on degradability are available – either experimentally determined or estimated data – the substance should be regarded as not rapidly degradable.

General interpretation problems

Complex substances

The harmonised criteria for classification of chemicals as hazardous for the aquatic environment focus on single substances. Certain types of intrinsically complex substance are multi-component substances. They are typically of natural origin and need occasionally to be considered. This may be the case for chemicals that are produced or extracted from mineral oil or plant material. Such complex chemicals are normally considered as single substances in a regulatory context. In most cases they are defined as a homologous series of substances within a certain range of carbon chain length and/or degree of substitution. When this is the case, no major difference in degradability is foreseen and the degree of degradability can be established from tests of the complex chemical. One exception would be when a borderline degradation is found because in this case some of the individual substances may be rapidly degradable and other may be not rapidly degradable. This requires a more detailed assessment of the degradability of the individual components in the complex substance. When not-rapidly-degradable components constitute a significant part of the complex substance (for example, more than 20%, or for a hazardous component, an even lower content), the substance should be regarded as not rapidly degradable.

Availability of the substance

Degradation of organic substances in the environment takes place mostly in the aquatic compartments or in aquatic phases in soil or sediment. Hydrolysis, of course, requires the presence of water. The activity of micro-organisms depends on the presence of water. Moreover, biodegradation requires that the micro-organisms are directly in contact with the substance. Dissolution of the substance in the water phase that surrounds the micro-organisms is therefore the most direct way for contact between the bacteria and fungi and the substrate.

The present standard methods for investigating degradability of chemical substances are developed for readily soluble test compounds. However, many organic substances are only slightly soluble in water. As the standard tests require 2–100 mg/L of the test substance, sufficient availability may not be reached for substances with a low water solubility. Tests with continuous mixing and/or an increased exposure time, or tests with a special design where concentrations of the test substance lower than the water solubility have been employed, may be available on slightly soluble compounds.

Test duration less than 28 days

Sometimes degradation is reported for tests terminated before the 28-day period specified in the standards (for example, the MITI, 1992). These data are of course directly applicable when a degradation greater than or equal to the pass level is obtained. When a lower degradation level is reached, the results need to be interpreted with caution. One possibility is that the duration of the test was too short and that the chemical structure would probably have been degraded in a 28-day biodegradability test. If substantial degradation occurs within a short time period, the situation may be compared with the criterion $BOD_5/COD > 0.5$ or with the requirements on degradation within the 10-days time window. In these cases, a substance may be considered readily degradable (and hence rapidly degradable), if:

- the ultimate biodegradability exceeds 50% within 5 days; or
- the ultimate degradation rate constant in this period is greater than 0.1 day⁻¹ corresponding to a half-life of 7 days.

These criteria are proposed in order to ensure that rapid mineralisation did occur, although the test was ended before 28 days and before the pass level was attained. Interpretation of test data that do not comply with the prescribed pass levels must be made with great caution. It is mandatory to consider whether biodegradability below the pass level was due to a partial degradation of the substance and not a complete mineralisation. If partial degradation is the probable explanation for the observed biodegradability, the substance should be considered not readily biodegradable.

Primary biodegradation

In some tests, only the disappearance of the parent compound (that is, primary degradation) is determined for example by following the degradation by specific or group specific chemical analyses of the test substance. Data on primary biodegradability may be used for demonstrating rapid degradability only when it can be satisfactorily demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

Conflicting results from screening tests

The situation where more degradation data are available for the same substance introduces the possibility of conflicting results. In general, conflicting results for a substance that has been tested several times with an appropriate biodegradability test could be interpreted by a 'weight-of-evidence approach'. This implies that if both positive (that is, higher degradation than the pass level) and negative results have been obtained for a substance in ready biodegradability tests, then the data of the highest quality and the best documentation should be used for determining the ready biodegradability of the substance. However, positive results in

ready biodegradability tests could be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, that is, guideline criteria are fulfilled, including the use of non-pre-exposed (non-adapted) inoculum. None of the various screening tests is suitable for the testing of all types of substances, and results obtained by the use of a test procedure that is not suitable for the specific substance should be evaluated carefully before a decision on the use is taken.

Thus, there are a number of factors may explain conflicting biodegradability data from screening tests, including:

- inoculum;
- toxicity of test substance;
- test conditions;
- solubility of the test substance; and
- volatilisation of the test substance.

The suitability of the inoculum for degrading the test substance depends on the presence and amount of competent degraders. When the inoculum is obtained from an environment that has previously been exposed to the test substance, the inoculum may be adapted as evidenced by a degradation capacity, which is greater than that of an inoculum from a non-exposed environment. As far as possible the inoculum must be sampled from an unexposed environment, but for substances that are used ubiquitously in high volumes and released widespread or more or less continuously, this may be difficult or impossible. When conflicting results are obtained, the origin of the inoculum should be checked in order to clarify whether or not differences in the adaptation of the microbial community may be the reason.

As mentioned above, many substances may be toxic or inhibitory to the inoculum at the relatively high concentrations tested in ready biodegradability tests. Especially in the Modified MITI (I) test (OECD Test Guideline 301C) and the Manometric Respirometry test (OECD Test Guideline 301F) where high concentrations (100 mg/L) are prescribed. The lowest test substance concentrations are prescribed in the Closed Bottle test (OECD Test Guideline 301D) where 2–10 mg/L is used. The possibility of toxic effects may be evaluated by including a toxicity control in the ready biodegradability test or by comparing the test concentration with toxicity test data on micro-organisms, for example, the respiration inhibition tests (OECD Test Guideline 209), the nitrification inhibition test (ISO 9509) or, if other microbial toxicity tests are not available, the bioluminescence inhibition test (ISO 11348). When conflicting results are found, this may be caused by the toxicity of the test substance. If the substance is not inhibitory at environmentally realistic concentrations, the greatest degradation measured in screening tests may be used as a basis for classification. If simulation test data are available in such cases, consideration of these data may be especially important, because a low non-inhibitory concentration of the substance may have been employed, thus giving a more reliable indication of the biodegradation half-life of the substance under environmentally realistic conditions.

When the solubility of the test substance is lower than the concentrations employed in a test, this parameter may be the limiting factor for the actual degradation measured. In these cases, results from tests employing the lowest concentrations of test substance should prevail, that is, often the Closed Bottle test (OECD Test

Guideline 301D). In general, the DOC Die-Away test (OECD Test Guideline 301A) and the Modified OECD Screening test (OECD Test Guideline 301E) are not suitable for testing the biodegradability of poorly soluble substances (for example, OECD Test Guideline 301).

Volatile substances should only be tested in closed systems as the Closed Bottle test (OECD Test Guideline 301D), the MITI I test (OECD Test Guideline 301C) and the Manometric Respirometry test (OECD Test Guideline 301F). Results from other tests should be evaluated carefully and only considered if it can be demonstrated, for example, by mass balance estimates, that the removal of the test substance is not a result of volatilisation.

Variation in simulation test data

A number of simulation test data may be available for certain high priority chemicals. Often such data provide a range of half-lives in environmental media such as soil, sediment and/or surface water. The observed differences in half-lives from simulation tests performed on the same substance may reflect differences in test conditions, all of which may be environmentally relevant. A suitable half-life in the higher end of the observed range of half-lives from such investigations should be selected for classification by employing a weight-of-evidence approach and taking the realism and relevance of the employed tests into account in relation to environmental conditions. In general, simulation test data of surface water are preferred relative to aquatic sediment or soil simulation test data in relation to the evaluation of rapid degradability in the aquatic environment.

Decision scheme

The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment. A substance is considered to be not rapidly degradable unless at least one of the following is fulfilled.

- The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70% DOC removal or 60% theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data. If this is not possible, then the pass level should be evaluated within a 14-days time window if possible, or after the end of the test.
- The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of > 70% within 28 days) Simulations tests should reflect realistic environmental conditions such as low concentration of the chemical, realistic temperature and employment of ambient microbial biomass not pre-exposed to the chemical.
- The substance is demonstrated to be primarily degraded (biotically or abiotically) in the aquatic environment with a half-life < 16 days (corresponding to a degradation of > 70% within 28 days) and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. When these data are not available, rapid degradation may be demonstrated if one of the following criteria is justified.

- The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life of < 16 days (corresponding to a degradation of > 70% within 28 days).
- In those cases where only BOD5 and COD data are available, the ratio of BOD5/COD is greater than or equal to 0.5. The same criterion applies to ready biodegradability tests of a shorter duration than 28 days, if the half-life < 7 days.
- If none of the above types of data are available then the substance is considered as not rapidly degradable. This decision may be supported by fulfilment of at least one of the following criteria.
 - The substance is not inherently degradable in an inherent biodegradability test.
 - The substance is predicted to be slowly biodegradable by scientifically valid QSARs, for example, for the Biodegradation Probability Program, the score for rapid degradation (linear or non-linear model) < 0.5.
 - The substance is considered to be not rapidly degradable based on indirect evidence, for example, knowledge from structurally similar substances.
 - No other data regarding degradability are available.

19D.4 Bioaccumulation

Introduction

Bioaccumulation is one of the important intrinsic properties of chemical substances that determine the potential environmental hazard. Bioaccumulation of a substance into an organism is not a hazard in itself, but bioconcentration and bioaccumulation will result in a body burden, which may or may not lead to toxic effects. In the harmonised integrated hazard classification system for human health and environmental effects of chemical substances (OECD, 1998), the wording “potential for bioaccumulation” is given. A distinction should, however, be drawn between bioconcentration and bioaccumulation. Here bioconcentration is defined as the net result of uptake, transformation, and elimination of a substance in an organism due to waterborne exposure, whereas bioaccumulation includes all routes of exposure (that is, via air, water, sediment or soil, and food). Finally, biomagnification is defined as accumulation and transfer of substances via the food chain, resulting in an increase of internal concentrations in organisms on higher levels of the trophic chain. For most organic chemicals uptake from water (bioconcentration) is believed to be the predominant route of uptake. Only for very hydrophobic substances does uptake from food becomes important. Also, the harmonised classification criteria use the bioconcentration factor (BCF) (or the octanol/water partition coefficient) as the measure of the potential for bioaccumulation. For these reasons, the present guidance document only considers bioconcentration and does not discuss uptake via food or other routes.

Classification of a chemical substance is primarily based on its intrinsic properties. However, the degree of bioconcentration also depends on factors such as the degree of bioavailability, the physiology of test organism, maintenance of constant exposure concentration, exposure duration, metabolism inside the body of the target organism and excretion from the body. The interpretation of the bioconcentration potential in a

chemical classification context therefore requires an evaluation of the intrinsic properties of the substance, as well as of the experimental conditions under which BCF has been determined. Based on the guide, a decision scheme for application of bioconcentration data or log KOW data for classification purposes has been developed. The emphasis of the present section is organic substances and organo-metals. Bioaccumulation of metals is also discussed in section 19D.6.

Data on bioconcentration properties of a substance may be available from standardised tests or may be estimated from the structure of the molecule. The interpretation of such bioconcentration data for classification purposes often requires detailed evaluation of test data.

See Appendix 19F for more detailed guidance.

Interpretation of bioconcentration data

Environmental hazard classification of a chemical substance is normally based on existing data on its environmental properties. Test data will only seldom be produced with the main purpose of facilitating a classification. Often a diverse range of test data is available which does not necessarily match the classification criteria. Consequently, guidance is needed on interpretation of existing test data in the context of hazard classification.

Bioconcentration of an organic substance can be experimentally determined in bioconcentration experiments, during which BCF is measured as the concentration in the organism relative to the concentration in water under steady-state conditions and/or estimated from the uptake rate constant (k_1) and the elimination rate constant (k_2) (OECD 305). In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A measure of lipophilicity is the n-octanol-water partition coefficient (K_{OW}) which, for lipophilic non-ionic organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the BCF. Therefore, K_{OW} is often used for estimating the bioconcentration of organic substances, based on the empirical relationship between log BCF and log K_{OW} . For most organic substances, estimation methods are available for calculating the K_{OW} . Data on the bioconcentration properties of a substance may thus be (i) experimentally determined, (ii) estimated from experimentally determined K_{OW} , or (iii) estimated from KOW values derived by use of Quantitative Structure Activity Relationships (QSARs). Guidance for interpretation of such data is given below together with guidance on assessment of chemical classes, which need special attention.

Bioconcentration factor

The BCF is defined as the ratio on a weight basis between the concentration of the chemical in biota and the concentration in the surrounding medium; here water, at steady state. The BCF can thus be experimentally derived under steady-state conditions, on the basis of measured concentrations. However, the BCF can also be calculated as the ratio between the first-order uptake and elimination rate constants; a method which does not require equilibrium conditions.

Different test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted; the most generally applied being the OECD test guideline (OECD 305, 1996).

Experimentally derived BCF values of high quality are ultimately preferred for classification purposes as such data override surrogate data, for example, K_{OW} .

High quality data are defined as data where the validity criteria for the test method applied are fulfilled and described, for example, maintenance of constant exposure concentration; oxygen and temperature variations, and documentation that steady-state conditions have been reached, etc. The experiment will be regarded as a high-quality study, if a proper description is provided (for example, by Good Laboratory Practice (GLP)) allowing verification that validity criteria are fulfilled. In addition, an appropriate analytical method must be used to quantify the chemical and its toxic metabolites in the water and fish tissue.

BCF values of low or uncertain quality may give a false and too low BCF value; for example, application of measured concentrations of the test substance in fish and water, but measured after a too short exposure period in which steady-state conditions have not been reached (compare with OECD 306, 1996, regarding estimation of time to equilibrium). Therefore, such data should be carefully evaluated before use and consideration should be given to using K_{OW} instead.

If there is no BCF value for fish species, high-quality data on the BCF value for other species may be used (for example, BCF determined on blue mussel, oyster, or scallop (ASTM E 1022-94)). Reported BCFs for microalgae should be used with caution.

For highly lipophilic substances, for example, with $\log K_{OW}$ above 6, experimentally derived BCF values tend to decrease with increasing $\log K_{OW}$. Conceptual explanations of this non-linearity mainly refer to either reduced membrane permeation kinetics or reduced biotic lipid solubility for large molecules. A low bioavailability and uptake of these substances in the organism will thus occur. Other factors comprise experimental artefacts, such as equilibrium not being reached, reduced bioavailability due to sorption to organic matter in the aqueous phase, and analytical errors. Special care should thus be taken when evaluating experimental data on the BCF for highly lipophilic substances as these data will have a much higher level of uncertainty than BCF values determined for less lipophilic substances.

Bioconcentration factor in different test species

BCF values used for classification are based on whole body measurements. As stated previously, the optimal data for classification are BCF values derived using the OECD 305 test method or internationally equivalent methods, which uses small fish. Due to the higher gill surface to weight ratio for smaller organisms than larger organisms, steady-state conditions will be reached sooner in smaller organisms than in larger ones. The size of the organisms (fish) used in bioconcentration studies is thus of considerable importance in relation to the time used in the uptake phase, when the reported BCF value is based solely on measured concentrations in fish and water at steady-state. Thus, if large fish, for example, adult salmon, have been used in bioconcentration studies, it should be evaluated whether the uptake period was sufficiently long for steady state to be reached or to allow for a kinetic uptake rate constant to be determined precisely.

Furthermore, when using existing data for classification, it is possible that the BCF values could be derived from several different fish or other aquatic species (for example, clams) and for different organs in the fish.

Thus, to compare these data to each other and to the criteria, some common basis or normalisation will be required. It has been noted that there is a close relationship between the lipid content of a fish or an aquatic organism and the observed BCF value. Therefore, when comparing BCF values across different fish species or when converting BCF values for specific organs to whole body BCFs, the common approach is to express the BCF values on a common lipid content. If, for example, whole body BCF values or BCF values for specific organs are found in the literature, the first step is to calculate the BCF on a percentage lipid basis using the relative content of fat in the fish (compare with the literature/test guideline for typical fat content of the test species) or the organ. In the second step the BCF for the whole body for a typical aquatic organism (that is, small fish) is calculated assuming a common default lipid content. A default value of 5% is most commonly used (Pedersen et al, 1995) as this represents the average lipid content of the small fish used in OECD 305 (1996).

Generally, the highest valid BCF value expressed on this common lipid basis is used to determine the wet weight based BCF-value in relation to the cut off value for the BCF of 500 of the HSNO Act classification criteria.

Use of radiolabelled substances

The use of radiolabelled test substances can facilitate the analysis of water and fish samples. However, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of the parent substance as well as possible metabolite(s) and possible metabolised carbon, which have been incorporated in the fish tissue in organic molecules. BCF values determined by use of radiolabelled test substances are therefore normally overestimated.

When using radiolabelled substances, the labelling is most often placed in the stable part of the molecule, for which reason the measured BCF value includes the BCF of the metabolites. For some substances it is the metabolite which is the most toxic and which has the highest bioconcentration potential. Measurements of the parent substance as well as the metabolites may thus be important for the interpretation of the aquatic hazard (including the bioconcentration potential) of such substances.

In experiments where radiolabelled substances have been used, high radiolabel concentrations are often found in the gall bladder of fish. This is interpreted to be caused by biotransformation in the liver and subsequently by excretion of metabolites in the gall bladder (Comotto et al, 1979; Goodrich et al, 1991; Toshima et al, 1992; Wakabayashi et al, 1987). When fish do not eat, the content of the gall bladder is not emptied into the gut, and high concentrations of metabolites may build up in the gall bladder. The feeding regime may thus have a pronounced effect on the measured BCF. In the literature many studies are found where radiolabelled compounds are used, and where the fish are not fed. As a result high concentrations of radioactive material are found in the gall bladder. In these studies the bioconcentration may in most cases have been overestimated. Thus when evaluating experiments, in which radiolabelled compounds are used, it is essential to evaluate the feeding regime as well.

If the BCF in terms of radiolabelled residues is documented to be $\geq 1,000$, identification and quantification of degradation products, representing $\geq 10\%$ of total residues in fish tissues at steady-state, are for, for

example, pesticides strongly recommended in the OECD Test Guideline 305 (1996). If no identification and quantification of metabolites are available, the assessment of bioconcentration should be based on the measured radiolabelled BCF value. If, for highly bioaccumulative substances ($BCF \geq 500$), only BCFs based on the parent compound and on radiolabelled measurements are available, the latter should thus be used in relation to classification.

Octanol-water-partitioning coefficient

For organic substances experimentally derived high-quality K_{OW} values, or values that are evaluated in reviews and assigned as the 'recommended values', are preferred over other determinations of K_{OW}. When no experimental data of high quality are available, validated QSARs for log K_{OW} may be used in the classification process. Such validated QSARs may be used without modification to the agreed criteria if they are restricted to chemicals for which their applicability is well characterised. For substances like strong acids and bases, substances that react with the eluent, or surface-active substances, a QSAR estimated value of K_{OW} or an estimate based on individual n-octanol and water solubilities should be provided instead of an analytical determination of K_{OW} (EEC A8; OECD 117). Measurements should be taken on ionisable substances in their non-ionised form (free acid or free base) only by using an appropriate buffer with pH below pK for free acid or above the pK for free base.

Experimental determination of K_{OW}

For experimental determination of K_{OW} values, several different methods, Shake-flask, and High Performance Liquid Chromatography (HPLC), are described in standard guidelines, see Appendix 19A. The shake-flask method is recommended when the log K_{OW} value falls within the range from -2 to 4. The shake-flask method applies only to essential pure substances soluble in water and n-octanol. For highly lipophilic substances, which slowly dissolve in water, data obtained by employing a slow-stirring method are generally more reliable. Furthermore, the experimental difficulties, associated with the formation of microdroplets during the shake-flask experiment, can to some degree be overcome by a slow-stirring method where water, octanol, and test compound are equilibrated in a gently stirred reactor. With the slow-stirring method (OECD Test Guideline 123) a precise and accurate determination of K_{OW} of compounds with log K_{OW} of up to 8.2 is allowed. As for the shake-flask method, the slow-stirring method applies only to essentially pure substances soluble in water and n-octanol. The HPLC method, which is performed on analytical columns, is recommended when the log K_{OW} value falls within the range 0 to 6. The HPLC method is less sensitive to the presence of impurities in the test compound compared to the shake-flask method. Another technique for measuring log K_{OW} is the generator column method (USEPA, 1996b). As an experimental determination of the K_{OW} is not always possible, for example, for very water soluble substances, very lipophilic substances, and surfactants, a QSAR-derived K_{OW} may be used.

Use of Quantitative Structure Activity Relationships for determination of log K_{OW}

When an estimated K_{OW} value is found, the estimation method has to be taken into account. Numerous QSARs have been and continue to be developed for the estimation of K_{OW}. Four commercially available computer programs (CLOGP, LOGKOW (KOWWIN), AUTOLOGP, and SPARC) are frequently used for risk

assessment if no experimentally derived data are available. CLOGP, LOGKOW, and AUTOLOGP are based upon the addition of group contributions, while SPARC is based upon a more fundamental chemical structure algorithm. SPARC can only be employed in a general way for inorganic or organometallic compounds. Special methods are needed for estimating log KOW for surface-active compounds, chelating compounds and mixtures. CLOGP is recommended in the USEPA/EC joint project on validation of QSAR estimation methods. Pedersen et al (1995) recommended the CLOGP and the LOGKOW programs for classification purposes because of their reliability, commercial availability, and convenience of use. The estimation methods in Table 19D.2 are recommended for classification purposes.

Table 19D.2: Recommended Quantitative Structure Activity Relationships (QSARs) for estimating the n-octanol-water partition coefficient (K_{OW})

| Model | Log K_{OW} range | Substance utility |
|-----------------|--|--|
| CLOGP | $0 < \log K_{OW} < 9^*$ | The program calculates log K_{OW} for organic compounds containing C, H, N, O, Hal, P, and/or S. |
| LOGKOW (KOWWIN) | $-4 < \log K_{OW} < 8^\dagger$ | The program calculates log K_{OW} for organic compounds containing C, H, N, O, Hal, Si, P, Se, Li, Na, K, and/or Hg. Some surfactants (eg, alcohol ethoxylates, dyestuffs, and dissociated substances) may be predicted by the program as well. |
| AUTOLOGP. | $\log K_{OW} > 5$ | The program calculates log K_{OW} for organic compounds containing C, H, N, O, Hal, P, and S. Improvements are in progress in order to extend the program's applicability. |
| SPARC | Provides improved results over KOWWIN and CLOGP for compounds with $\log K_{OW} > 5$ | The program is a mechanistic model based on chemical thermodynamic principles rather than a deterministic model rooted in knowledge obtained from observational data. Therefore, SPARC differs from models that use QSARs (ie, KOWWIN, CLOGP, and AUTOLOGP) in that no measured log K_{OW} data are needed for a training set of chemicals. Only SPARC can be used in a general way for inorganic or organometallic compounds. |

Notes

- * A validation study performed by Niemelä, who compared experimental determined log KOW values with estimated values, showed that the program precisely predicts the log KOW for a great number of organic chemicals in the log KOW range from below 0 to above 9 ($n = 501$, $r^2 = 0.967$) (Pedersen et al, 1995, p 581).
- † Based on a scatter plot of estimated compared with experimental log KOW (Syracuse Research Corporation, 1999), where 13,058 compounds have been tested, the LOGKOW is evaluated being valid for compounds with a log KOW in the interval -4–8.

Chemical classes that need special attention with respect to the bioconcentration factor and octanol-water-partition coefficient values

There are certain physico-chemical properties, which can make the determination of the BCF or its measurement difficult. These may be substances, which do not bioconcentrate in a manner consistent with their other physico-chemical properties, for example, steric hindrance or substances that make the use of

descriptors inappropriate, for example, surface activity, which makes both the measurement and use of log K_{OW} inappropriate.

Difficult substances

Some chemical substances are difficult to test in aquatic systems and guidance has been developed to assist in testing these materials (OECD, 2000). This document is a good source of information on the types of substances that are difficult to test for bioconcentration and the steps needed to ensure valid conclusions from tests with these substances. Difficult to test substances may be poorly soluble, volatile, or subject to rapid degradation due to such processes as phototransformation, hydrolysis, oxidation, or biotic degradation.

To bioconcentrate organic compounds, a substance needs to be soluble in lipids, present in the water, and available for transfer across the fish gills. Properties that alter this availability will thus change the actual bioconcentration of a substance, when compared with the prediction. For example, readily biodegradable substances may only be present in the aquatic compartment for short periods. Similarly, volatility, and hydrolysis will reduce the concentration and the time during which a substance is available for bioconcentration. A further important parameter, which may reduce the actual exposure concentration of a substance, is adsorption, either to particulate matter or to surfaces in general. There are a number of substances, which have shown to be rapidly transformed in the organism, thus leading to a lower BCF value than expected. Substances that form micelles or aggregates may bioconcentrate to a lower extent than would be predicted from simple physico-chemical properties. This is also the case for hydrophobic substances that are contained in micelles formed as a consequence of the use of dispersants. Therefore, the use of dispersants in bioaccumulation tests is discouraged.

In general, for difficult to test substances, measured BCF and KOW values – based on the parent substance – are a prerequisite for the determination of the bioconcentration potential. Furthermore, proper documentation of the test concentration is a prerequisite for the validation of the given BCF value.

Poorly soluble and complex substances

Special attention should be paid to poorly soluble substances. Frequently the solubility of these substances is recorded as less than the detection limit, which creates problems in interpreting the bioconcentration potential. For such substances the bioconcentration potential should be based on experimental determination of log K_{OW} or QSAR estimations of log K_{OW} . When a multi-component substance is not fully soluble in water, it is important to attempt to identify the components of the mixture as far as practically possible and to examine the possibility of determining its bioaccumulation potential using available information on its components. When bioaccumulating components constitute a significant part of the complex substance (for example, more than 20% or for hazardous components an even lower content), the complex substance should be regarded as being bioaccumulating.

High molecular weight substances

Above certain molecular dimensions, the potential of a substance to bioconcentrate decreases. This is possibly due to steric hindrance of the passage of the substance through gill membranes. It has been

proposed that a cut-off limit of 700 for the molecular weight could be applied. However, this cut-off has been subject to criticism and an alternative cut-off of 1000 has been proposed in relation to exclusion of consideration of substances with possible indirect aquatic effects (CSTEE, 1999). In general, bioconcentration of possible metabolites or environmental degradation products of large molecules should be considered. Data on bioconcentration of molecules with a high molecular weight should therefore be carefully evaluated and only be used if such data are considered to be fully valid in respect to both the parent compound and its possible metabolites and environmental degradation products.

Surface-active agents

Surfactants consist of a lipophilic (most often an alkyl chain) and a hydrophilic part (the polar headgroup). According to the charge of the headgroup, surfactants are subdivided into classes of anionic, cationic, non-ionic, or amphoteric surfactants. Due to the variety of different headgroups, surfactants are a structurally diverse class of compounds, which is defined by surface activity rather than by chemical structure. The bioaccumulation potential of surfactants should thus be considered in relation to the different subclasses (anionic, cationic, non-ionic, or amphoteric) instead of to the group as a whole. Surface-active substances may form emulsions, in which the bioavailability is difficult to ascertain. Micelle formation can result in a change of the bioavailable fraction even when the solutions are apparently formed, thus giving problems in interpretation of the bioaccumulation potential.

Experimentally derived bioconcentration factors

Measured BCF values on surfactants show that the BCF may increase with increasing alkyl chain length and be dependant of the site of attachment of the head group, and other structural features.

Octanol-water-partition coefficient

The octanol-water partition coefficient for surfactants can not be determined using the shakeflask or slow stirring method because of the formation of emulsions. In addition, the surfactant molecules will exist in the water phase almost exclusively as ions, whereas they will have to pair with a counter-ion in order to be dissolved in octanol. Therefore, experimental determination of KOW does not characterise the partition of ionic surfactants (Tolls, 1998). On the other hand, it has been shown that the bioconcentration of anionic and non-ionic surfactants increases with increasing lipophilicity (Tolls, 1998). Tolls (1998) showed that for some surfactants, an estimated log KOW value using LOGKOW could represent the bioaccumulation potential; however, for other surfactants some 'correction' to the estimated log KOW value using the method of Roberts (1989) was required. These results illustrate that the quality of the relationship between log KOW estimates and bioconcentration depends on the class and specific type of surfactants involved. Therefore, the classification of the bioconcentration potential based on log KOW values should be used with caution.

Conflicting data and lack of data

Conflicting bioconcentration factor data

In situations where multiple BCF data are available for the same substance, the possibility of conflicting results might arise. In general, conflicting results for a substance, which has been tested several times with

an appropriate bioconcentration test, should be interpreted by a 'weight-of-evidence approach'. This implies that if experimental determined BCF data, both \geq and $<$ 500, have been obtained for a substance the data of the highest quality and with the best documentation should be used for determining the bioconcentration potential of the substance. If differences still remain, if, for example, high-quality BCF values for different fish species are available, generally the highest valid value should be used as the basis for classification. When larger data sets (that is, with four or more values) are available for the same species and life stage, the geometric mean of the BCF values may be used as the representative BCF value for that species.

Conflicting log K_{OW} data

The situations, where multiple log K_{OW} data are available for the same substance, the possibility of conflicting results might arise. If log K_{OW} data both \geq and $<$ 4 have been obtained for a substance, then the data of the highest quality and the best documentation should be used for determining the bioconcentration potential of the substance. If differences still exist, generally the highest valid value should take precedence. In such situation, QSAR-estimated log K_{OW} could be used as a guidance.

Expert judgement

If no experimental BCF or log K_{OW} data or no predicted log KOW data are available, the potential for bioconcentration in the aquatic environment may be assessed by expert judgement. This may be based on a comparison of the structure of the molecule with the structure of other substances for which experimental bioconcentration or log K_{OW} data or predicted KOW are available.

Decision scheme

Based on the above discussions and conclusions, a decision scheme has been elaborated which may facilitate decisions as to whether or not a substance has the potential for bioconcentration in aquatic species.

Experimentally derived BCF values of high quality are ultimately preferred for classification purposes. BCF values of low or uncertain quality should not be used for classification purposes if data on log KOW are available because they may give a false and too low BCF value, for example, due to a too short exposure period in which steady-state conditions have not been reached. If no BCF is available for fish species, high quality data on the BCF for other species (for example, mussels) may be used.

For organic substances, experimentally derived high quality KOW values, or values that are evaluated in reviews and assigned as the 'recommended values', are preferred. If no experimentally data of high quality are available validated QSARs for log KOW may be used in the classification process. Such validated QSARs may be used without modification in relation to the classification criteria, if restricted to chemicals for which their applicability is well characterised. For substances like strong acids and bases, metal complexes, and surface-active substances a QSAR-estimated value of KOW or an estimate based on individual n-octanol and water solubilities should be provided instead of an analytical determination of KOW.

If data are available but not validated, expert judgement should be used.

Whether or not a substance has a potential for bioconcentration in aquatic organisms could thus be decided in accordance with the following scheme.

- a. Valid/high quality experimentally determined BCF value = YES:
BCF \geq 500: The substance has a potential for bioconcentration.
BCF < 500: The substance does not have a potential for bioconcentration.
- b. Valid/high quality experimentally determined BCF value = NO:
Valid/high quality experimentally determined log K_{OW} value = YES:
Log K_{OW} \geq 4: The substance has a potential for bioconcentration.
Log K_{OW} < 4: The substance does not have a potential for bioconcentration.
- c. Valid/high quality experimentally determined BCF value = NO:
Valid/high quality experimentally determined log K_{OW} value = NO:
Use of validated QSAR for estimating a log K_{OW} value = YES:
Log K_{OW} \geq 4: The substance has a potential for bioconcentration
Log K_{OW} < 4: The substance does not have a potential for bioconcentration.

19D.5 Use of Quantitative Structure Activity Relationships

History

QSARs in aquatic toxicology can be traced to the work of Overton in Zürich (Lipnick, 1986) and Meyer in Marburg (Lipnick, 1989). They demonstrated that the potency of substances producing narcosis in tadpoles and small fish is in direct proportion to their partition coefficients measured between olive oil and water. Overton postulated in his 1901 monograph *Studien über die Narkose* that this correlation reflects toxicity taking place at a standard molar concentration or molar volume within some molecular site within the organism (Lipnick, 1991a). In addition, he concluded that this corresponds to the same concentration or volume for a various organisms, regardless of whether uptake is from water or via gaseous inhalation. This correlation became known in anaesthesia as the Meyer-Overton theory.

Corwin Hansch and co-workers at Pomona College proposed the use of n-octanol/water as a standard partitioning system, and found that these partition coefficients were an additive, constitutive property that can be directly estimated from chemical structure. In addition, they found that regression analysis could be used to derive QSAR models, providing a statistical analysis of the findings. Using this approach, in 1972 these workers reported 137 QSAR models in the form $\log(1/C) = A \log K_{OW} + B$, where K_{OW} is the n-octanol/water partition coefficient, and C is the molar concentration of a chemical yielding a standard biological response for the effect of simple non-electrolyte non-reactive organic compounds on whole animals, organs, cells, or even pure enzymes. Five of these equations, which relate to the toxicity of five simple monohydric alcohols to five species of fish, have almost identical slopes and intercepts and are in fact virtually the same as those found by Könemann in 1981, who appears to have been unaware of Hansch's earlier work. Könemann and others have demonstrated that such simple non-reactive non-electrolytes all act

by a narcosis mechanism in an acute fish toxicity test, giving rise to minimum or baseline toxicity (Lipnick, 1989b).

Experimental artifacts causing underestimation of hazard

Other non-electrolytes can be more toxic than predicted by such a QSAR, but not less toxic, except as a result of a testing artefact. Such testing artefacts include data obtained for compounds such as hydrocarbons which tend to volatilise during the experiment, as well as very hydrophobic compounds for which the acute testing duration may be inadequate to achieve steady state equilibrium partitioning between the concentration in the aquatic phase (aquarium test solution), and the internal hydrophobic site of narcosis action. A QSAR plot of log KOW vs log C for such simple non-reactive non-electrolytes exhibits a linear relationship so long as such equilibrium is established within the test duration. Beyond this point, a bilinear relationship is observed, with the most toxic chemical being the one with the highest log KOW value for which such equilibrium is established (Lipnick, 1995).

Another testing problem is posed by water solubility cut-off. If the toxic concentration required to produce the effect is above the compound's water solubility, no effect will be observed even at water saturation.

Compounds for which the predicted toxic concentration is close to water solubility will also show no effect if the test duration is insufficient to achieve equilibrium partitioning. A similar cut-off is observed for surfactants if toxicity is predicted at a concentration beyond the critical micelle concentration. Although such compounds may show no toxicity under these conditions when tested alone, their toxic contributions to mixtures are still present. For compounds with the same log KOW value, differences in water solubility reflect differences in enthalpy of fusion related to melting point. Melting point is a reflection of the degree of stability of the crystal lattice and is controlled by intermolecular hydrogen bonding, lack of conformational flexibility, and symmetry. The more highly symmetric a compound, the higher the melting point (Lipnick, 1990).

Quantitative Structure Activity Relationship modelling issues (aquatic toxicity)

Choosing an appropriate QSAR implies that the model will yield a reliable prediction for the toxicity or biological activity of an untested chemical. Generally speaking, reliability decreases with increasing complexity of chemical structure, unless a QSAR has been derived for a narrowly defined set of chemicals similar in structure to the candidate substance. QSAR models derived from narrowly defined classes of chemicals are commonly employed in the development of pharmaceuticals once a new lead compound is identified and there is a need to make minor structural modifications to optimise activity (and decrease toxicity). Overall, the objective is make estimates by interpolation rather than extrapolation.

For example, if 96-h LC₅₀ test data for fathead minnow are available for ethanol, n-butanol, n-hexanol, and n-nonanol, there is some confidence in making a prediction for this endpoint for n-propanol and n-pentanol. In contrast, there is would have less confidence in making such a prediction for methanol, which is an extrapolation, with fewer carbon atoms than any of the tested chemicals. In fact, the behaviour of the first member of such a homologous is typically the most anomalous, and should not be predicted using data from remaining members of the series. Even the toxicity of branched chain alcohols may be an unreasonable extrapolation, depending upon the endpoint in question. Such extrapolation becomes more unreliable to the

extent that toxicity is related to production of metabolites for a particular endpoint, as opposed to the properties of the parent compound. Also, if toxicity is mediated by a specific receptor binding mechanism, dramatic effects may be observed with small changes in chemical structure.

What ultimately governs the validity of such predictions is the degree to which the compounds used to derive the QSAR for a specific biological endpoint, are acting by a common molecular mechanism. In many and perhaps most cases, a QSAR does not represent such a mechanistic model, but merely a correlative one. A truly valid mechanistic model must be derived from a series of chemicals all acting by a common molecular mechanism, and fit to an equation using one or more parameters that relate directly to one or more steps of the mechanism in question. Such parameters or properties are more generally known as molecular descriptors. It is also important to keep in mind that many such molecular descriptors in common use may not have a direct physical interpretation. For a correlative model, the statistical fit of the data are likely to be poorer than a mechanistic one given these limitations. Mechanisms are not necessarily completely understood, but enough information may be known to provide confidence in this approach. For correlative models, the predictive reliability increases with the narrowness with which each is defined, for example, categories of electrophiles, such as acrylates, in which the degree of reactivity may be similar and toxicity can be estimated for a 'new' chemical using a model based solely on the log KOW parameter.

As an example, primary and secondary alcohols containing a double or triple bond that is conjugated with the hydroxyl function (that is, allylic or propargylic) are more toxic than would be predicted for a QSAR for the corresponding saturated compounds. This behaviour has been ascribed to a proelectrophile mechanism involving metabolic activation by the ubiquitous enzyme alcohol dehydrogenase to the corresponding α,β -unsaturated aldehydes and ketones that can act as electrophiles via a Michael-type acceptor mechanism (Veith et al, 1989). In the presence of an alcohol dehydrogenase inhibitor, these compounds behave like other alcohols and do not show excess toxicity, consistent with the mechanistic hypothesis.

The situation quickly becomes more complex once one goes beyond such a homologous series of compounds. Consider, for example, simple benzene derivatives. A series of chlorobenzenes may be viewed as similar to a homologous series. Not much difference is likely in the toxicities of the three isomeric dichlorobenzenes, so that a QSAR for chlorobenzenes based upon test data for one of these isomers is likely to be adequate. What about the substitution of other functional groups on benzene ring? Unlike an aliphatic alcohol, the addition of a hydroxyl functionality to a benzene ring produces a phenol that is no longer neutral, but an ionisable acidic compound, due to the resonance stabilisation of the resulting negative charge. For this reason, phenol does not act as a true narcotic agent. With the addition of electron withdrawing substituents to phenol (for example, chlorine atoms), there is a shift to these compounds acting as uncouplers of oxidative phosphorylation (for example, the herbicide dinoseb). Substitution of an aldehyde group leads to increased toxicity via an electrophile mechanism for such compounds react with amino groups, such as the lysine ϵ -amino group to produce a Schiff Base adduct. Similarly, a benzylic chloride acts as an electrophile to form covalent adducts with sulfhydryl groups. In tackling a prediction for an untested compound, the chemical reactivity of these and many other functional groups and their interaction with one

another should be carefully studied, and attempts made to document these from the chemical literature (Lipnick, 1991b).

Given these limitations in using QSARs for making predictions, it is best employed as a means of establishing testing priorities, rather than as a means of substituting for testing, unless some mechanistic information is available on the untested compound itself. In fact, the inability to make a prediction along with known environmental release and exposure may in itself be adequate to trigger testing or the development of a new QSAR for a class of chemicals for which such decisions are needed. A QSAR model can be derived by statistical analysis, for example, regression analysis, from such a data set. The most commonly employed molecular descriptor, log KOW, may be tried as a first attempt.

By contrast, derivation of a mechanism based QSAR model requires an understanding or working hypothesis of molecular mechanism and what parameter or parameters would appropriately model these actions. It is important to keep in mind that this is different from a hypothesis regarding mode of action, which relates to biological/physiological response, but not molecular mechanism.

Use of Quantitative Structure Activity Relationships in aquatic classification

The inherent properties of substances relevant for classification purposes concerning the aquatic environment are:

- the partition coefficient n-octanol-water (log KOW);
- the BCF;
- degradability – abiotic and biodegradation;
- acute aquatic toxicity for fish, Daphnia, and algae; and
- prolonged toxicity for fish and Daphnia.

Test data always take precedence over QSAR predictions, providing the test data are valid, with QSARs used for filling data gaps for purposes of classification. Since the available QSARs are of varying reliability and application range, different restrictions apply for the prediction of each of these endpoints. Nevertheless, if a tested compound belongs to a chemical class or structure type (see above) for which there is some confidence in the predictive utility of the QSAR model, it is worthwhile to compare this prediction with the experimental data, as it is not unusual to use this approach to detect some of the experimental artefacts (volatilisation, insufficient test duration to achieve equilibrium, and water solubility cut-off) in the measured data, which would mostly result in classifying substances as lower than actual toxicity.

When two or more QSARs are applicable or appear to be applicable, it is useful to compare the predictions of these various models in the same way that predicted data should be compared with measured (as discussed above). If there is no discrepancy between these models, the result provides encouragement of the validity of the predictions. Of course, it may also mean that the models were all developed using data on similar compounds and statistical methods. On the other hand, if the predictions are quite different, this result needs to be examined further. There is always the possibility that none of the models used provides a valid prediction. As a first step, the structures and properties of the chemicals used to derive each of the predictive models should be examined to determine if any models are based upon chemicals similar in both of these

respects to the one for which a prediction is needed. If one data set contains such an appropriate analogue used to derive the model, the measured value in the database for that compound vs model prediction should be tested. If the results fit well with the overall model, it is likely the most reliable one to use. Likewise, if none of the models contain test data for such an analogue, testing of the chemical in question is recommended.

Octanol-water-partition coefficient (K_{OW})

Computerised methods such as CLOGP (USEPA, 1999), LOGKOW (USEPA, 2000a) and SPARC (USEPA, 2000b) are available to calculate log K_{OW} directly from chemical structure. CLOGP and LOGKOW are based upon the addition of group contributions, while SPARC is based upon a more fundamental chemical structure algorithm. Caution should be used in using calculated values for compounds that can undergo hydrolysis in water or some other reaction, since these transformations need to be considered in the interpretation of aquatic toxicity test data for such reactive chemicals. Only SPARC can be employed in a general way for inorganic or organometallic compounds. Special methods are needed in making estimates of log KOW or aquatic toxicity for surface-active compounds, chelating compounds, and mixtures.

Values of log KOW can be calculated for pentachlorophenol and similar compounds, both for the ionised and unionised (neutral) forms. These values can potentially be calculated for certain reactive molecules (for example, benzotrichloride), but the reactivity and subsequent hydrolysis also need to be considered. Also, for such ionisable phenols, pKa is a second parameter. Specific models can be used to calculate log KOW values for organometallic compounds, but they need to be applied with caution since some of these compounds really exist in the form of ion pairs in water.

For compounds of extremely high lipophilicity, measurements up to about 6 to 6.5 can be made by shake flask, and can be extended up to about log KOW of 8 using the slow stirring approach (De Bruijn et al, 1989). Calculations are considered useful even in extrapolating beyond what can be measured by either of these methods. Of course, it should be kept in mind that if the QSAR models for toxicity, etc. are based on chemicals with lower log K_{OW} values, the prediction itself will also be an extrapolation; in fact, it is known that in the case of bioconcentration, the relationship with log K_{OW} becomes non-linear at higher values. For compounds with low log K_{OW} values, the group contribution can also be applied, but this is not very useful for hazard purposes since for such substances, particularly with negative log K_{OW} values, little if any partitioning can take place into lipophilic sites and as Overton reported, these substances produce toxicity through osmotic effects (Lipnick, 1986).

Bioconcentration factor

If experimentally determined BCF values are available, these values should be used for classification. Bioconcentration measurements must be performed using pure samples at test concentrations within water solubility, and for an adequate test duration to achieve steady state equilibrium between the aqueous concentration and that in the fish tissue. Moreover, with bioconcentration tests of extended duration, the correlation with log KOW levels off and ultimately decreases. Under environmental conditions, bioconcentration of highly lipophilic chemicals takes place by a combination of uptake from food and water, with the switch to food taking place at log KOW \approx 6. Otherwise log KOW values can be used with a QSAR

model as a predictor of the bioaccumulation potential of organic compounds. Deviations from these QSARs tend to reflect differences in the extent to which the chemicals undergo metabolism in the fish. Thus, some chemicals, such as phthalate, can bioconcentrate significantly less than predicted for this reason. Also, caution should be applied in comparing predicted BCF values with those using radiolabelled compounds, where the tissue concentration thus detected may represent a mix of parent compound and metabolites or even covalently bound parent or metabolite.

Experimental log KOW values are to be used preferentially. However, older shake flask values above 5.5 are not reliable and in many cases it is better to use some average of calculated values or to have these remeasured using the slow stirring method (de Bruijn et al, 1989). If there is reasonable doubt about the accuracy of the measured data, calculated log KOW values shall be used.

Degradability – abiotic and biodegradation

QSARs for abiotic degradation in water phases are narrowly defined linear free energy relationships (LFERs) for specific classes of chemicals and mechanisms. For example, such LFERs are available for hydrolysis of benzylic chlorides with various substituents on the aromatic ring. Such narrowly defined LFER models tend to be very reliable if the needed parameters are available for the substituent(s) in question. Photo degradation, that is, reaction with ultra-violet (UV) produced reactive species, may be extrapolated from estimates for the air compartment. While these abiotic processes do not usually result in complete degradation of organic compounds, they are frequently significant starting points, and may be rate limiting. QSARs for calculating biodegradability are either compound specific or group contribution models like the BIODEG program (Boethling et al, 1994; Hansch and Leo, 1995; Hilal et al, 1994; Howard et al, 1992; Howard and Meylan, 1992; Loonen et al, 1999; Meylan and Howard, 1995). While validated compound class specific models are very limited in their application range, the application range of group contribution models is potentially much broader, but limited to compounds containing the model substructures. Validation studies have suggested that the biodegradability predictions by currently available group contribution models may be used for prediction of 'not ready biodegradability' (Langenberg et al, 1996; Pedersen et al, 1995; USEPA, 1993) – and thus in relation to aquatic hazard classification 'not rapid degradability'.

Acute aquatic toxicity for fish, Daphnia, and algae

The acute aquatic toxicity of non-reactive, non-electrolyte organic chemicals (baseline toxicity) can be predicted from their log KOW value with a quite high level of confidence, provided the presence of electrophile, proelectrophile, or special mechanism functional groups (see above) were not detected. Problems remain for such specific toxicants, for which the appropriate QSAR has to be selected in a prospective manner. Since straightforward criteria for the identification of the relevant modes of action are still lacking, empirical expert judgement needs to be applied for selecting a suitable model. Thus, if an inappropriate QSAR is employed, the predictions may be in error by several orders of magnitude, and in the case of baseline toxicity, will be predicted less toxic, rather than more.

Prolonged toxicity for fish and Daphnia

Calculated values for chronic toxicity to fish and Daphnia should not be used to overrule classification based on experimental acute toxicity data. Only a few validated models are available for calculating prolonged toxicity for fish and Daphnia. These models are based solely on log KOW correlations and are limited in their application to non-reactive, non-electrolyte organic compounds, and are not suitable for chemicals with specific modes of action under prolonged exposure conditions. The reliable estimation of chronic toxicity values depends on the correct discrimination between non-specific and specific chronic toxicity mechanisms; otherwise, the predicted toxicity can be wrong by orders of magnitude. It should be noted that although for many compounds, excess toxicity in a chronic test correlates with excess toxicity in an acute test, this is not always the case; where:

Excess toxicity = (Predicted baseline toxicity)/Observed toxicity

19D.6 Classification of metals and metal compounds

Introduction

The harmonised system for classifying chemical substances is a hazard-based system, and the basis of the identification of hazard is the aquatic toxicity of the substances, and information on the degradation and bioaccumulation behaviour (OECD, 1998). Since this document deals only with the hazards associated with a given substance when the substance is dissolved in the water column, exposure from this source is limited by the solubility of the substance in water and bioavailability of the substance in species in the aquatic environment. Thus, the hazard classification schemes for metals and metal compounds are limited to the hazards posed by metals and metal compounds when they are available (that is, exist as dissolved metal ions, for example, as M⁺ when present as M-NO₃), and do not take into account exposures to metals and metal compounds that are not dissolved in the water column but may still be bioavailable, such as metals in foods. This section does not take into account the non-metallic ion (for example, CN⁻) of metal compounds which may be toxic or which may be organic and may pose bioaccumulation or persistence hazards. For such metal compounds the hazards of the non-metallic ions must also be considered.

The level of the metal ion that may be present in solution following the addition of the metal and/or its compounds, will largely be determined by two processes: the extent to which it can be dissolved, that is, its water solubility, and the extent to which it can react with the media to transform to water soluble forms. The rate and extent at which this latter process, known as 'transformation' for the purposes of this guidance, takes place can vary extensively between different compounds and the metal itself, and is an important factor in determining the appropriate hazard class. Where data on transformation are available, they should be taken into account in determining the classification. The protocol for determining this rate is in Appendix 19G.

Generally speaking, the rate at which a substance dissolves is not considered relevant to the determination of its intrinsic toxicity. However, for metals and many poorly soluble inorganic metal compounds, the

difficulties in achieving dissolution through normal solubilisation techniques is so severe that the two processes of solubilisation and transformation become indistinguishable. Thus, where the compound is sufficiently poorly soluble that the levels dissolved following normal attempts at solubilisation do not exceed the available L(E)C50, it is the rate and extent of transformation, which must be considered. The transformation will be affected by a number of factors, not least of which will be the properties of the media with respect to pH, water hardness, temperature, etc. In addition to these properties, other factors such as the size and specific surface area of the particles which have been tested, the length of time over which exposure to the media takes place and, of course the mass or surface area loading of the substance in the media will all play a part in determining the level of dissolved metal ions in the water. Transformation data can generally, therefore, only be considered as reliable for the purposes of classification if conducted according to the standard protocol in Appendix 19G.

This protocol aims at standardising the principal variables such that the level of dissolved ion can be directly related to the loading of the substance added. It is this loading level that yields the level of metal ion equivalent to the available L(E)C50 that can then be used to determine the hazard category appropriate for classification. The strategy to be adopted in using the data from the testing protocol, and the data requirements needed to make that strategy work, will be described.

In considering the classification of metals and metal compounds, both readily and poorly soluble, recognition has to be paid to a number of factors. As defined in section 19D.3, the term 'degradation' refers to the decomposition of organic molecules. For inorganic compounds and metals, clearly the concept of degradability, as it has been considered and used for organic substances, has limited or no meaning. Rather, the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally, the log KOW cannot be considered as a measure of the potential to accumulate. Nevertheless, the concepts that a substance, or a toxic metabolite/reaction product may not be rapidly lost from the environment and/or may bioaccumulate are as applicable to metals and metal compounds as they are to organic substances.

Speciation of the soluble form can be affected by pH, water hardness, and other variables, and may yield particular forms of the metal ion that are more or less toxic. In addition, metal ions could be made non-available from the water column by a number of processes (for example, mineralisation and partitioning). Sometimes these processes can be sufficiently rapid to be analogous to degradation in assessing chronic classification. However, partitioning of the metal ion from the water column to other environmental media does not necessarily mean that it is no longer bioavailable, nor does it mean that the metal has been made permanently unavailable.

Information pertaining to the extent of the partitioning of a metal ion from the water column, or the extent to which a metal has been or can be converted to a form that is less toxic or non-toxic is frequently not available over a sufficiently wide range of environmentally relevant conditions, and thus, a number of assumptions will need to be made as an aid in classification. These assumptions may be modified if available data show otherwise. In the first instance it should be assumed that the metal ions, once in the water, are not rapidly partitioned from the water column and thus these compounds do not meet the criteria.

Underlying this is the assumption that, although speciation can occur, the species will remain available under environmentally relevant conditions. This may not always be the case, as described above, and any evidence available that would suggest changes to the bioavailability over the course of 28 days, should be carefully examined. The bioaccumulation of metals and inorganic metal compounds is a complex process and bioaccumulation data should be used with care. The application of bioaccumulation criteria will need to be considered on a case-by-case basis taking due account of all the available data.

A further assumption that can be made, which represents a cautious approach, is that, in the absence of any solubility data for a particular metal compound, either measured or calculated, the substance will be sufficiently soluble to cause toxicity at the level of the L(E)C50, and thus may be classified in the same way as other soluble salts. Again, this is clearly not always the case, and it may be wise to generate appropriate solubility data.

This section deals with metals and metal compounds. Within the context of this guidance document, metals and metal compounds are characterised as follows, and therefore, organo-metals are outside the scope of this section.

- Metals, M0, in their elemental state are not soluble in water but may transform to yield the available form. This means that a metal in the elemental state may react with water or a dilute aqueous electrolyte to form soluble cationic or anionic products, and in the process the metal will oxidise, or transform, from the neutral or zero oxidation state to a higher one.
- In a simple metal compound, such as an oxide or sulphide, the metal already exists in the oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium. However, while oxidation may not change, interaction with the media may yield more soluble forms. A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield a small amount of the available form by dissolution. However, it should be recognised that the final solution concentration may be influenced by a number of factors, including the solubility product of some metal compounds precipitated during the transformation/dissolution test, for example, aluminium hydroxide.

Application of aquatic toxicity data and solubility data for classification of metals and metal compounds

Interpretation of aquatic toxicity data

Aquatic toxicity studies carried out according to a recognised protocol should normally be acceptable as valid for the purposes of classification. Section A9.3 should also be consulted for generic issues that are common to assessing any aquatic toxicity data point for the purposes of classification.

Metal complexation and speciation

The toxicity of a particular metal in solution, appears to depend primarily on (but is not strictly limited to) the level of dissolved free metal ions. Abiotic factors including alkalinity, ionic strength and pH can influence the toxicity of metals in two ways. By influencing the:

- chemical speciation of the metal in water (and hence affecting the availability); and
- the uptake and binding of available metal by biological tissues.

Where speciation is important, it may be possible to model the concentrations of the different forms of the metal, including those that are likely to cause toxicity. Analysis methods for quantifying exposure concentrations, which are capable of distinguishing between the complexed and uncomplexed fractions of a test substance, may not always be available or economic.

Complexation of metals to organic and inorganic ligands in test media and natural environments can be estimated from metal speciation models. Speciation models for metals, including pH, hardness, DOC, and inorganic substances such as MINTEQA2 (Brown and Allison, 1987), WHAM (Tipping, 1994) and CHESS (Santore and Driscoll, 1995) can be used to calculate the uncomplexed and complexed fractions of the metal ions. Alternatively, the Biotic Ligand Model (BLM), allows for the calculation of the concentration of metal ion responsible for the toxic effect at the level of the organism. The BLM model has at present only been validated for a limited number of metals, organisms, and end-points (Santore et al, 1999). The models and formula used for the characterisation of metal complexation in the media should always be clearly reported, allowing for their translation back to natural environments (OECD, 2000).

Interpretation of solubility data

When considering the available data on solubility, their validity and applicability to the identification of the hazard of metal compounds should be assessed. In particular, the pH at which the data were generated should be known.

Assessment of existing data

Existing data will be in one of three forms. For some well-studied metals, there will be solubility products and/or solubility data for the various inorganic metal compounds. It is also possible that the pH relationship of the solubility will be known. However, for many metals or metal compounds, it is probable that the available information will be descriptive only, for example, poorly soluble. Unfortunately there appears to be very little (consistent) guidance about the solubility ranges for such descriptive terms. Where these are the only information available it is probable that solubility data will need to be generated using the Transformation/Dissolution Protocol (see Appendix 19G).

Screening test for assessing solubility of metal compounds

In the absence of solubility data, a simple 'screening test' for assessing solubility, based on the high rate of loading for 24 h, can be used for metal compounds as described in the Transformation/Dissolution Protocol. The function of the screening test is to identify those metal compounds that undergo either dissolution or rapid transformation such that they are indistinguishable from soluble forms, and hence may be classified based on the dissolved ion concentration. Where data are available from the screening test detailed in the Transformation/Dissolution Protocol, the maximum solubility obtained over the tested pH range should be used. Where data are not available over the full pH range, a check should be made that this maximum

solubility has been achieved by reference to suitable thermodynamic speciation models or other suitable methods. It should be noted that this test is only intended to be used for metal compounds.

Full test for assessing solubility of metals and metal compounds

The first step in this part of the study is, as with the screening test, an assessment of the pH(s) at which the study should be conducted. Normally, the Full Test should have been carried out at the pH that maximises the concentration of dissolved metal ions in solution. In such cases, the pH may be chosen following the same guidance as given for the screening test. Based on the data from the Full Test, it is possible to generate a concentration of the metal ions in solution after 7 days for each of the three loadings (that is, 1 mg/L as 'low', 10 mg/L as 'medium', and 100 mg/L as 'high') used in the test. If the purpose of the test is to assess the long-term hazard of the substance, then the test at the low loading may be extended to 28 days, at an appropriate pH.

Comparison of aquatic toxicity data and solubility data

A decision whether or not the substance is classified will be made by comparing aquatic toxicity data and solubility data. If the L(E)C50 is exceeded, irrespective of whether the toxicity and dissolution data are at the same pH and if this is the only data available then the substance should be classified. If other solubility data are available to show that the dissolution concentration would not exceed the L(E)C50 across the entire pH range then the substance should not be classified on its soluble form. This may involve the use of additional data either from ecotoxicological testing or from applicable bioavailability effect models.

Assessment of environmental transformation

Environmental transformation of one species of a metal to another species of the same does not constitute degradation as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. However as a result of naturally occurring geochemical processes metal ions can partition from the water column. Data on water column residence time, the processes involved at the water – sediment interface (that is, deposition and re-mobilisation) are fairly extensive, but have not been integrated into a meaningful database. Nevertheless, using the principles and assumptions discussed above, it may be possible to incorporate this approach into classification.

Such assessments are very difficult to give guidance for and will normally be addressed on a case by case approach. However, the following may be taken into account.

- Changes in speciation if they are to non-available forms. However, the potential for the reverse change to occur must also be considered.
- Changes to a metal compound that is considerably less soluble than that of the metal compound being considered.

Bioaccumulation

While log K_{OW} is a good predictor of BCF for certain types of organic compounds for example, non-polar organic substances, it is of course irrelevant for inorganic substances such as inorganic metal compounds.

The mechanisms for uptake and depuration rates of metals are very complex and variable and there is at present no general model to describe this. Instead the bioaccumulation of metals according to the classification criteria should be evaluated on a case-by-case basis using expert judgement.

While BCFs are indicative of the potential for bioaccumulation there may be a number of complications in interpreting measured BCF values for metals and inorganic metal compounds. For some metals and inorganic metal compounds there is an inverse relationship between water concentration and BCF in some aquatic organisms, and bioconcentration data should be used with care. This is particularly relevant for metals that are biologically essential. Metals that are biologically essential are actively regulated in organisms in which the metal is essential. Since nutritional requirement of the organisms can be higher than the environmental concentration, this active regulation can result in high BCFs and an inverse relationship between BCFs and the concentration of the metal in water. When environmental concentrations are low, high BCFs may be expected as a natural consequence of metal uptake to meet nutritional requirements and in these instances can be viewed as a normal phenomenon. Additionally, if internal concentration is regulated by the organism, then measured BCFs may decline as external concentration increases. When external concentrations are so high that they exceed a threshold level or overwhelm the regulatory mechanism this can cause harm to the organism. Also, while a metal may be essential in a particular organism, it may not be essential in other organisms. Therefore, where the metal is not essential or when the bioconcentration of an essential metal is above nutritional levels special consideration should be given to the potential for bioconcentration and environmental concern.

Application of classification criteria to metals and metal compounds

Introduction to the classification strategy for metals and metal compounds

The schemes for the classification of metals and metal compounds are described below and summarised diagrammatically in Figure 19D.1. There are several stages in these schemes where data are used for decision purposes. It is not the intention of the classification schemes to generate new data. In the absence of valid data, it will be necessary to use all available data and expert judgement. In the following sections, the reference to the L(E)C50 refers to the data point(s) that will be used to assign the classification for the metal or metal compound.

When considering L(E)C50 data for metal compounds, it is important to ensure that the data point to be used as the justification for the classification is expressed in the weight of the molecule of the metal compound to be classified. This is known as correcting for molecular weight. Thus while most metal data is expressed in, for example, mg/L of the metal, this value will need to be adjusted to the corresponding weight of the metal compound. Thus:

L(E)C50 metal compound =

$$\text{L(E)C50 of metal} \times \frac{\text{Molecular weight of metal compound}}{\text{Atomic weight of metal}}$$

Chronic NOEC data may also need to be adjusted to the corresponding weight of the metal compounds.

Classification strategy for metals

Where the L(E)C₅₀ for the metal ions of concern is > 100 mg/L, the metals need not be considered further in the classification scheme.

Where the L(E)C₅₀ for the metal ions of concern is \leq 100 mg/L, consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Appendix 19G).

Where such data are unavailable, that is, there is no clear data of sufficient validity to show that the transformation to metal ions will not occur, the safety net classification (9.1D) should be applied since the known classifiable toxicity of these soluble forms is considered to produce sufficient concern.

Where data from dissolution protocol are available, then, the results should be used to aid classification according to the following rules.

Seven-day transformation test

If the dissolved metal ion concentration after a period of 7 days (or earlier) exceeds that of the L(E)C₅₀, then the default classification for the metals is replaced by the following classification.

- If the dissolved metal ion concentration at the low loading rate is greater than or equal to the L(E)C₅₀, then classify as 9.1A.
- If the dissolved metal ion concentration at the medium loading rate is greater than or equal to the L(E)C₅₀, then classify 9.1B unless there is evidence of both rapid partitioning from the water column and no bioaccumulation, in which case classify as 9.1D.
- If the dissolved metal ion concentration at the high loading rate is greater than or equal to the L(E)C₅₀, then classify as 9.1C unless there is evidence of both rapid partitioning from the water column and no bioaccumulation, in which case classify as 9.1D.

Twenty-eight-day transformation test

If the process described in for the first step of the 7-day transformation test results in the classification of 9.1A, no further assessment is required, as the metal will be classified irrespective of any further information.

In all other cases, further data may have been generated through the transformation test in order to show that the classification may be amended. If for substances classified as 9.1B, 9.1C, or 9.1D the dissolved metal ion concentration at the low loading rate after a total period of 28 days is less than or equal to the of the long-term NOECs, then the classification is removed.

Classification strategy for metal compounds

Where the L(E)C₅₀ for the metal ions of concern is greater than 100 mg/L, the metal compounds need not be considered further in the classification scheme.

If solubility > L(E)C₅₀, classify on the basis of soluble ion

All metal compounds with a water solubility (either measured for example, through 24-hour Dissolution Screening test or estimated for example, from the solubility product) greater or equal to the L(E)C₅₀ of the dissolved metal ion concentration are considered as readily soluble metal compounds. Care should be exercised for compounds whose solubility is close to the acute toxicity value as the conditions under which solubility is measured could differ significantly from those of the acute toxicity test. In these cases the results of the Dissolution Screening Test are preferred.

Readily soluble metal compounds are classified on the basis of the L(E)C₅₀ (corrected where necessary for molecular weight).

- If the L(E)C₅₀ of the dissolved metal ion is ≥ 1 mg/L, then classify as 9.1A.
- If the L(E)C₅₀ of the dissolved metal ion is > 1 mg/L and ≤ 10 mg/L, then classify as 9.1B unless there is evidence of both rapid partitioning from the water column and no bioaccumulation, in which case classify as 9.1D.
- If the L(E)C₅₀ of the dissolved metal ion is > 10 mg/L and ≤ 100 mg/L, then classify as 9.1C unless there is evidence of both rapid partitioning from the water column and no bioaccumulation in which case classify as 9.1D.

If solubility < L(E)C₅₀, classify default 9.1D

In the context of the classification criteria, poorly soluble compounds of metals are defined as those with a known solubility (either measured for example, through 24-hour Dissolution Screening test or estimated for example, from the solubility product) less than the L(E)C₅₀ of the soluble metal ion. In those cases when the soluble forms of the metal of poorly soluble metal compounds have a L(E)C₅₀ less than or equal to 100 mg/L and the substance can be considered as poorly soluble the default safety net classification (9.1D) should be applied.

Seven-day transformation test

For poorly soluble metal compounds classified with the default safety net classification further information that may be available from the 7-day transformation/dissolution test can also be used. Such data should include transformation levels at low, medium and high loading levels. If the dissolved metal ion concentration after a period of 7 days (or earlier) exceeds that of the L(E)C₅₀, then the default classification for the metals is replaced by the following classification.

- If the dissolved metal ion concentration at the low loading rate is \geq L(E)C₅₀, then classify as 9.1A.
- If the dissolved metal ion concentration at the medium loading is \geq L(E)C₅₀, then classify as 9.1B unless there is evidence of both rapid partitioning from the water column and no bioaccumulation, in which case classify as 9.1D.
- If the dissolved metal ion concentration at the high loading rate is \geq L(E)C₅₀, then classify 9.1C unless there is evidence of both rapid partitioning from the water column and no bioaccumulation, in which case classify as 9.1D.

Twenty-eight-day transformation test

If the process described in for the 7-day transformation test results in the classification of 9.1A, no further assessment is required as the metal compound will be classified irrespective of any further information. In all other cases, further data may have been generated through the dissolution/transformation test for 28 days in order to show that the classification may be amended. If for poorly soluble metal compounds classified as 9.1B or 9.1C or 9.1D, the dissolved metal ion concentration at the low loading rate after a total period of 28 days is less than or equal to the long-term NOECs, then classification is removed.

Particle size and surface area

Particle size, or moreover surface area, is a crucial parameter in that any variation in the size or surface area tested may cause a significant change in the levels of metals ions released in a given time window. Thus, this particle size or surface area is fixed for the purposes of the transformation test, allowing the comparative classifications to be based solely on the loading level. Normally, the classification data would have used the smallest particle size marketed to determine the extent of transformation. There may be cases where data generated for a particular metal powder is not considered as suitable for classification of the massive forms. For example, where it can be shown that the tested powder is structurally a different material (for example, different crystallographic structure) and/or it has been produced by a special process and cannot be generated from the massive metal, classification of the massive can be based on testing of a more representative particle size or surface area, if such data are available. The powder may be classified separately based on the data generated on the powder. However, in normal circumstances it is not anticipated that more than two classification proposals would be made for the same metal.

Metals with a particle size smaller than the default diameter value of 1 mm can be tested on a case-by-case basis. One example of this is where metal powders are produced by a different production technique or where the powders give rise to a higher dissolution (or reaction) rate than the massive form leading to a more stringent classification.

The particle sizes tested depend on the substance being assessed and are shown in Table 19D.3.

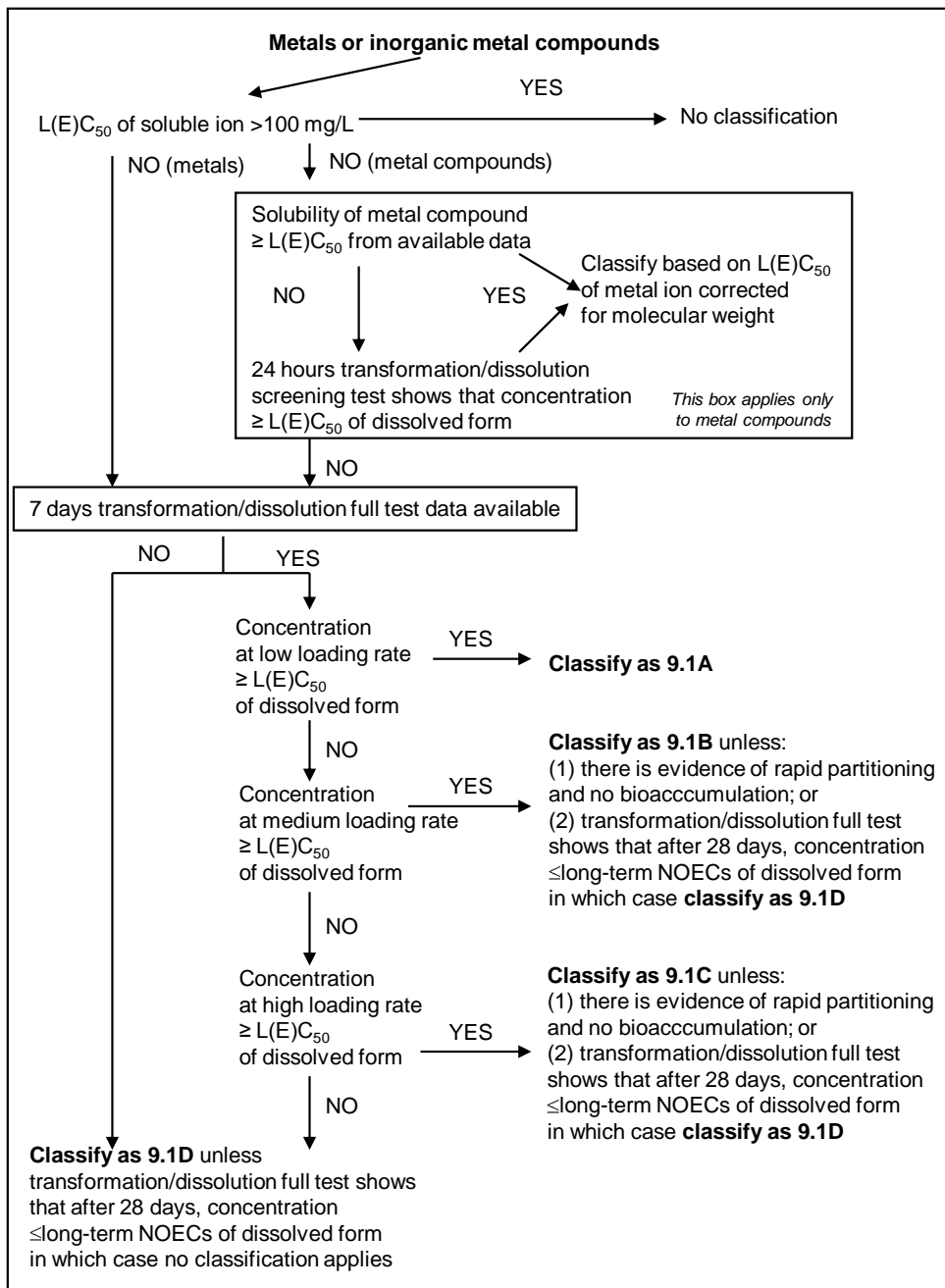
Table 19D.3: Particle sizes tested depend on the substance being assessed

| Type | Particle size | Comments |
|------------------|-----------------------------------|--|
| Metal compounds | Smallest representative size sold | Never larger than 1 mm |
| Metals – powders | Smallest representative size sold | May need to consider different sources if yielding different crystallographic/morphologic properties |
| Metals – massive | 1 mm | Default value may be altered if sufficient justification |

For some forms of metals, it may be possible, using the Transformation/Dissolution Protocol (see Appendix 19G), to obtain a correlation between the concentration of the metal ion after a specified time interval as a function of the surface area loadings of the forms tested. In such cases, it could then be possible to estimate

the level of dissolved metal ion concentration of the metal with different particles, using the critical surface area approach as proposed by Skeaff et al (2000). That is, from this correlation and a linkage to the appropriate toxicity data, it may be possible to determine a critical surface area of the substance that delivers the $L(E)C_{50}$ to the medium and then to convert the critical surface area to the low, medium and high mass loadings used in hazard identification. While this approach is not normally used for classification it may provide useful information for labelling and downstream decisions.

Figure 19D.1: Decision scheme for aquatic classification of metals and inorganic metal compounds



Note: $L(E)C_{50}$ = median lethal concentration or median effect concentration; NOEC = no observable effect concentration.

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Appendix 19E: Degradation of organic substances

19E.1 Introduction

The following appendix is an extract from the UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) guidance document Annex 9 (Appendices I and II) (United Nations, 2007) with minor changes where necessary to refer to the Hazardous Substances and New Organisms Act 1996 (HSNO Act) criteria.

19E.2 Principles

Introduction

Organic substances may be degraded by abiotic or biotic processes or by a combination of these. A number of standard procedures or tests for determination of the degradability are available. The general principles of some of these are described below. It is by no way the intention to present a comprehensive review of degradability test methods, but only to place the methods in the context of aquatic hazard classification.

Abiotic degradability

Abiotic degradation comprises chemical transformation and photochemical transformation. Usually abiotic transformations will yield other organic compounds but will not cause a full mineralisation (Schwarzenbach et al, 1993). Chemical transformation is defined as transformation that happens without light and without the mediation of organisms whereas photochemical transformations require light.

Examples of relevant chemical transformation processes in aqueous environment are hydrolysis, nucleophilic substitution, elimination, oxidation and reduction reactions (Schwarzenbach et al, 1993). Of these, hydrolysis is often considered the most important and it is the only chemical transformation process for which international test guidelines are generally available. The tests for abiotic degradation of chemicals are generally in the form of determination of transformation rates under standardised conditions.

Hydrolysis

Hydrolysis is the reaction of the nucleophiles H₂O or OH⁻ with a chemical where a (leaving) group of the chemical is exchanged with an OH group. Many compounds, especially acid derivatives, are susceptible to hydrolysis. Hydrolysis can both be abiotic and biotic, but in regard to testing only abiotic hydrolysis is considered. Hydrolysis can take place by different mechanisms at different pHs, neutral, acid or base-catalysed hydrolysis, and hydrolysis rates may be very dependent on pH.

Currently two guidelines for evaluating abiotic hydrolysis are available, the Organisation for Economic Co-operation and Development (OECD) Test Guideline 111 Hydrolysis as a function of pH (corresponding to Office of Prevention, Pesticides and Toxic Substances (OPPTS) 835.2110) and OPPTS 835.2130 Hydrolysis as a function of pH and temperature. In OECD Test Guideline 111, the overall hydrolysis rate at different pHs in pure buffered water is determined. The test is divided in two, a preliminary test that is performed for

chemicals with unknown hydrolysis rates and a more detailed test that is performed for chemicals that are known to be hydrolytically unstable and for chemicals for which the preliminary test shows fast hydrolysis. In the preliminary test the concentration of the chemical in buffered solutions at pHs in the range normally found in the environment (pHs of 4, 7 and 9) at 50°C is measured after 5 days. If the concentration of the chemical has decreased less than 10% it is considered hydrolytically stable, otherwise the detailed test may be performed. In the detailed test, the overall hydrolysis rate is determined at three pHs (4, 7 and 9) by measuring the concentration of the chemical as a function of time. The hydrolysis rate is determined at different temperatures so that interpolations or extrapolations to environmentally relevant temperatures can be made. The OPPTS 835.2130 test is almost identical in design to the OECD Test Guideline 111, the difference mainly being in the treatment of data.

It should be noted that apart from hydrolysis the hydrolysis rate constants determined by the tests include all other abiotic transformations that may occur without light under the given test conditions. Good agreement has been found between hydrolysis rates in natural and in pure waters (OPPTS 835.2110).

Photolysis

At present, there is a draft OECD guideline on aqueous photodegradation, and a guidance document, concerning aquatic direct photolysis, is available (OECD, 1997). The guidance document is supposed to form the basis for the scheduled guideline. According to the definitions set out in this guidance document, phototransformation of compounds in water can be in the form of primary or secondary phototransformation, where the primary phototransformation (photolysis) can be divided further into direct and indirect photolysis. Direct phototransformation (photolysis) is the case where the chemical absorbs light and as a direct result hereof undergoes transformation. Indirect phototransformation is the case where other excited species transfer energy, electrons or H-atoms to the chemical and thereby induces a transformation (sensitised photolysis). Secondary phototransformation is the case where chemical reactions occur between the chemical and reactive short lived species like hydroxy radicals, peroxy radicals or singlet oxygen that are formed in the presence of light by reactions of excited species like excited humic or fulvic acids or nitrate.

The only currently available guidelines on phototransformation of chemicals in water are therefore OPPTS 835.2210 Direct photolysis rate in water by sunlight and OPPTS 835.5270 Indirect photolysis screening test. The OPPTS 835.2210 test uses a tiered approach. In Tier 1 the maximum direct photolysis rate constant (minimum half-life) is calculated from a measured molar absorptivity. In Tier 2 there are two phases. In Phase 1 the chemical is photolysed with sunlight and an approximate rate constant is obtained. In Phase 2, a more accurate rate constant is determined by using an actinometer that quantifies the intensity of the light that the chemical has actually been exposed to. From the parameters measured, the actual direct photodegradation rate at different temperatures and for different latitudes can be calculated. This degradation rate will only apply to the uppermost layer of a water body, for example, the first 50 cm or less and only when the water is pure and air saturated which may clearly not be the case in environment. However, the results can be extended over other environmental conditions by the use of a computer program incorporating attenuation in natural waters and other relevant factors.

The OPPTS 835.5270 screening test concerns indirect photolysis of chemicals in waters that contain humic substances. The principle of the test is that in natural waters exposed to natural sunlight a measured phototransformation rate will include both direct and indirect phototransformation, whereas only direct phototransformation will take place in pure water. Therefore, the difference between the direct photodegradation rate in pure water and the total photodegradation in natural water is the sum of indirect photolysis and secondary photodegradation. In the practical application of the test, commercial humic substances are used to make up a synthetic humic water, which mimics a natural water. It should be noted that the indirect phototransformation rate determined is only valid for the season and latitude for which it is determined and it is not possible to transfer the results to other latitudes and seasons.

Biotic degradability

Only a brief overview of the test methods is given below. For more information, consult the comprehensive paper Detailed Review Paper on Biodegradability Testing (OECD, 1995).

Ready biodegradability

Standard tests for determination of the ready biodegradability of organic substances are developed by a number of organisations including OECD (OECD Test Guidelines 301A-F), European Union (EU) (C.4 tests), OPPTS (835.3110) and International Organization for Standardization (ISO) (9408, 9439, 10707).

The ready biodegradability tests are stringent tests, which provide limited opportunity for biodegradation and acclimatisation to occur. The basic test conditions ensuring these specifications are:

- high concentration of test substance (2–100 mg/L);
- the test substance is the sole carbon and energy source;
- low to medium concentration of inoculum (10⁴–10⁸ cells/mL);
- no pre-adaptation of inoculum is allowed;
- 28-day test period with a 10-days time window (except for the MITI I method (OECD Test Guideline 301C)) for degradation to take place;
- test temperature < 25°C; and
- pass levels of 70% (DOC removal) or 60% (O₂ demand or CO₂ evolution) demonstrating complete mineralisation (as the remaining carbon of the test substance is assumed to be built into the growing biomass).

It is assumed that a positive result in one of the ready biodegradability tests demonstrates that the substance will degrade rapidly in the environment (OECD 301 Test Guidelines).

Also the traditional BOD₅ tests (for example, the EU C.5 test) may demonstrate whether a substance is readily biodegradable. In this test, the relative biochemical oxygen demand in a period of 5 days is compared to the theoretical oxygen demand (ThOD) or, when this is not available, the chemical oxygen demand (COD). The test is completed within 5 days and consequently, the pass level defined in the proposed hazard classification criteria at 50% is lower than in the ready biodegradability tests.

The screening test for biodegradability in seawater (OECD Test Guideline 306) may be seen as seawater parallel to the ready biodegradability tests. Substances that reach the pass level in OECD Test Guideline 306 (that is, > 70% DOC removal or > 60 theoretical oxygen demand) may be regarded as readily biodegradable, since the degradation potential is normally lower in seawater than in the freshwater degradation tests.

Inherent biodegradability

Tests for inherent biodegradability are designed to assess whether a substance has any potential for biodegradation. Examples of such tests are the OECD Test Guidelines 302A-C tests, the EU C.9 and C.12 tests, and the ASTM E 1625-94 test.

The basic test conditions favouring an assessment of the inherent biodegradation potential are a:

- prolonged exposure of the test substance to the inoculum allowing adaptation within the test period;
- high concentration of micro-organisms; and
- favourable substance/biomass ratio.

A positive result in an inherent test indicates that the test substance will not persist indefinitely in the environment, however a rapid and complete biodegradation can not be assumed. A result demonstrating more than 70% mineralisation indicates a potential for ultimate biodegradation, a degradation of more than 20% indicates inherent, primary biodegradation, and a result of less than 20% indicates that the substance is persistent. Thus, a negative result means that non-biodegradability (persistence) should be assumed.

In many inherent biodegradability tests only the disappearance of the test substance is measured. Such a result only demonstrates a primary biodegradability and not a total mineralisation. Thus, more or less persistent degradation products may have been formed. Primary biodegradation of a substance is no indication of ultimate degradability in the environment.

The OECD inherent biodegradation tests are very different in their approach and especially, the MITI II test (OECD Test Guideline 302C) employs a concentration of inoculum that is only three times higher than in the corresponding MITI I ready biodegradability test (OECD Test Guideline 301C). Also the Zahn-Wellens test (OECD Test Guideline 302B) is a relatively 'weak' inherent test. However, although the degradation potential in these tests is not very much stronger than in the ready biodegradability tests, the results can not be extrapolated to conditions in the ready biodegradability tests and in the aquatic environment.

Aquatic simulation tests

A simulation test attempts to simulate biodegradation in a specific aquatic environment. As examples of a standard test for simulation of degradation in the aquatic environment may be mentioned the ISO/DS14592 Shake flask batch test with surface water or surface water/sediment suspensions (Nyholm and Toräng, 1999), the ASTM E 1279-89(95) test on biodegradation by a shake-flask die-away method and the similar OPPTS 835.3170 test. Such test methods are often referred to as river die-away tests.

The features of the tests that ensure simulation of the conditions in the aquatic environment are:

- use of a natural water (and sediment) sample as inoculum; and

- low concentration of test substance (1–100 µg/L) ensuring first-order degradation kinetics.

The use of a radiolabelled test compound is recommended as this facilitates the determination of the ultimate degradation. If only the removal of the test substance by chemical analysis is determined, only the primary degradability is determined. From observation of the degradation kinetics, the rate constant for the degradation can be derived. Due to the low concentration of the test substance, first order degradation kinetics are assumed to prevail.

The test may also be conducted with natural sediment simulating the conditions in the sediment compartment. Moreover, by sterilising the samples, the abiotic degradation under the test conditions can be determined.

STP simulation tests

Tests are also available for simulating the degradability in a sewage treatment plant (STP), for example, the OECD Test Guideline 303A Coupled Unit test, ISO 11733 Activated sludge simulation test, and the EU C.10 test. Recently, a new simulation test employing low concentrations of organic pollutants has been proposed (Nyholm et al, 1996).

STP simulation tests are not relevant to classifying substances under the HSNO Act as the test conditions are too dissimilar to the natural environment.

Anaerobic degradability

Test methods for anaerobic biodegradability determine the intrinsic potential of the test substance to undergo biodegradation under anaerobic conditions. Examples of such tests are the ISO11734:1995(E) test, ASTM E 1196-92 test, and OPPTS 835.3400 test.

The potential for anaerobic degradation is determined during a period of up to eight weeks and with the test conditions indicated below:

- performance of the test in sealed vessels in the absence of O₂ (initially in a pure N₂ atmosphere);
- use of digested sludge;
- a test temperature of 35°C; and
- determination of head-space gas pressure (CO₂ and CH₄ formation).

The ultimate degradation is determined by determining the gas production. However, also primary degradation may be determined by measuring the remaining parent substance.

Anaerobic degradability tests are not relevant to classifying substances under the HSNO Act as organisms of concern generally live in aerobic conditions.

Degradation in soil and sediment

Many chemical substances end up in the soil or sediment compartments and an assessment of their degradability in these environments may therefore be of importance.

OECD guidelines are available on aerobic and anaerobic transformation in soil (OECD 307 (2002)) and in aquatic sediment systems (OECD 308), respectively. The experiments are performed to determine the rate of transformation of the test substance and the nature and rates of formation and decline of transformation products under environmentally realistic conditions including a realistic concentration of the test substance. Either complete mineralisation or primary degradability may be determined depending on the analytical method employed for determining the transformation of the test substance.

Standard methods for inherent degradability in soil include the OECD Test Guideline 304A, which corresponds to the OPPTS 835.3300 test.

The special test characteristics ensuring the determination of the inherent degradability in soil are:

- natural soil samples are used without additional inoculation;
- radiolabelled test substance is used; and
- evolution of radiolabelled CO₂ is determined.

A standard method for determining the biodegradation in sediment is the OPPTS 835.3180 Sediment/water microcosm biodegradation test. Microcosms containing sediment and water are collected from test sites and test compounds are introduced into the system. Disappearance of the parent compound (that is, primary biodegradation) and, if feasible, appearance of metabolites or measurements of ultimate biodegradation may be made.

Methods for estimating biodegradability

In recent years, possibilities for estimating environmental properties of chemical substances have been developed and, among these, also methods for predicting the biodegradability potential of organic substances (for example, the Syracuse Research Corporation's Biodegradability Probability Program, BLOWIN). Reviews of methods have been performed by OECD (1993) and by Langenberg et al (1996). They show that group contribution methods seem to be the most successful methods. Of these, the BLOWIN seems to have the broadest application. It gives a qualitative estimate of the probability of slow or fast biodegradation in the presence of a mixed population of environmental microorganisms. The applicability of this program has been evaluated by the United States Environmental Protection Agency/European Commission (USEPA/EC) Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships ((Q)SARs) (OECD, 1994), and by Pedersen et al (1995). The latter is briefly referred to below.

A validation set of experimentally determined biodegradation data was selected among the data from MITI (1992), but excluding substances for which no precise degradation data were available and substances already used for development of the program. The validation set then consisted of 304 substances. The biodegradability of these substances were estimated by use of the program's non-linear estimation module (the most reliable) and the results compared with the measured data. One hundred and sixty-two substances were predicted to degrade 'fast', but only 41 (25%) were actually readily degradable in the MITI I test. One hundred and forty-two substances were predicted to degrade 'slowly', which was confirmed by 138 (97%) substances being not readily degradable in the MITI I test. Thus, it was concluded that the program may be used for classification purposes only when no experimental degradation data can be obtained, and when the

program predicts a substance to be degraded 'slowly'. In this case, the substance can be regarded as not rapidly degradable.

The same conclusion was reached in the US EPA/EC Joint Project on the Evaluation of (Q)SARs by use of experimental and QSAR data on new substances in the EU. The evaluation was based on an analysis of QSAR predictions on 115 new substances also tested experimentally in ready biodegradability tests. Only 9 of the substances included in this analysis were readily biodegradable. The employed QSAR methodology is not fully specified in the final report of the joint US EPA/EC project (OECD, 1994), but it is likely that the majority of predictions were made by using methods which later have been integrated in the Biodegradation Probability Program.

Also in the EU technical guidance document (EC, 2003) it is recommended that estimated biodegradability by use of the Biodegradation Probability Program is used only in a conservative way, that is, when the program predicts fast biodegradation, this result should not be taken into consideration, whereas predictions of slow biodegradation may be considered.

Thus, the use of results of the Biodegradability Probability Program in a conservative way may fulfil the needs for evaluating biodegradability of some of the large number of substances for which no experimental degradation data are available.

19E.3 Factors influencing degradability in the aquatic environment

Introduction

Interpretation of test results on biodegradability of organic substances has been considered in Detailed Review Paper on Biodegradability Testing (OECD, 1995).

The conditions in the environment are typically very different from the conditions in the standardised test systems, which make the extrapolation of degradation data from laboratory tests to the environment difficult. Among the differences, the following have significant influence on the degradability:

- organism-related factors (presence of competent micro-organisms);
- substrate-related factors (concentration of the substance and presence of other substrates); and
- environment-related factors (physico-chemical conditions, presence of nutrients, bioavailability of the substance).

These aspects are discussed further below.

Presence of competent micro-organisms

Biodegradation in the aquatic environment is dependent on the presence of competent microorganisms in sufficient numbers. The natural microbial communities consist of a very diverse biomass and when a 'new' substance is introduced in a sufficiently high concentration, the biomass may be adapted to degrade this substance. Frequently, the adaptation of the microbial population is caused by the growth of specific

degraders that by nature are competent to degrade the substance. However, also other processes as enzyme induction, exchange of genetic material and development of tolerance to toxicity may be involved.

Adaptation takes place during a 'lag' phase, which is the time period from the onset of the exposure until a significant degradation begins. It seems obvious that the length of the lag phase will depend on the initial presence of competent degraders. This will again depend on the history of the microbial community, that is, whether the community formerly has been exposed to the substance. This means that when a xenobiotic substance has been used and emitted ubiquitously in a number of years, the likelihood of finding competent degraders will increase. This will especially be the case in environments receiving emissions for example, biological wastewater treatment plants. Often more consistent degradation results are found in tests where inocula from polluted waters are used compared to tests with inocula from unpolluted water (Nyholm and Ingerslev, 1997; OECD, 1995).

A number of factors determine whether the potential for adaptation in the aquatic environment is comparable with the potential in laboratory tests. Among other things adaptation depends on:

- initial number of competent degraders in the biomass (fraction and number);
- presence of surfaces for attachment;
- concentration and availability of substrate; and
- presence of other substrates.

The length of the lag phase depends on the initial number of competent degraders and, for toxic substances, the survival and recovery of these. In standard ready biodegradability tests, the inoculum is sampled in sewage treatment plants. As the load with pollutants is normally higher than in the environment, both the fraction and the number of competent degraders may be higher than in the less polluted aquatic environment. It is, however, difficult to estimate how much longer the lag phase will be in the aquatic environment than in a laboratory test due to the likely lower initial number of competent degraders.

Over long periods, the initial concentration of competent degraders is not important as they will grow up when a suitable substrate is present in sufficient concentrations. However, if the degradability in a short period is of concern, the initial concentration of competent degrading microorganisms should be considered (Scow, 1982).

The presence of flocs, aggregates, and attached micro-organisms may also enhance adaptation by, for example, the development of microbial niches with consortia of micro-organisms. This is of importance when considering the capability of adaptation in the diverse environments in sewage treatment plants or in sediment or soil. However, the total number of micro-organisms in ready biodegradability tests and in the aquatic environment are of the same orders of magnitude (10⁴–10⁸ cells/mL in ready biodegradability tests and 10⁵–10⁶ cells/mL or more in surface water (Scow, 1982). Thus, this factor is probably of minor importance.

When discussing the extrapolation to environmental conditions it may be valuable to discriminate between oligotrophic and eutrophic environments. Micro-organisms thriving under oligotrophic conditions are able to mineralise organic substrates at low concentrations (fractions of mg C/L), and they normally have a greater

affinity for the substrate but lower growth rates and higher generation times than eutrophic organisms (OECD, 1995). Moreover, oligotrophs are unable to degrade chemicals in concentrations higher than 1 mg/L and may even be inhibited at high concentrations. Opposite to that, eutrophs require higher substrate concentrations before mineralisation begins and they thrive at higher concentrations than oligotrophs. Thus, the lower threshold limit for degradation in the aquatic environment will depend on whether the microbial population is an oligotroph or an eutroph population. It is, however, not clear whether oligotrophs and eutrophs are different species or whether there is only an oligotrophic and an eutrophic way of life (OECD, 1995). Most pollutants reach the aquatic environment directly through discharge of wastewater and consequently, these recipients are mostly eutrophic.

From the above discussion it may thus be concluded that the chance of presence of competent degraders is greatest in highly exposed environments, that is, in environments continuously receiving substances (which more frequently occurs for high production volume chemicals than for low production volume chemicals). These environments are often eutrophic and therefore, the degradation may require relatively high concentrations of substances before onset. On the other hand, in pristine waters competent species may be lacking, especially species capable of degradation of chemicals only occasionally released as low production volume chemicals.

Substrate-related factors

Concentration of test substance

In most laboratory tests, the test substance is applied in very high concentrations (2–100 mg/L) compared to the concentrations in the lower $\mu\text{g/L}$ range that may be expected in the aquatic environment. In general, growth of micro-organisms is not supported when a substrate is present in concentrations below a threshold level of around 10 $\mu\text{g/L}$ and at lower concentrations, even the energy requirement for maintenance is not met (OECD, 1995). The reason for this lower threshold level is possibly a lack of sufficient stimulus to initiate an enzymatic response (Scow, 1982). This means in general that the concentrations of many substances in the aquatic environment are at a level where they are too low to be the primary substrate for degrading micro-organisms.

Moreover, the degradation kinetics depends on substance concentration (S_0) compared with the saturation constant (K_s) as described in the Monod equation. The saturation constant is the concentration of the substrate resulting in a specific growth rate of 50% of the maximum specific growth rate. At substrate concentrations much lower than the saturation constant, which is the normal situation in most of the aquatic environment, the degradation can be described by first order or logistic kinetics (OECD, 1995). When a low density of micro-organisms (lower than 10³–10⁵ cells/mL) prevails (for example, in oligotrophic waters), the population grows at ever decreasing rates which is typical of logistic kinetics. At a higher density of microorganisms (for example, in eutrophic waters), the substrate concentration is not high enough to support growth of the cells and first order kinetics apply, that is, the degradation rate is proportional with the substance concentration. In practice, it may be impossible to distinguish between the two types of degradation kinetics due to uncertainty of the data (OECD, 1995).

In conclusion, substances in low concentrations (that is, below 10 µg/L) are probably not degraded as primary substrates in the aquatic environment. At higher concentrations, readily degradable substances will probably be degraded as primary substrates in the environment at a degradation rate more or less proportional with the concentration of the substance. The degradation of substances as secondary substrates is discussed below.

Presence of other substrates

In the standard tests, the test substance is applied as the sole substrate for the microorganisms while in the environment, a large number of other substrates are present. In natural waters, concentrations of dissolved organic carbon are often found in the range 1–10 mg C/L, that is, up to a factor 1,000 higher than a pollutant. However, much of this organic carbon is relatively persistent with an increasing fraction of persistent matter the longer the distance from the shore.

Bacteria in natural waters are primarily nourishing on exudates from algae. These exudates are mineralised very quickly (within minutes) demonstrating that there is a high degradation potential in the natural micro-organism communities. Thus, as micro-organisms compete for the variety of substrates in natural waters, there is a selection pressure among micro-organisms resulting in growth of opportunistic species capable of nourishing on quickly mineralised substrates, while growth of more specialised species is suppressed. Experiences from isolation of bacteria capable of degrading various xenobiotics have demonstrated that these organisms are often growing relatively slowly and survive on complex carbon sources in competition with more rapidly growing bacteria. When competent micro-organisms are present in the environment, their numbers may increase if the specific xenobiotic substrate is continuously released and reach a concentration in the environment sufficient to support growth. However, most of the organic pollutants in the aquatic environment are present in low concentrations and will only be degraded as secondary substrates not supporting growth.

On the other hand, the presence of quickly mineralised substrates in higher concentrations may facilitate an initial transformation of the xenobiotic molecule by co-metabolism. The co-metabolised substance may then be available for further degradation and mineralisation. Thus, the presence of other substrates may increase the possibilities for a substance to be degraded.

It may then be concluded that the presence of a variety of substrates in natural waters and among them quickly mineralised substrates, may on the one hand cause a selection pressure suppressing growth of micro-organisms competent of degrading micro-pollutants. On the other hand it may facilitate an increased degradation by an initial co-metabolism followed by a further mineralisation. The relative importance of these processes under natural conditions may vary depending on both the environmental conditions and the substance and no generalisation can yet be established.

Environment related factors

The environmental variables control the general microbial activity rather than specific degradation processes. However, the significance of the influence varies between different ecosystems and microbial species (Scow, 1982).

Redox potential

One of the most important environment related factors influencing the degradability is probably the presence of oxygen. The oxygen content and the related redox potential determines the presence of different types of micro-organisms in aquatic environments with aerobic organisms present in the water phase, in the upper layer of sediments and in parts of sewage treatment plants, and anaerobic organisms present in sediments and parts of sewage treatment plants. In most parts of the water phase, aerobic conditions are prevailing and the prediction of the biodegradability should be based on results from aerobic tests. However, in some aquatic environments the oxygen content may be very low in periods of the year due to eutrophication and the following decay of produced organic matter. In these periods, aerobic organisms will not be able to degrade the chemical, but anaerobic processes may take over if the chemical is degradable under anaerobic conditions.

Temperature

Another important parameter is the temperature. Most laboratory tests are performed at 20–25°C (standard aerobic ready biodegradability tests), but anaerobic tests may be performed at 35°C as this better mimics the conditions in a sludge reactor. Microbial activity is found in the environment at temperatures ranging from below 0 °C to 100 °C. However, optimum temperatures are probably in the range from 10°C to 30°C and roughly, the degradation rate doubles for every 10 °C increase of temperature in this range (de Henau, 1993). Outside this optimum range the activity of the degraders is reduced drastically although some specialised species (thermo- and psycro-philic bacteria) may thrive. When extrapolating from laboratory conditions, it should be considered that some aquatic environments are covered by ice in substantial periods of the year and that only minor or even no degradation can be expected during the winter season.

pH

Active micro-organisms are found in the entire pH range found in the environment. However, for bacteria as a group, slightly alkaline conditions favour the activity and the optimum pH range is 6–8. At a pH lower than 5, the metabolic activity in bacteria is significantly decreased. For fungi as a group, slightly acidic conditions favour the activity with an optimum pH range of 5–6 (Scow, 1982). Thus, an optimum for the degrading activity of micro-organisms will probably be within the pH range of 5–8, which is the range most often prevailing in the aquatic environment.

Presence of nutrients

The presence of inorganic nutrients (nitrogen and phosphorus) is often required for microbial growth. However, these are only seldom the activity limiting factors in the aquatic environment where growth of micro-organisms is often substrate limited. However, the presence of nutrient influences the growth of primary producers and then again the availability of readily mineralised exudates.

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Appendix 19F: Globally Harmonized System of Classification and Labelling of Chemicals – guidance on the bioconcentration factor

19F.1 Introduction

This appendix is largely the same as the Globally Harmonized System of Classification and Labelling of Chemicals, Annex 9.(United Nations, 2007) Section 19F.2 reflects Appendix III and section 19F.3 reflects Appendix IV of Annex 9.

19F.2 Basic principles of the experimental and estimation methods for determination of bioconcentration factor and n-octanol-water partition coefficient of organic substances

Bioconcentration factor

Definition

The bioconcentration factor (BCF) is defined as the ratio between the concentration of the chemical in biota and the concentration in the surrounding medium, here water, at steady state. The BCF can be measured experimentally directly under steady-state conditions or calculated by the ratio of the first-order uptake and elimination rate constants, a method that does not require equilibrium conditions.

Appropriate methods for experimental determination of bioconcentration factor

Different test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted; the most generally applied being the Organisation for Economic Cooperation and Development (OECD) test guideline (OECD 305, 1996) and the ASTM standard guide (ASTM E 1022-94) (see Appendix 19A for sources of these guidelines). OECD 305 (1996) was revised and replaced the previous version OECD 305A-E, (1981). Although flow-through test regimes are preferred (OECD 305, 1996), semistatic regimes are allowed (ASTM E 1022-94), provided that the validity criteria on mortality and maintenance of test conditions are fulfilled. For lipophilic substances ($\log KOW > 3$, where KOW means the n-octanol-water partition coefficient), flow-through methods are preferred.

The principles of the OECD 305 and the ASTM guidelines are similar, but the experimental conditions described are different, especially concerning:

- the method of test water supply (static, semi-static or flow through);
- the requirement for carrying out a depuration study;
- the mathematical method for calculating BCF;
- sampling frequency: number of measurements in water and number of samples of fish;
- requirement for measuring the lipid content of the fish; and
- the minimum duration of the uptake phase.

In general, the test consists of two phases: The exposure (uptake) and post-exposure (depuration) phases. During the uptake phase, separate groups of fish of one species are exposed to at least two concentrations of the test substance. A 28-day exposure phase is obligatory unless a steady state has been reached within this period. The time needed for reaching steady-state conditions may be set on the basis of K_{OW} – k₂ correlations (for example, $\log k_2 = 1.47 - 0.41 \log K_{OW}$ (Spacie and Hamelink, 1982) or $\log k_2 = 1.69 - 0.53 \log K_{OW}$ (Gobas et al, 1989)). The expected time (d) for, for example, 95% steady state may thus be calculated by $-\ln(1-0.95)/k_2$, provided that the bioconcentration follows first order kinetics. During the depuration phase the fish are transferred to a medium free of the test substance. The concentration of the test substance in the fish is followed through both phases of the test. The BCF is expressed as a function of the total wet weight of the fish. As for many organic substances, there is a significant relationship between the potential for bioconcentration and the lipophilicity, and furthermore, there is a corresponding relationship between the lipid content of the test fish and the observed bioconcentration of such substances. Therefore, to reduce this source of variability in the test results for the substances with high lipophilicity, bioconcentration should be expressed in relation to the lipid content in addition to whole body weight (OECD 305; ECETOC (1995)). The guidelines mentioned are based on the assumption that bioconcentration may be approximated by a first-order process (one-compartment model) and thus that $BCF = k_1/k_2$ (k₁: first-order uptake rate, k₂: first-order depuration rate, described by a log-linear approximation). If the depuration follows biphasic kinetics, that is, two distinct depuration rates can be identified, the approximation k₁/k₂ may significantly underestimate the BCF. If a second order kinetic has been indicated, the BCF may be estimated from the relation: C_{Fish}/C_{Water} , provided that 'steady-state' for the fish-water system has been reached.

Together with details of sample preparation and storage, an appropriate analytical method of known accuracy, precision, and sensitivity must be available for the quantification of the substance in the test solution and in the biological material. If these are lacking it is impossible to determine a true BCF. The use of radiolabelled test substance can facilitate the analysis of water and fish samples. However, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of parent substance, possible metabolite(s), and possible metabolised carbon, which have been incorporated in the fish tissue in organic molecules. For the determination of a true BCF it is essential to clearly discriminate the parent substance from possible metabolites. If radiolabelled materials are used in the test, it is possible to analyse for total radio label (that is, parent and metabolites) or the samples may be purified so that the parent compound can be analysed separately.

In the log K_{OW} range above 6, the measured BCF data tend to decrease with increasing log K_{OW}. Conceptual explanations of non-linearity mainly refer to either biotransformation, reduced membrane permeation kinetics or reduced biotic lipid solubility for large molecules. Other factors consider experimental artefacts, such as equilibrium not being reached, reduced bioavailability due to sorption to organic matter in the aqueous phase, and analytical errors. Moreover, care should be taken when evaluating experimental data on BCF for substances with log K_{OW} above 6, as these data will have a much higher level of uncertainty than BCF values determined for substances with log K_{OW} below 6.

Log n-octanol-water partition coefficient

Definition and general considerations

The log K_{OW} is a measure of the lipophilicity of a substance. As such, log K_{OW} is a key parameter in the assessment of environmental fate. Many distribution processes are driven by log K_{OW} , for example, sorption to soil and sediment and bioconcentration in organisms.

The basis for the relationship between bioconcentration and log K_{OW} is the analogy for the partition process between the lipid phase of fish and water and the partition process between n-octanol and water. The reason for using KOW arises from the ability of octanol to act as a satisfactory surrogate for lipids in fish tissue. Highly significant relationships between log KOW and the solubility of substances in cod liver oil and triolin exist (Niimi, 1991). Triolin is one of the most abundant triacylglycerols found in freshwater fish lipids (Henderson and Tocher, 1987).

The determination of the n-octanol-water partition coefficient (KOW) is a requirement of the base data set to be submitted for notified new and priority existing substances within the European Union (EU). As the experimental determination of the KOW is not always possible, for example, for very water-soluble and for very lipophilic substances, a Quantitative Structure Activity Relationship (QSAR) derived KOW may be used. However, extreme caution should be exercised when using QSARs for substances where the experimental determination is not possible (as for, for example, surfactants).

Appropriate methods for experimental determination of KOW values

For experimental determination of KOW values, two different methods, Shake-flask and High Performance Liquid Chromatography (HPLC), have been described in standard guidelines for example, OECD 107 and OECD 117. For highly lipophilic substances, which are slowly soluble in water, data obtained by employing a slow-stirring method are generally more reliable (De Bruijn et al, 1989; Tolls and Sijm, 1993; OECD Test Guideline 123, 2006).

Shake-flask method

The basic principle of the method is to measure the dissolution of the substance in two different phases, water and n-octanol. In order to determine the partition coefficient, equilibrium between all interacting components of the system must be achieved after which the concentration of the substances dissolved in the two phases is determined. The shake-flask method is applicable when the log KOW value falls within the range from -2 to 4 (OECD 107, 1995). The shake-flask method applies only to essential pure substances soluble in water and n-octanol and should be performed at a constant temperature ($\pm 1^\circ\text{C}$) in the range 20–25°C.

High Performance Liquid Chromatography method

HPLC is performed on analytical columns packed with a commercially available solid phase containing long hydrocarbon chains (for example, C8, C18) chemically bound onto silica. Chemicals injected onto such a column move along at different rates because of the different degrees of partitioning between the mobile aqueous phase and the stationary hydrocarbon phase. The HPLC method is not applicable to strong acids

and bases, metals complexes, surface-active materials, or substances that react with the eluent. The HPLC method is applicable when the log K_{OW} value falls within the range 0 to 6 (OECD 117, 1989). The HPLC method is less sensitive to the presence of impurities in the test compound compared to the shake-flask method.

Slow stirring method

With the slow-stirring method provides a precise and accurate determination of K_{OW} of compounds with log K_{OW} up to 8.2 (De Bruijn et al, 1989). For highly lipophilic compounds the shake-flask method is prone to produce artefacts (formation of micro-droplets), and with the HPLC method K_{OW} needs to be extrapolated beyond the calibration range to obtain estimates of K_{OW}. In order to determine a partition coefficient, water, n-octanol, and test compound are equilibrated with each other after which the concentration of the test compound in the two phases is determined. The experimental difficulties associated with the formation of micro-droplets during the shake-flask experiment can to some degree be overcome in the slow-stirring experiment as water, n-octanol, and the test compound are equilibrated in a gently stirred reactor. The stirring creates a more or less laminar flow between the n-octanol and the water, and exchange between the phases is enhanced without micro-droplets being formed.

Generator column method

Another very versatile method for measuring log K_{OW} is the generator column method. In this method, a generator column method is used to partition the test substance between the n-octanol and water phases. The column is packed with a solid support and is saturated with a fixed concentration of the test substance in n-octanol. The test substance is eluted from the n-octanol -saturated generator column with water. The aqueous solution exiting the column represents the equilibrium concentration of the test substance that has partitioned from the octanol phase into the water phase. The primary advantage of the generator column method over the shake flask method is that the former completely avoids the formation of micro-emulsions. Therefore, this method is particularly useful for measuring K_{OW} for substances values over 4.5 (Doucette and Andren, 1987; 1988; Shiu et al, 1988) as well as for substances having log K_{OW} < 4.5. A disadvantage of the generator column method is that it requires sophisticated equipment.

Use of QSARs for determination of log K_{OW}

(See also section 19D.5 in Appendix 19D.)

Numerous QSARs have been and continue to be developed for the estimation of K_{OW}. Commonly used methods are based on fragment constants. The fragmental approaches are based on a simple addition of the lipophilicity of the individual molecular fragments of a given molecule. Three commercially available PC programs are recommended in the European Commission's technical guidance document (EC, 1996) for risk assessment, part III, if no experimentally derived data are available.

CLOGP (Daylight Chemical Information Systems, 1995) was initially developed for use in drug design. The model is based on the Hansch and Leo calculation procedure (Hansch and Leo, 1979). The program calculates log K_{OW} for organic compounds containing C, H, N, O, Hal, P, and/or S. Log K_{OW} for salts and for

compounds with formal charges cannot be calculated (except for nitro compounds and nitrogen oxides). The calculation results of $\log K_{OW}$ for ionisable substances, like phenols, amines, and carboxylic acids, represent the neutral or unionised form and will be pH dependent. In general, the program results in clear estimates in the range of $\log K_{OW}$ between 0 and 5 (EC, 2003, part III). However a validation study performed by Niemelä (1993), who compared experimental determined $\log K_{OW}$ values with estimated values, showed that the program precisely predicts the $\log K_{OW}$ for a great number of organic chemicals in the $\log K_{OW}$ range from below 0 to above 9 ($n = 501$, $r^2 = 0.967$). In a similar validation study on more than 7000 substances the results with the CLOGP program (PC version 3.32, EPA version 1.2) were $r^2 = 0.89$, $s.d. = 0.58$, $n = 7221$. These validations show that the CLOGP program may be used for estimating reliable $\log K_{OW}$ values when no experimental data are available. For chelating compounds and surfactants the CLOGP program is stated to be of limited reliability (OECD, 1993). However, as regards anionic surfactants (LAS) a correction method for estimating adjusted CLOGP values has been proposed (Roberts, 1989).

LOGKOW or KOWWIN (Syracuse Research Corporation) uses structural fragments and correction factors. The program calculates $\log K_{OW}$ for organic compounds containing the following atoms: C, H, N, O, Hal, Si, P, Se, Li, Na, K, and/or Hg. $\log K_{OW}$ for compounds with formal charges (like nitrogenoxides and nitro compounds) can also be calculated. The calculation of $\log K_{OW}$ for ionisable substances, like phenols, amines and carboxylic acids, represent the neutral or unionised form, and the values will thus be pH dependent. Some surfactants (for example, alcohol ethoxylates (Tolls, 1998), dyestuffs, and dissociated substances may be predicted by the LOGK_{OW} program (Pedersen et al, 1995). In general, the program gives clear estimates in the range of $\log K_{OW}$ between 0 and 9 (Pedersen et al, 1995). Like the CLOGP-program, LOGK_{OW} has been validated and is recommended for classification purposes because of its reliability, commercial availability, and convenience of use.

AUTOLOGP (Devillers et al, 1995) has been derived from a heterogeneous data set, comprising 800 organic chemicals collected from literature. The program calculates $\log K_{OW}$ values for organic chemicals containing C, H, N, O, Hal, P, and S. The $\log K_{OW}$ values of salts cannot be calculated. Also the $\log K_{OW}$ of some compounds with formal charges cannot be calculated, with the exception of nitro compounds. The $\log K_{OW}$ values of ionisable chemicals like phenols, amines, and carboxylic acids can be calculated although pH-dependencies should be noted. Improvements are in progress in order to extend the applicability of AUTOLOGP. According to the presently available information, AUTOLOGP gives accurate values especially for highly lipophilic substances ($\log K_{OW} > 5$) (EC, 1996).

SPARC is a mechanistic model based on chemical thermodynamic principles rather than a deterministic model rooted in knowledge obtained from observational data. Therefore, SPARC differs from models that use QSARs (that is, KOWWIN, LOGP) in that no measured $\log K_{OW}$ data are needed for a training set of chemicals. The Environmental Protection Agency (EPA) does occasionally run the model for a list of CAS numbers, if requested. SPARC provides improved results over KOWWIN and CLOGP only for compounds with $\log K_{OW}$ values greater than 5. Only SPARC can be employed in a general way for inorganic or organometallic compounds. In Table 19F.1, an overview of $\log K_{OW}$ estimation methods based on

fragmentation methodologies is presented. Also, other methods for the estimation of log K_{OW} values exist, but they should only be used on a case-by-case basis and only with appropriate scientific justification.

Table 19F.1: Overview of Quantitative Structure Activity Relationship methods for estimation of log n-octanol-water partition coefficient (K_{OW}) based on fragmentation methodologies

| Method | Methodology | Statistics |
|--|---|--|
| CLOGP Hansch and Leo (1979), CLOGP Daylight (1995) | Fragments + correction factors | Total n = 8942, $r^2 = 0,917$, sd = 0,482 Validation: n = 501, $r^2 = 0,967$ Validation: n = 7221, $r^2 = 0,89$, sd = 0,58 |
| LOGKOW (KOWWIN) Meylan and Howard (1995), SRC | 140 fragments 260 correction factors | Calibration: n = 2430, $r^2 = 0,981$, sd = 0,219, me = 0,161 Validation: n = 8855, $r^2 = 0,95$, sd = 0,427, me = 0,327 |
| AUTOLOGP Devillers et al (1996) | 66 atomic and group contributions from Rekker and Manhold (1992) | Calibration: n = 800, $r^2 = 0,96$, sd = 0,387 |
| SPARC Under development by EPA, Athens, Georgia. | Based upon fundamental chemical structure algorithm. | No measured log K_{OW} data are needed for a training set of chemicals. |
| Rekker and De Kort (1979) | Fragments + correction factors | Calibration n = 1054, $r^2 = 0,99$ Validation: n = 20, $r^2 = 0,917$, sd = 0,53, me = 0,40 |
| Niemi et al (1992) | MCI | Calibration n = 2039, $r^2 = 0,77$ Validation: n = 2039, $r^2 = 0,49$ |
| Klopman et al (1994) | 98 fragments + correction factors | Calibration n = 1663, $r^2 = 0,928$, sd = 0,3817 |
| Suzuki and Kudo (1990) | 424 fragments | Total: n= 1686, me = 0,35 Validation: n = 221, me = 0,49 |
| ATOMLOGP Ghose et al (1988) | 110 fragments | Calibration: n = 830, $r^2 = 0,93$, sd = 0,47 Validation: n = 125, $r^2 = 0,87$, sd = 0,52 |
| Bodor and Huang (1992) | Molecule orbital | Calibration: n = 302, $r^2 = 0,96$, sd = 0,31, me = 0,24 Validation: n = 128, sd = 0,38 |
| ProLogP Broto et al (1984) | 110 fragments | Calibration: n = 1868, me= ca. 0,4 |

Source: Howard and Meylan (1997).

19F.3 Influence of external and internal factors on the bioconcentration potential of organic substances

Factors influencing the uptake

The uptake rate for lipophilic compounds is mainly a function of the size of the organism (Sijm and Linde, 1995). External factors such as the molecular size, factors influencing the bioavailability, and different environmental factors are of great importance to the uptake rate as well.

Size of organism

Since larger fish have a relatively lower gill surface to weight ratio, a lower uptake rate constant (k_1) is to be expected for large fish compared to small fish (Opperhuizen and Sijm, 1990; Sijm and Linde, 1995). The uptake of substances in fish is further controlled by the water flow through the gills; the diffusion through aqueous diffusion layers at the gill epithelium; the permeation through the gill epithelium; the rate of blood flow through the gills, and the binding capacity of blood constituents (ECETOC, 1995).

Molecular size

Ionised substances do not readily penetrate membranes; as aqueous pH can influence the substance uptake. Loss of membrane permeability is expected for substances with a considerable cross sectional area (Anliker et al, 1988; Opperhuizen et al, 1985) or long chain length (> 4.3 nm) (Opperhuizen, 1986). Loss of membrane permeability due to the size of the molecules will thus result in total loss of uptake. The effect of molecular weight on bioconcentration is due to an influence on the diffusion coefficient of the substance, which reduces the uptake rate constants (Gobas et al, 1986).

Availability

Before a substance is able to bioconcentrate in an organism it needs to be present in water and available for transfer across fish gills. Factors, which affect this availability under both natural and test conditions, will alter the actual bioconcentration in comparison to the estimated value for BCF. As fish are fed during bioconcentration studies, relatively high concentrations of dissolved and particulate organic matter may be expected, thus reducing the fraction of chemical that is actually available for direct uptake via the gills. McCarthy and Jimenez (1985) have shown that adsorption of lipophilic substances to dissolved humic materials reduces the availability of the substance, the more lipophilic the substance the larger reduction in availability (Schrap and Opperhuizen, 1990). Furthermore, adsorption to dissolved or particulate organic matter or surfaces in general may interfere during the measurement of BCF (and other physical/chemical properties) and thus make the determination of BCF or appropriate descriptors difficult. As bioconcentration in fish is directly correlated with the available fraction of the chemical in water, it is necessary for highly lipophilic substances to keep the available concentration of the test chemical within relatively narrow limits during the uptake period. Substances, which are readily biodegradable, may only be present in the test water for a short period, and bioconcentration of these substances may thus be insignificant. Similarly, volatility and hydrolysis will reduce the concentration and time in which the substance is available for bioconcentration.

Environmental factors

Environmental parameters influencing the physiology of the organism may also affect the uptake of substances. For instance, when the oxygen content of the water is lowered, fish have to pass more water over their gills in order to meet respiratory demands (McKim and Goeden, 1982). However, there may be species dependency as indicated by Opperhuizen and Schrap (1987). It has, furthermore, been shown that the temperature may have an influence on the uptake rate constant for lipophilic substances (Sijm et al, 1993), whereas other authors have not found any consistent effect of temperature changes (Black et al, 1991).

Factors influencing the elimination rate

The elimination rate is mainly a function of the size of the organism, the lipid content, the biotransformation process of the organism, and the lipophilicity of the test compound.

Size of organism

As for the uptake rate the elimination rate is dependent on the size of the organism. Due to the higher gill surface to weight ratio for small organisms (for example, fish larvae) than that of large organisms, steady-state and thus 'toxic dose equilibrium' has shown to be reached sooner in early life stages than in juvenile/adult stages of fish (Petersen and Kristensen, 1998). As the time needed to reach steady-state conditions is dependent on k_2 , the size of fish used in bioconcentration studies has thus an important bearing on the time required for obtaining steady-state conditions.

Lipid content

Due to partitioning relationships, organisms with a high fat content tend to accumulate higher concentrations of lipophilic substances than lean organisms under steady-state conditions. Body burdens are therefore often higher for 'fatty' fish such as eel, compared to 'lean' fish such as cod. In addition, lipid 'pools' may act as storage of highly lipophilic substances. Starvation or other physiological changes may change the lipid balance and release such substances and result in delayed impacts.

Metabolism

In general, metabolism or biotransformation leads to the conversion of the parent compound into more water-soluble metabolites. As a result, the more hydrophilic metabolites may be more easily excreted from the body than the parent compound. When the chemical structure of a compound is altered, many properties of the compound are altered as well. Consequently the metabolites will behave differently within the organism with respect to tissue distribution, bioaccumulation, persistence, and route and rate of excretion. Biotransformation may also alter the toxicity of a compound. This change in toxicity may either be beneficial or harmful to the organism. Biotransformation may prevent the concentration in the organism from becoming so high that a toxic response is expressed (detoxification). However, a metabolite may be formed which is more toxic than the parent compound (bioactivation) as known for, for example, benzo(a)pyrene.

Terrestrial organisms have a developed biotransformation system, which is generally better than that of organisms living in the aquatic environment. The reason for this difference may be the fact that

biotransformation of xenobiotics may be of minor importance in gill breathing organisms as they can relatively easily excrete the compound into the water (Van Den Berg et al, 1995). Concerning the biotransformation capacity in aquatic organisms the capacity for biotransformation of xenobiotics increases in general as follows: Molluscs < crustaceans < fish (Wofford et al, 1981).

Lipophilicity of substance

A negative linear correlation between k_2 (depuration constant) and log KOW (or BCF) has been shown in fish by several authors (for example, Spacie and Hamelink, 1982; Gobas et al, 1989; Petersen and Kristensen, 1998), whereas k_1 (uptake rate constant) is more or less independent of the lipophilicity of the substance (Connell, 1990). The resultant BCF will thus generally increase with increasing lipophilicity of the substances, that is, log BCF and log KOW correlate for substances that do not undergo extensive metabolism.

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Appendix 19G: Globally Harmonized System of Classification and Labelling of Chemicals – guidance on transformation and dissolution of metals and metal compounds in aqueous media

19G.1 Introduction

The full guidance document on the transformation and dissolution of metals and metal compounds in aqueous media series on testing and assessment is *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media* (OECD, 2001).

19G.2 Test guidance

This test guidance is designed to determine the rate and extent to which metals and sparingly soluble metal compounds can produce soluble available ionic and other metal-bearing species in aqueous media under a set of standard laboratory conditions representative of those generally occurring in the environment. Once determined, this information can be used to evaluate the short-term and long-term aquatic toxicity of the metal or sparingly soluble metal compound from which the soluble species came.

This test guidance is the outcome of an international effort under the OECD to develop an approach for the toxicity testing and data interpretation of metals and sparingly soluble inorganic metal compounds (SSIMs). As a result of recent meetings and discussions held within the OECD and European Union (EU), the experimental work on several metals and metal compounds on which this test guidance is based has been conducted and reported.

The evaluation of the short-term and long-term aquatic toxicity of metals and sparingly soluble metal compounds is to be accomplished by comparison of (a) the concentration of the metal ion in solution, produced during transformation or dissolution in a standard aqueous medium with (b) appropriate standard ecotoxicity data as determined with the soluble metal salt (acute and chronic values).

This document gives guidance for performing the transformation and dissolution tests. The strategy to derive an environmental hazard classification using the results of the transformation and dissolution protocol is not within the scope of this guidance document and can be found in Annex 9, section A9.7.

For this test guidance, the transformations of metals and sparingly soluble metal compounds are, within the context of the test, defined and characterised as follows.

- Metals, M₀, in their elemental state are not soluble in water but may transform to yield the available form. This means that a metal in the elemental state may react with the media to form soluble cationic or anionic products, and in the process the metal will oxidize, or transform, from the neutral or zero oxidation state to a higher one.
- In a simple metal compound, such as an oxide or sulphide, the metal already exists in an oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium. However, while oxidation state may not change, interaction with the media may yield more

soluble forms. A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield small amount of the available form by dissolution. However, it should be recognised that the final solution concentration may be influenced by a number of factors, including the solubility product of some metal compounds precipitated during the transformation/dissolution test, for example, aluminium hydroxide.

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20. Soil Ecotoxicity – Subclass 9.2

20.1. Basic elements and general considerations

The basic elements to consider in determining hazard classification under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) for effects on the soil environment are:

- acute toxicity to soil-dwelling organisms; and
- degradation of the substance in soil.

While data from internationally harmonised test methods are preferred, in practice, data from national methods may also be used where they are considered equivalent. In general, test data are to be derived using Test Guidelines from the Organisation for Economic Co-operation and Development (OECD) or equivalent according to the principles of Good Laboratory Practice (GLP). Where such data are not available, classification should be based on the best available data.

See section 18.6 in chapter 18 for definitions of the key terms used in this chapter.

See section 1.3 in chapter 1 for information about assessing data quality.

See Appendix 20A for a detailed list of acceptable test methods.

20.1.1. Acute toxicity to soil organisms

The toxicity of substances to soil-dwelling organisms is assessed by mixing the test substance in soil. Some standard international guidelines use aqueous solutions as the exposure medium or foliar application. The results from these tests are generally not applicable for assessment against the HSNO Act classification criteria.

The usual acute tests for effects on soil organisms used for HSNO Act classification are:

- 14-day EC₅₀ for earthworms (OECD 207 or equivalent);
- 14-day EC₅₀ for terrestrial plants (OECD 208 or equivalent) when soil is used as the exposure medium; and
- 28-day EC₂₅ for soil microbial function (for example, OECD 216 nitrogen transformation and OECD 217 carbon transformation).

The lowest value from these tests, with the results expressed in terms of milligrams of substance per kilogram of dry weight of soil is used to classify the substance. Acute toxicity tests on other soil-dwelling organisms may be used if conducted according to international guidelines. See chapter 18 when judgements are required on the weight-of-evidence approach to the selection of the most sensitive species and highest quality studies.

Results from chronic studies are not used for classification purposes but are used for risk assessment.

Metals

The assessment of the toxicity of metals and metal compounds to soil organisms is complicated by the interactions of the metal with the soil matrix.

For a detailed discussion on testing the toxicity of metals and metal compounds, see Fairbrother et al (2002).

Conversion of data

Earthworm tests

The OECD protocol does not derive an EC₅₀ value, with reference given only to a LC₅₀. While a single conversion factor will not be accurate for all chemicals, some conservative guidance can be obtained from standardised risk assessment procedures that include a conversion factor of 10 when comparing lethal concentrations and 'safe' concentrations. While the EC₅₀ defines an effect concentration rather than the 'safe' concentration, the factor of 10 would nevertheless represent the conservative end of the range of values for extrapolating from an LC₅₀ to EC₅₀. When evidence for a specific substance demonstrates that a reduced factor is valid, the reduced value should be acceptable. As the OECD earthworm test requires a description of obvious physical or pathological symptoms or distinct changes in behaviour observed in the test animals, evidence for a lower factor could include the absence of obvious physical, pathological, or behavioural changes.

Field application rate to milligrams of active ingredient per kilogram of dry soil

Data for pesticides can be derived from studies where the test substance has been surface applied to soil at a field application rate. The results are usually expressed in terms of milligrams active ingredient per hectare, or pounds of active ingredient per acre and must be converted to milligrams of active ingredient per kilogram of dry soil (mg active/kg dry soil).

The EPA default values to be used in converting field application rates to units of mg active/kg dry soil are in Table 20.1. See also the example calculation under the table.

Table 20.1: EPA default values for converting field application rates to units of milligrams of active ingredient per kilogram of dry soil

| Soil depth | 5 cm when the substance is surface applied 20 cm when the substance is incorporated into the soil after application (eg, by ploughing) |
|-----------------------------|---|
| Soil density | 1.5 g/cm ³ |
| Soil mass in 1 hectare (ha) | 5 cm deep = 750,000 kg 20 cm deep = 3,000,000 kg |
| lb/acre to kg/ha | Multiply lb/acre by 1.121 |

Example

An earthworm EC₅₀ is 5 lb active ingredient per acre for a substance that is surface applied to soil. The conversion to mg active/kg dry soil is as follows.

- Convert to kg/ha ($\times 1.121$) = 5.605 kg/ha.
- Convert to mg/ha ($\times 1,000,000$) = 5,605,000 mg/ha.
- At 5 cm depth = 5,605,000 mg/750,000 kg dry soil.

- $EC_{50} = 7.47$ mg active/kg dry soil.

20.1.2. Degradability in soil

The HSNO Act classification criterion (in Schedule 6 of the Hazardous Substances (Classification) Regulations 2001) includes consideration of the half-life of the substance in soil, where:

Soil DT_{50} is the half-life in soil, which is the time required to reduce the original concentration of the substance in the soil by 50%.

Unlike the HSNO Act criteria for rapid degradation in aquatic systems, the regulations have no further details to assist with interpretation of the above criterion. The EPA policy is to consider only degradation (abiotic and biotic) when determining the applicability of a DT_{50} value for use in hazard classification. Other processes such as dissipation, volatilisation, or leaching are not relevant for the classification of the substance, but are used for risk assessment.

The most commonly used guidelines for testing soil degradation are OECD 307 and United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) 835.3300 (see Appendix 20A for acceptable test methods). Generally, freshly sampled representative soils are characterised with regard to common soil properties (for example, texture, pH, and organic carbon content) and incubated under static soil moisture and temperature conditions in the dark.

The use of ^{14}C -labelled material is preferred. During incubation soil samples are taken and analysed for active substance, metabolites, volatile components, and bound residues. The time taken for degradation of 50% and 90% of the active substance and major metabolites is derived from the formation and decline curves. The degradation pathway must be reported for one soil. The rate of degradation is also investigated in a minimum of three additional soils at 20°C. Further evaluation of the rate of degradation is also undertaken at 10°C.

Multiple DT_{50} values

Where degradation data are available from several acceptable studies and a single value study is needed for modelling or a trigger value, usually an average of the kinetic parameters is sufficient. However, in some circumstances, such as when degradation rates are strong functions of soil properties such as pH, averaging is not appropriate.

The geometric mean should normally be used as the average of degradation parameters because it provides the best representation of the average of different first-order degradation curves over the entire period. Using the geometric mean also has the advantage that the same result is obtained from averaging first-order degradation rates and averaging the corresponding half-lives (FOCUS, 2006).

Metals: bioavailability in soil

As noted in chapter 19, the property of degradation has limited relevance to metals and inorganic metal compounds. Numerous interactions with the soil matrix will reduce the bioavailability of a metal to soil organism. Poorly soluble metal compounds may release toxic species over time, resulting in the

underestimation of the hazard in acute tests; the availability of soluble metal compounds can decrease rapidly after addition to soil. Strongly sorbed metals are less available than weakly sorbed metals. For metal salts, the counter-ion may influence the toxicity of the metal, for example, sulphate or chloride ions. Organic anions may reduce metal toxicity. A transformation test is needed to ensure that such effects are adequately evaluated (Fairbrother et al, 2002).

See Fairbrother et al (2002) for guidance on testing the toxicity of poorly soluble metals in soil, including a suggested transformation protocol.

20.1.3. Default classification in absence of data on degradation in soil

Where there are no data on the degradation of a substance, the default position is that the substance will attract the same classification as if those data were available and indicate that the substance has a DT50 > 30 days.

20.1.4. Metabolites

Data on metabolites in soil come from soil degradation studies, including information on the time course of appearance and concentration. These metabolites are relevant for soil organisms and ground-dwelling arthropods.

Where the parent substance degrades to a more hazardous metabolite, the rate at which it is formed should be taken into consideration when assigning a classification to the parent substance.

See chapter 18 for further information on the evaluation of metabolites.

20.1.5. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions are clearly and completely articulated.

See section 18.4.3 in chapter 18 [above](#) for a discussion on the assessment of multiple tests on the same species.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

20.2. Hazard threshold and classification criteria for the soil environment

20.2.1. Hazard threshold criteria for the soil environment

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 Minimum degrees of hazard

(1) A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—

...

(b) the substance is ecotoxic to soil organisms because—

- i. data for the substance indicates that a plant or soil invertebrate EC₅₀ is 100 milligrams or less of the substance per kilogram of dry weight of soil over a 14-day exposure period, as a result of exposure to the substance; or
- ii. data for the substance indicates that a 25% reduction in microbial respiration or microbial nitrification at 100 milligrams or less of the substance per kilogram of dry weight of soil after a 28-day exposure period, as a result of exposure to the substance.

20.2.2. Classification criteria for the soil environment

The HSNO Act classification criteria for substances with ecotoxic properties under Schedule 6 of the Hazardous Substances (Classification) Regulations 2001 identify four classification categories for substances that are ecotoxic to the soil environment (subclass 9.2).

A subclass 9.2 classification and the subsequent category apply to any substance that meets the following criteria.

- Category 9.2A – substances that are very ecotoxic in the soil environment
A substance for which data indicate a soil ecotoxicity value ≤ 1 milligram of the substance per kilogram dry weight of soil.
- Category 9.2B – substances that are ecotoxic in the soil environment
A substance for which data indicate a soil ecotoxicity value > 1 but ≤ 10 milligrams, of the substance per kilogram dry weight of soil.
- Category 9.2C – substances that are harmful in the soil environment
A substance for which data indicate a soil ecotoxicity value > 10 but ≤ 100 milligrams of the substance per kilogram dry weight of soil, where the soil DT₅₀ is > 30 days.
- Category 9.2D – substances that are slightly harmful in the soil environment
A substance for which data indicate a soil ecotoxicity value > 10 but ≤ 100 milligrams of the substance per kilogram dry weight of soil, where the soil DT₅₀ is ≤ 30 days.

The classification criteria for single component substances are summarised in Table 20.2 and Figure 20.1. The application of the criteria to mixtures is set out in more detail in section 20.3.

Table 20.2: Soil classification of a single substance

| Acute EC ₅₀ of the tested mixture | Soil DT ₅₀ >30 days* | Classification of substance |
|--|---------------------------------|---|
| ≤ 1 mg/kg | Not applicable | 9.2A |
| >1 and ≤ 10 mg/kg | Not applicable | 9.2B |
| >10 and ≤ 100 mg/kg | Yes | 9.2C |
| >10 and ≤ 100 mg/kg | No | 9.2D |
| >100 mg/kg | | Not classified as hazardous to the soil environment |

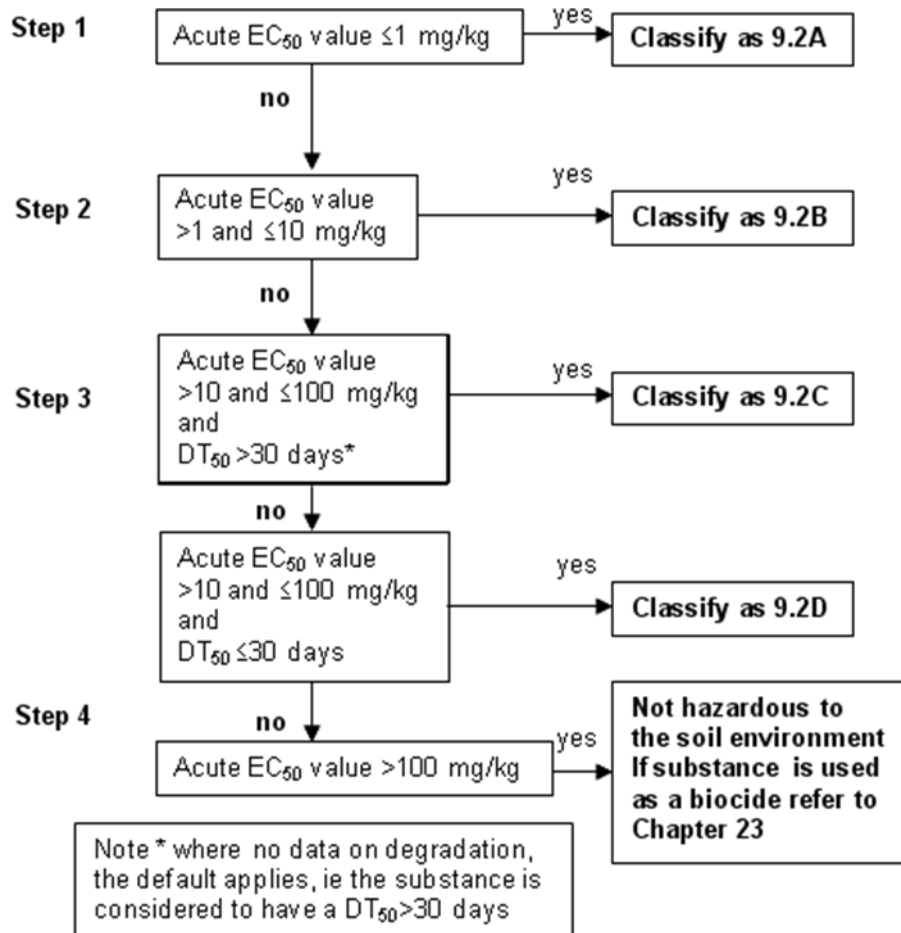
Notes:

*where no data on degradation, the default applies, ie the substance is considered to have DT₅₀ >30 days;

EC₅₀ = median effect concentration;

DT₅₀ = time required to reduce the concentration of the original substance by 50%. [where appropriate, ie for microbial function data, the EC₂₅ value can be used]

Figure 20.1: Soil classification of a single substance



20.3. Classification of mixtures

To make use of all available data to classify the hazards of the mixture to the soil environment, the following assumption has been made and is applied where appropriate.

The 'relevant components' of a mixture are those that are present in a concentration of 1% (by weight – w/w) or greater, unless there is a presumption (for example, in the case of highly toxic components) that a component present at less than 1% can still be relevant for classifying the mixture for aquatic environmental hazards.

The approach for classifying hazards to the soil environment is tiered, and depends on the type of information available for the mixture itself and for its components. Elements of the tiered approach include classification based on:

- tested mixtures (see section 20.3.1);
- bridging principles (see section 20.3.2); and
- a summation approach using the classifications of components (see section 20.3.3).

20.3.1. Tested mixtures

When a mixture has been directly tested, this result should be used in determining whether the substance as a mixture triggers a soil ecotoxicity classification.

It should be noted, however, that degradation time in soil cannot be directly tested for mixtures. Therefore, the degradation time of components of the mixture need to be considered when determining whether a substance is classified as 9.2C or 9.2D, as set out in Table 20.3. Refer to Table 20.6 for a worked example of calculating the weighted sum of components.

Table 20.3: Classification using test data on the mixture

| Acute EC ₅₀ of the tested mixture | Components in mixture with DT ₅₀ > 30 days* | Classification of mixture |
|--|--|---------------------------------------|
| ≤1 mg/kg | Not applicable | 9.2A |
| >1 and ≤ 10 mg/kg | Not applicable | 9.2B |
| >10 and ≤ 100 mg/kg | Yes (weighted sum ≥ 25%) | 9.2C |
| >10 and ≤ 100 mg/kg | No or weighted sum < 25% | 9.2D |
| >100 mg/kg | | Not hazardous to the soil environment |

Note:

*where no data on degradation, the default applies, ie the substance is considered to have DT₅₀ >30 days; EC₅₀ = median effect concentration;

DT₅₀ = time required to reduce the concentration of the original substance by 50%. [where appropriate, ie for microbial function data, the EC₂₅ value can be used]

If the mixture is used as a biocide and does not trigger classification under subclass 9.2, see also chapter 23 [below](#).

20.3.2. Bridging principles

Guidance on the bridging principles for the classification of mixtures without test data is in chapter 18 [above](#).

20.3.3. Classification of mixture based on classifications of components: summation approach

When test data on the mixture are not available and the bridging principles are not applicable, the summation approach is used to derive a soil hazard classification for the mixture.

Rationale

The toxicity criteria for the soil hazard classification categories differ by a factor of 10 from one category to another. Substances with a classification in a high toxicity band may, therefore, contribute to the classification of a mixture in a lower band. The calculation of these classification categories, therefore, needs to consider the contribution of all substances that are classified for toxicity to the soil environment.

When components are classified as 9.2A and their acute toxicity is well below the cut-off value (that is, $\ll 1$ mg/kg) they contribute to the toxicity of the mixture even at a low concentration. Active ingredients in pesticides often possess such high toxicity but so do some other substances such as organometallic compounds. Under these circumstances, the application of the normal cut-off values or concentration limits may lead to an 'under-classification' of the mixture. Therefore, multiplying factors are applied to account for highly toxic components, as described in 'Mixtures with highly toxic components' under 'Classification procedure' below.

Classification procedure

Degradability in soil

When classifying a mixture for hazards to the soil environment, separate consideration must be given the degradability of the components of the mixture. In general, a mixture cannot be directly tested for this property. The classification criteria for 9.2C require that the mixture includes components with a half-life in soil of > 30 days.

If the mixture is classified as 9.2C using the summation approach and the weighted sum of components with a soil half-life > 30 days is $< 25\%$, the classification is reduced to 9.2D. To calculate the weighted sum of components use the 'classification procedure' using components with DT50 > 30 days or with no data on degradation. See Table 20.6 for a worked example.

The steps to follow in applying the summation approach to soil hazard classification are set out below and summarised in Table 20.4 and Figure 20.2.

Mixtures with no highly toxic components

The steps to follow in applying the summation approach to soil hazard classification for mixtures with no highly toxic components are set out below.

- Step 1: Consider all components classified as 9.2A.
A mixture is classified as 9.2A if the sum of the components is $\geq 25\%$, and then the classification process is complete.
- Step 2: Where the mixture is not classified as 9.2A, consider classification of the mixture as 9.2B.
A mixture is classified as 9.2B if:

$$\sum(9.2A)\% \times 10 + \sum(9.2B)\% \geq 25\%$$
 If so, the classification process is complete.
- Step 3: Where the mixture is not classified as 9.2A or 9.2B, consider classification of the mixture as 9.2C.

A mixture is classified as 9.2C if:

$$\sum (9.2A)\% \times 100 + \sum (9.2B)\% \times 10 + \sum (9.2C)\% \geq 25\%$$

However, if the weighted sum of components with soil DT50 values ≤ 30 days is $<25\%$, then the mixture is classified as 9.2D.

- Step 4: Where the mixture is not classified as 9.2A, 9.2B, or 9.2C, consider classification of the mixture as 9.2D.

A mixture is classified as 9.2D if:

$$\sum (9.2A)\% + \sum (9.2B)\% + \sum (9.2C)\% + \sum (9.2D)\% \geq 25\%$$

If so, the classification process is complete.

If the sum is $< 25\%$, then the substance is not classified as hazardous to the soil environment. If the substance is used as a biocide, refer to Chapter 23.

Mixtures with highly toxic components

Components with toxicities well below the cut-off for 9.2A classification ($<< 1$ mg/kg) may influence the toxicity of the mixture and are given increased weight in applying the summation of classification approach.

The multiplying factors to be applied to these components are defined using the toxicity value, as summarised in Table 20.5. Therefore, in order to classify a mixture containing highly toxic components, the classifier needs to apply the multiplying factor (M) in assigning a soil hazard classification to the mixture.

See Table 20.6 and the following text for a worked example.

The steps for classifying mixtures for hazards to the soil environment are summarised in Table 20.4 and Figure 20.2.

Table 20.4: Classification of a mixture for ecotoxicity to the soil environment: summation approach

| Sum of components classified as | Cut-off | Mixture classified as |
|--|-------------|-----------------------|
| 9.2A x M | $\geq 25\%$ | 9.2A |
| $(M \times 10 \times 9.2A) + 9.2B$ | $\geq 25\%$ | 9.2B |
| $(M \times 100 \times 9.2A) + (10 \times 9.2B) + 9.2C$ | $\geq 25\%$ | 9.2C* |
| $9.2A + 9.2B + 9.2C + 9.2D\%$ | $\geq 25\%$ | 9.2D |

Notes: M = multiplying factor for highly ecotoxic components.

* Unless the weighted sum of components with DT50 > 30 days (or no data on degradation) is $<25\%$, in which case classify as 9.2D.

Table 20.5: Multiplying factors for highly ecotoxic components of mixtures

| EC ₅₀ value (mg/kg dry weight soil) | Multiplying factor (M) |
|--|------------------------|
| $0.1 < EC_{50} \leq 1$ | 1 |
| $0.01 < EC_{50} \leq 0.1$ | 10 |
| $0.001 < EC_{50} \leq 0.01$ | 100 |

| | |
|-----------------------------------|--------|
| $0.0001 < EC_{50} \leq 0.001$ | 1,000 |
| $0.00001 < EC_{50} \leq 0.0001$ | 10,000 |
| (continue in factor 10 intervals) | |

Note: EC_{50} = median effect concentration. [where appropriate, ie for microbial function data, the EC_{25} value can be used]

Table 20.6: Example calculation for soil hazard classification of mixture Y containing one highly toxic component

| Component | L(E) C_{50} (mg/kg) | Soil classification of individual component | Component with DT_{50} >30 days* | Concentr ation of compone nt in mixture (%) | Multiplying factor (M) (from Error! Reference source not found.) | Adjusted concentration of component in mixture (M x %) |
|-----------|--------------------------|--|--|---|---|--|
| B | 5 | 9.2B | No | 5 | 1 | 5 |
| P | 0.02 | 9.2A | Yes | 0.05 | 10 | 0.5 |
| Q | 0.9 | 9.2A | No | 1 | 1 | 1 |
| T | 50 | 9.2C | Yes | 40 | 1 | 40 |
| U | 200 | Not classified | No | 53.95 | 1 | 53.95 |

Note:

*where no data on degradation, the default applies, ie the substance is considered to have $DT_{50} > 30$ days

L(E) C_{50} = median lethal concentration or median effect concentration. [where appropriate, ie for microbial function data, the EC_{25} value can be used]

The steps to follow in applying the summation approach to soil hazard classification for mixtures with highly toxic components are set out below, using the information from Table 20.6.

- Step 1

Component P is highly ecotoxic and attracts a multiplier of 10, resulting in a weighted concentration of that component of 0.5%.

Component Q, although classified as 9.2A does not attract a multiplier, that is:

$$\Rightarrow (10 \times P) + Q$$

$$\Rightarrow (10 \times 0.05\%) + 1\% = 1.5\%, \text{ which is } < 25\%$$

Therefore mixture Y is not classified as 9.2A.

- Step 2: Consider components classified as 9.2A and 9.2B.

$$\Rightarrow 10((10 \times P) + Q) + B$$

$$\Rightarrow 10((10 \times 0.05\%) + 1\%) + 5\% = 15\% + 5\% = 20\%$$

which is <25% therefore mixture Y is not classified as 9.2B.

- Step 3: Consider components classified as 9.2A, 9.2B and 9.2C.

$$\Rightarrow 100((10 \times P) + Q) + (10 \times B) + T$$

$$\Rightarrow 100((10 \times 0.05\%) + 1\%) + (10 \times 5) + 40 = 240\% \text{ which is } \geq 25\%$$

So mixture Y is classified as 9.2C unless

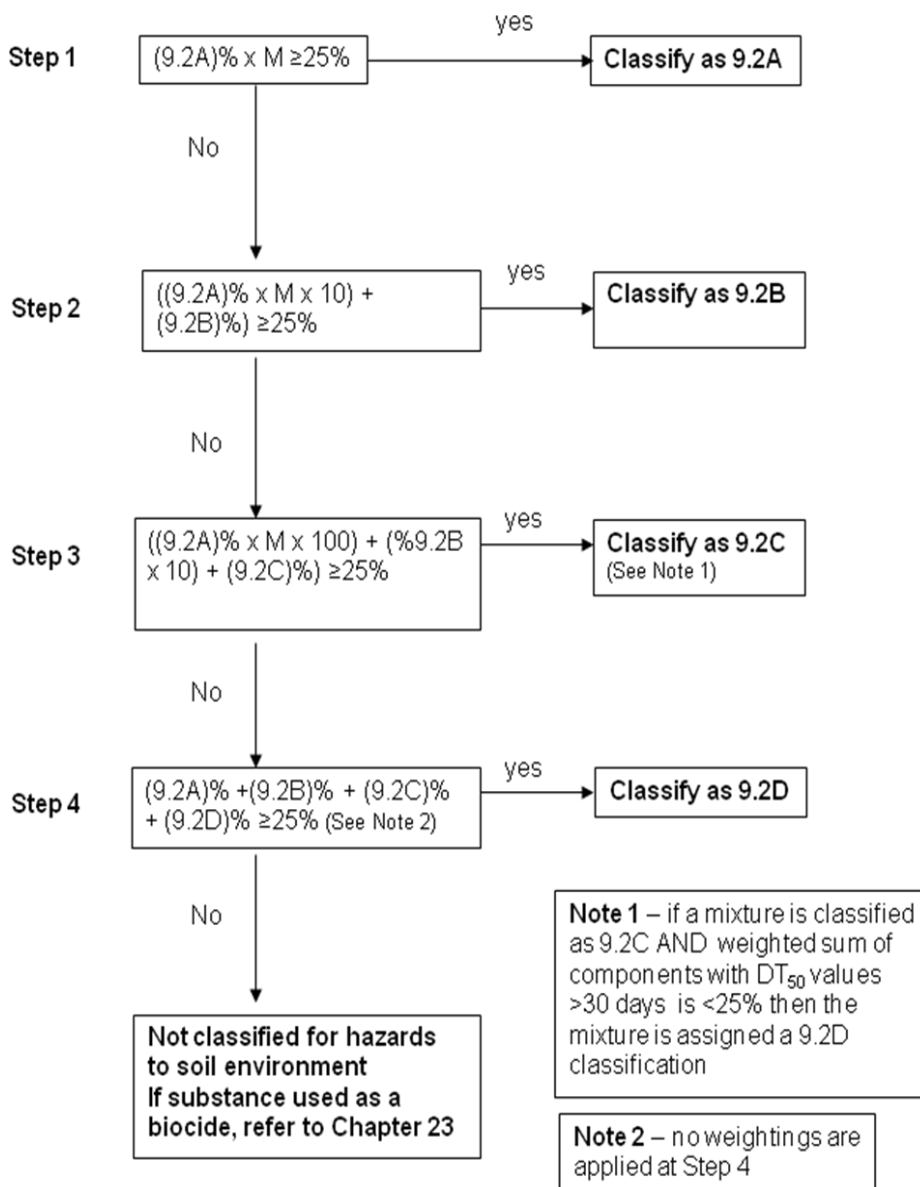
Consider the weighted sum of components with $DT_{50} > 30$ days

Mixture Y contains two components with a DT_{50} of > 30 days (components P and T). The weighted sum for these components

$\Rightarrow 100(0.05 \times 10) + 40 = 90\%$, which is $> 25\%$

therefore the mixture retains the 9.2C classification.

Figure 20.2: Classification of mixtures for hazards to the soil environment



Notes: M = multiplying factor.

References

Fairbrother, A, Glazebrook, PW, van Straalen, NM, Tarazona, JV (eds) 2002. *Test Methods to Determine the Hazards of Sparingly Soluble Metals in Soil*. SETAC Press, Pensacola FL.

FOCUS (2006) *Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration*, report of the FOCUS Work Group on Degradation Kinetics. <http://viso.jrc.it/focus/dk/docs/finalreportFOCDegKin04June06linked.pdf> Retrieved 8 October 2007.

Appendix 20A: Acceptable test methodologies for assessing toxicity to soil organisms and degradation in soil

20A.1 Introduction

Most of the guidelines mentioned in the tables in this appendix are found in compilations from the organisation issuing them. The main references are as follows, but other guidelines may be used where appropriate.

- European Commission (EC) guidelines:
European Commission 1997. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- International Organization for Standardization (ISO) guidelines:
Guidelines are available from the national standardisation organisations or ISO website (<http://www.iso.ch> Retrieved 14 August 2007).
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 18 September 2007.

20A.2 Soil organism toxicity test guidelines

The guidelines in Table 20A.1 are primarily relevant to substances which are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides, which include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in the aquatic environment.

For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011..

See also Table 20.8.

Table 20A.1: Test guidelines for assessing the acute toxicity of chemicals to soil-dwelling organisms

| Test protocol | Guideline number | | |
|---------------------------------------|--|---|---|
| | OECD | USEPA OPPTS | EC |
| Acute earthworm toxicity | 207: Earthworm, Acute Toxicity Tests | – | C.8 Toxicity for earthworms: artificial soil test |
| Terrestrial plant, growth test | 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test | 850.4230 Early seedling growth toxicity test (soil exposure only) | |
| Terrestrial plant, seedling emergence | | 850.4100 Terrestrial plant toxicity, Tier I (seedling emergence) | |
| Soil microbial community test | | 850.5100 Soil microbial community toxicity test | |
| Nitrogen transformation test | 216: Soil Micro-organisms: Nitrogen Transformation Test | – | C.21 Soil micro-organisms: nitrogen transformation test |
| Carbon transformation test | 217: Soil Micro-organisms: Carbon Transformation Test | – | C.22 Soil micro-organisms: carbon transformation test |

Table 20A.2: United States Environmental Protection Agency test guidelines for assessing toxicity of biopesticides to soil-dwelling organisms

| |
|--|
| 885.5000 Background for microbial pesticides testing |
| 885.4000 Background for non-target organism testing of microbial pest control agents |
| 885.4300 Non-target plant studies, Tier I |
| 885.4340 Non-target insect testing, Tier I |
| 885.5200 Expression in a terrestrial environment |

20A.3 Soil degradation test guidelines

Table 20A.3: Test guidelines for assessing the degradation of chemicals in soil

| Test protocol | Test guideline number | | |
|---------------------|---|------------------------------|---|
| | OECD | USEPA OPPTS | EC |
| Degradation in soil | 307: Aerobic and Anaerobic Transformation in Soil | 835.3300 Soil biodegradation | C.23 Aerobic and anaerobic transformation in soil |
| | 304A: Inherent Biodegradability in Soil | | |

Photolysis in soil

Phototransformation of
Chemicals on Soil
Surfaces Draft New
Guideline (2002 version)

Appendix 20B: Additional guidance – degradation in soil

20B.1 Methods for calculating DT_{50/90} values

General recommendations

These recommendations are from the European Commission (EC, 2000). For the calculations of DT₅₀ and DT₉₀ values in laboratory as well as field dissipation studies the following recommendations are given.

- For a sound regression analysis in calculating DT₅₀, at least five sampling times are required, including zero time.
- Care should be taken when using time points from near the end of soil degradation/field dissipation studies for the calculation of DT_{50/90} values, when the concentration of the remaining active substance is low (< 2–5% initial concentration), especially when concentrations are approaching the limit of quantification for the method of analysis for non-radiolabelled studies.
- Experience shows that DT₅₀ can usually be calculated from first-order kinetics, and this is the preferred method. The determination coefficient r² should be in a range between 0.85 and 1.0. In practice there will be many cases where r² will be lower than 0.85. In such situations it is advisable to distinguish whether a DT₅₀ is needed for modelling purposes or as a trigger value for further (field) studies. Since most models can handle only first-order kinetics, for pragmatic reasons the determination coefficient r² ≥ 0.7 can still be accepted. In order to trigger further studies a DT₅₀ value can be calculated according to the best fit. If the use of first-order kinetics to calculate degradation rates results in a determination coefficient of r² < 0.7, then other methods can be tested and used.
- As a first option, the approximation of two degradation or dissipation rates to first-order kinetics (one for the initial part and one for the later part of the degradation or dissipation process) should be tested, which may be shown up by a hinge point in the curve. Rather simple statistical methods are available in standard statistical software to show this. A hinge point can arise as a result of a change in the contribution to degradation of various processes over a period. For example, a hinge point may be caused by a significant decline of microbial activity or bioavailability in the soil or by adaptation. Therefore, the hinge point does not represent an instantaneous change in the degradation process but is the product of the limitations of sampling intensity, and does not reflect gradual changes in processes and possible bioavailability.
- The results of the fit give the first-order rate coefficient as one of the two regression coefficients. The DT₅₀ and DT₉₀ are calculated using the formulae:

$$DT_{50} = \frac{\ln 2}{k} \text{ and } DT_{90} = \frac{\ln 10}{k}$$

When there is a hinge point in the degradation curve, the calculation of the DT₉₀ is less simple, the complication may be taken into account on a case-by-case basis.

- Mathematical models that fit the data may be used (for example, Gustafson and Holden (1990); ModelMaker (no date); TopFit).

From the shape of the curve of concentration against time, one can decide whether a lag phase has to be taken into account. A lag phase may be assumed where at least three measurement points are more or less on a horizontal line. The length of the lag phase has to be reported. The DT₅₀ is then calculated by leaving out the experimental results within the lag phase. At least five sampling times (including zero time) must be available after excluding the three points of the lag phase.

Special aspects of laboratory studies

The following aspects should be taken into account when considering soil degradation studies in the laboratory.

- Often the DT_{90lab} is difficult or impossible to obtain for persistent compounds, because of the obvious problems with extrapolation beyond the end of study periods and the general problem with extremely long study durations making statistical analysis of the data very inaccurate. When first-order kinetics is applicable, then mathematically the DT_{90lab} can be estimated as three times DT_{50lab}. In addition to these points, loss of microbial activity of the soil might result in a decrease in the rate of degradation after approximately 2 to 4 months of incubation.
- Effect of temperature on the degradation rate, where relevant.
The Arrhenius equation is a validated relationship that can be used to describe temperature effects on transformation. As a guide, the DT₅₀ approximately doubles for each 10°C decrease in temperature. A Q10 value of 2.20 could reasonably be used to extrapolate DT₅₀ data derived at 20°C to expected values at 10°C. A Q10 value can also be calculated, if degradation studies have been carried out at different temperatures. In every case, the method used for calculating the compound-specific Q10 value should be clearly described.
(See EFSA (2006) for detailed guidance on using Q10 values for pesticides.)
- The methods described are also used for metabolites, breakdown or reaction products, where they are relevant from the toxicological, ecotoxicological, or environmental point of view, if separate studies with these substances are available.

References

EC 2000. *Guidance Document on Persistence in Soil*. European Commission.

http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkd0c11_en.pdf Retrieved 8 October 2007.

EFSA (European Food Safety Authority) 2006. Opinion of the scientific panel on plant protection products and their residues (PPR) related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. Question number: EFSA-Q-2005-058. *EFSA Journal* 322: 1–40.

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770752.htm

Gustafson, DI, Holden, LR 1990. Nonlinear pesticide dissipation in soil: a new model based on special variability. *Environmental Science and Technology* 24: 1032–1038.

ModelMaker No Date. Cherwell Scientific Publishing, Oxford, UK.

21. Terrestrial Vertebrate Ecotoxicity – Subclass 9.3

21.1. Basic elements and general considerations

The basic elements to consider in determining hazard classifications under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) for effects on terrestrial vertebrates are:

- acute mammalian toxicity (oral and dermal tests only);
- chronic mammalian toxicity;
- acute avian toxicity (oral gavage or short-term dietary test); and
- chronic avian toxicity.

See section 18.6 in chapter 18 [above](#) for definitions of the key terms used in this chapter.

21.1.1. Acute toxicity

Acute exposure to the substance is examined to determine the relationship between a single administered dose and the observed adverse effects to establish the substance's toxicity relative to other substances whose acute toxicity is known. By studying the effects, following administration by the most likely exposure routes (ingestion or absorption through the skin), the relative hazards of different pathways of exposure can be determined. Therefore, these studies identify highly toxic substances and provide information on the possible hazards that may occur where terrestrial organisms are exposed.

Ideally, acute toxicity data from both mammalian (oral and dermal tests) and avian sources (oral gavage or short-term dietary tests) will be available for classification purposes, with classification based on the most sensitive test result of either animal class.

21.1.2. Chronic toxicity

Chronic exposure to the substance is examined to determine the relationship between repeated administered doses and the observed long-term adverse effect to establish the substance's toxicity relative to other substances whose chronic toxicity is known. By studying the effects, following administration by the most likely exposure route (ingestion), the hazards can be determined. Therefore, these studies identify chronically toxic substances and provide information on the possible hazards that may occur where terrestrial organisms are exposed.

Conversion of data: Values expressed as no observable effect level, no observable adverse effect level, or no observable effect concentration rather than a maximum acceptable toxicant concentration

The maximum acceptable toxicant concentration (MATC) is the geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) that are derived from the same study.

- Qualitative prediction

As the NOEC gives a conservative estimate of the MATC, if the NOEC for a substance does not trigger the threshold, it can be assumed that the MATC will also not trigger the threshold.

- **Quantitative prediction**

The calculation of the MATC from a NOEC value can be problematic as none of the test guidelines requires doses to be in a specified concentration series. As a result, an up-front conversion factor cannot be used based on the maximum differences between the NOEC and LOEC.

Maximum acceptable toxicant concentration values expressed in units of parts per million diet or milligrams per kilogram body weight, while chronic threshold is limited to parts per million diet

The expression of the MATC in milligrams per kilogram (mg/kg) body weight is inconsistent with the chronic threshold (see 21.2.2 2(1)(c)(iii)). Equations to determine the average food intake per body weight for standard test species are provided by *Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC* (EC, 2002). To accurately determine the food intake for a species, the body weight and diet must be provided within the test report. Given these data, the units of dose can be converted from mg/kg body weight to ppm diet (mg/kg) following the equations, data tables, and examples found in Appendix 21C.

21.1.3. Metabolites

The substances may be transformed in the environment by abiotic or biotic processes. The potential hazards that these metabolites pose to terrestrial organisms must be evaluated when classifying the parent substance. An in-depth discussion of the classification of metabolites is in chapter 18 [above](#).

21.1.4. Weight of evidence

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Preferably, data should be derived using Organisation for Economic Co-operation and Development (OECD) test guidelines or equivalent, according to the principles of Good Laboratory Practice (GLP). Where such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

See Appendix 21B [below](#) for a detailed list of acceptable test methods for acute toxicity.

When experimental data for acute toxicity are available in several vertebrate species, scientific judgement should be used in selecting the most appropriate LD₅₀ or LC₅₀ value from among valid, well-performed tests.

21.2. Hazard thresholds and classification criteria for terrestrial vertebrate ecotoxicity

21.2.1. Thresholds



Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—

...

(c) the substance is ecotoxic to terrestrial vertebrates because—

- (i) *data for the substance indicates an acute avian or mammalian oral or dermal LD₅₀ of 2000 milligrams or less of the substance per kilogram of body weight, as a result of exposure to the substance; or*
- (ii) *data for the substance indicates an acute avian or mammalian LC₅₀ of 5000 parts or less of the substance per million in the diet, as a result of exposure to the substance; or*
- (iii) *data for the substance indicates a chronic avian or mammalian MATC of 100 parts or less of the substance per million in the diet, as a result of exposure to the substance.*

21.2.2. Classification

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 identifies three classification categories for substances that are ecotoxic to terrestrial vertebrates (subclass 9.3).

A subclass 9.3 classification and the subsequent category apply to any substance that meets the following criteria.

- Category 9.3A – substances that are very ecotoxic to terrestrial vertebrates
 - a. A substance for which data indicate an acute avian or mammalian (oral or dermal) LD₅₀ ≤ 50 milligrams of the substance per kilogram of bodyweight; or
 - b. A substance for which data indicate an acute avian or mammalian LC₅₀ ≤ 500 parts per million of the substance in the diet.
- Category 9.3B – substances that are ecotoxic to terrestrial vertebrates
 - a. A substance for which data indicate an acute avian or mammalian (oral or dermal) LD₅₀ > 50 milligrams, but ≤ 500 milligrams, of the substance per kilogram of bodyweight; or
 - b. A substance for which data indicate an acute avian or mammalian LC₅₀ > 500 parts per million, but ≤ 1,000 parts per million, of the substance in the diet.
- Category 9.3C – substances that are harmful to terrestrial vertebrates
 - a. A substance for which data indicate an acute avian or mammalian (oral or dermal) LD₅₀ > 500 milligrams, but ≤ 2,000 milligrams, of the substance per kilogram of bodyweight; or
 - b. A substance for which data indicate an acute avian or mammalian LC₅₀ > 1,000 parts per million, but ≤ 5,000 parts per million, in the diet; or
 - c. A substance for which data indicate a chronic avian or mammalian MATC ≤ 100 parts per million of the substance in the diet, but that does not meet the criteria for hazard classification 9.3A or 9.3B.

Note that assignment to category 9.1D due solely to biocidal action is discussed in chapter 23 [below](#).

21.2.3. Classification of single components

The terrestrial classification criteria for single component substances are summarised in Table 21.1 and Figure 21.1. The application of the criteria to mixtures is set out in more detail in section 21.2.

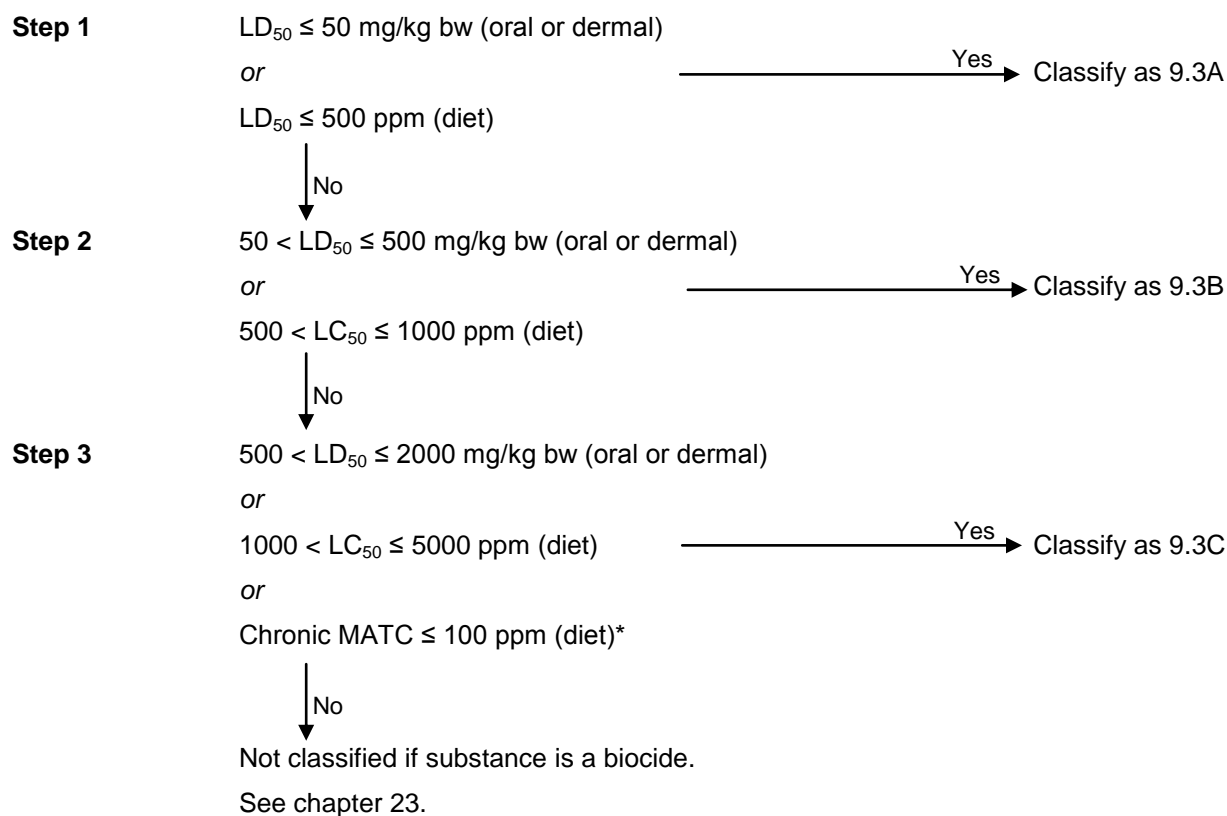
Table 21.1: Terrestrial vertebrate hazard classification of a single component substance

| Classification category | Measure |
|--|---|
| 9.3A (very ecotoxic to terrestrial vertebrates) | a. $LD_{50} \leq 50$ mg/kg bw (oral or dermal); or b. $LC_{50} \leq 500$ ppm (diet) |
| 9.3B (ecotoxic to terrestrial vertebrates) | a. $50 < LD_{50} \leq 500$ mg/kg bw (oral or dermal); or b. $500 < LC_{50} \leq 1,000$ ppm (diet) |
| 9.3C (harmful to terrestrial vertebrates) | a. $500 < LD_{50} \leq 2,000$ mg/kg bw (oral or dermal); or b. $1000 < LC_{50} \leq 5,000$ ppm (diet); or c. a chronic MATC ≤ 100 ppm (diet), but which does not meet the criteria for classifications 9.3A or 9.3B. |
| Substance classified as non-hazardous* | a. $>2,000$ mg/kg bw (oral or dermal); or b. $>5,000$ ppm (diet); or c. a chronic MATC > 100 ppm (diet). |

Notes: LC_{50} = median lethal concentration; LD_{50} = median lethal dose; MATC = maximum acceptable toxicant concentration; ppm = parts per million.

* Unless intended for biocidal use, in which case 9.1D applies (see chapter 23 [below](#))

Figure 21.1: Terrestrial vertebrate hazard classification of a single component



*Note 1: if substance does not meet criteria for 9.3A or 9.3B classification

Notes: LC_{50} = median lethal concentration; LD_{50} = median lethal dose; MATC = maximum acceptable toxicant concentration; ppm = parts per million.

21.3. Classification of mixtures

To make use of all available data for the purpose of classifying the terrestrial vertebrate hazards of a mixture, the following assumption has been made and is applied where appropriate.

The 'relevant components' of a mixture are those that are present in a concentration of 1% (by weight – w/w) or greater, unless there is a presumption (for example, in the case of highly toxic components) that a component present at less than 1% can still be relevant for classifying the mixture for terrestrial vertebrate hazards.

The approach for classifying terrestrial vertebrate hazards is tiered, and depends on the type of information available for the mixture itself and for its components. Elements of the tiered approach include classification based on:

- tested mixtures (see section 21.3.1);
- bridging principles (see section 21.3.2); and

- a summation approach, using the classifications of the mixture components (see section 21.3.3).

21.3.1. Tested mixtures

For terrestrial vertebrate hazard classification, the test data on the mixture can be used directly to assign a substance to a classification as indicated in Table 21.2. Where components of the mixture are toxic, the concentrations of components with these properties are summed to determine the classification of the mixture. Where the sum of these components is $\geq 25\%$, the more conservative classification applies.

Table 21.2: Terrestrial vertebrate hazard classification of tested mixtures

| Classification category | Acute L(D)C ₅₀ of the tested mixture | Chronic maximum acceptable toxicant concentration (MATC) of the tested mixture |
|-------------------------|--|--|
| 9.3A | 1. ≤ 50 mg/kg bw (oral or dermal); or 2. ≤ 500 ppm (diet) | |
| 9.3B | a. $50 < LD_{50} \leq 500$ mg/kg bw (oral or dermal); or b. $500 < LC_{50} \leq 1,000$ ppm (diet) | |
| 9.3C | a. $500 < LD_{50} \leq 2,000$ mg/kg bw (oral or dermal); or b. $1000 < LC_{50} \leq 5,000$ ppm (diet) | MATC ≤ 100 ppm (diet) but which does not meet the criteria for hazard classification 9.3A or 9.3B |
| Non-hazardous* | a. $LD_{50} > 2,000$ mg/kg bw (oral or dermal); or b. $LC_{50} > 5,000$ ppm (diet) | MATC > 100 ppm (diet) |

Notes: LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; L(D)C₅₀ = LD₅₀ or LC₅₀; ppm = parts per million.

* Unless intended for biocidal use, in which case 9.1D applies (see chapter 23 [below](#)).

21.3.2. Bridging principles

Guidance on the bridging principles for classifying mixtures without test data is in chapter 18 [above](#).

21.3.3. Classification of a mixture based on the classification of components: Summation approach

When test data of the mixture are not available and the bridging principles are not applicable, the summation approach is used to derive a terrestrial hazard classification for the mixture.

Rationale

The toxicity criteria for the terrestrial classification categories differ by a factor of 10 in moving from higher to lower categories. Substances with a classification in a high toxicity band may, therefore, contribute to the classification of a mixture in a lower band. The calculation of these classification categories, therefore, needs to consider the contribution of all substances that are classified for terrestrial toxicity.

When components are classified as 9.3A and their acute toxicity is well below the cut-off value (median lethal dose (LD_{50}) \ll 5 mg/kg bodyweight or median lethal concentration (LC_{50}) \ll 50 parts per million (ppm) diet) they contribute to the toxicity of the mixture even if they are present at a low concentration. Under these circumstances the application of the normal cut-off values/concentration limits may lead to an 'under-classification' of the mixture. Therefore, multiplying factors are applied to account for highly toxic components.

Classification procedure

In general, a more severe classification for mixtures overrides a less severe classification, for example, a 9.3A classification overrides a 9.3B classification. The classification is complete as a more severe classification than 9.3A is not possible.

First, all components classified as 9.3A are considered. If the sum of these components is $\geq 25\%$ the whole mixture is classified as 9.3A. If the result of the calculation is a classification of the mixture as 9.3A, the classification process is complete.

The steps to follow in applying the summation approach to terrestrial hazard classification are set out below and summarised in Table 21.3 [below](#) and Figure 21.2 [below](#).

Mixtures with no highly toxic components

The steps to follow in applying the summation approach to terrestrial hazard classification for mixtures with no highly toxic components are set out below.

- Step 1: Consider all components classified as 9.3A.
If:
$$\sum(9.3A)\% \geq 25\%$$
then the mixture is classified as 9.3A, and the classification process is complete.
- Step 2: Consider all components classified as 9.3A and 9.3B.
If:
$$(\sum(9.3A)\% \times 10) + \sum(9.3B)\% \geq 25\%$$
then the mixture is classified as 9.3B, and the classification process is complete.
- Step 3: Consider all components classified as 9.3A, 9.3B, and 9.3C.
If:
$$(\sum(9.3A)\% \times 100) + (\sum(9.3B)\% \times 10) + \sum(9.3C)\% \geq 25\%$$
then the mixture is classified as 9.3C, and the classification process is complete.

The exception to this is where the substance is used as a biocide. See chapter 23 [below](#) for further guidance.

Table 21.3: Classification of a mixture for terrestrial vertebrate ecotoxicity based on the summation of classified components

| Process | Summation formulae | Cut-off | Classification |
|---------|---------------------------------------|---------|----------------|
| Step 1 | 9.3A x M | ≥ 25% | 9.3A |
| Step 2 | (M x 10 x 9.3A) + 9.3B | ≥ 25% | 9.3B |
| Step 3 | (M x 100 x 9.3A) + (10 x 9.3B) + 9.3C | ≥ 25% | 9.3C |
| Step 4 | (M x 100 x 9.3A) + (10 x 9.3B) + 9.3C | < 25% | Not hazardous* |

Notes: M = multiplying factor.

* Unless intended for biocidal use, in which case 9.1D applies (see chapter 23 [below](#)).

Mixtures with highly toxic components

In applying the summation of classified components approach, more weight should be given to highly ecotoxic components. When a mixture contains components classified as 9.3A, the tiered approach described above should be applied using a weighted sum by multiplying the concentrations of 9.3A components by a factor, instead of merely adding up the percentages. The multiplying factors to be applied to the component are summarised in the Table 21.4. Therefore, to classify a mixture containing highly toxic components, the classifier needs to apply the multiplying factor (M) in assigning a terrestrial hazard classification to the mixture.

The multiplying factors to be applied to highly toxic components are set out in Table 21.4. See 'Mixtures with highly ecotoxic components (multiplication factors)' and Table 21.5 for a worked example.

Table 21.4: Multiplying factors for highly ecotoxic components of mixtures

| LD ₅₀ (mg/kg body weight)/LC ₅₀ (ppm) | Multiplying factor (M) |
|--|------------------------|
| 5 < LD ₅₀ ≤ 50 50 < LC ₅₀ ≤ 500 | 1 |
| 0.5 < LD ₅₀ ≤ 5 5 < LC ₅₀ ≤ 50 | 10 |
| 0.05 < LD ₅₀ ≤ 0.5 0.5 < LC ₅₀ ≤ 5 | 100 |
| 0.005 < LD ₅₀ ≤ 0.05 0.05 < LC ₅₀ ≤ 0.5 | 1,000 |
| 0.0005 < LD ₅₀ ≤ 0.005 0.005 < LC ₅₀ ≤ 0.05 | 10,000 |
| (continue in factor of 10 intervals) | |

Note: LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; ppm = parts per million.

Mixtures with highly ecotoxic components (multiplication factors)

The steps to follow in applying the summation approach to terrestrial hazard classification for mixtures with highly ecotoxic components are set out below Table 21.5.

Table 21.5: Example of the summation approach for a mixture containing highly toxic components

| Component | LD ₅₀ (mg/kg bw) | Individual substance (100%) classification | Concentration in mixture (%) | Multiplying factor | Weighted concentration of individual substance in mixture (%) |
|-----------|--------------------------------|---|------------------------------------|-----------------------|---|
| B | 55 | 9.3B | 5 | - | 5 |
| P | 0.2 | 9.3A | 0.05 | 100 | 5 |
| Q | 9 | 9.3A | 1 | - | 1 |
| T | 1,000 | 9.3C | 40 | - | 40 |
| U | Not classified | Not classified | 53.95 | - | 53.95 |

Note: bw = bodyweight; LD₅₀ = median lethal dose.

- Step 1

Component P is highly ecotoxic and attracts a multiplier of 100, resulting in a weighted concentration of that component of 5%.

Component Q, although classified as 9.3A is not given addition weighting, that is:

$$\Rightarrow (100 \times P) + Q$$

$$\Rightarrow (100 \times 0.05\%) + 1\% = 6\% , \text{ which is } < 25\%$$

so the mixture Z is not classified as 9.3A.

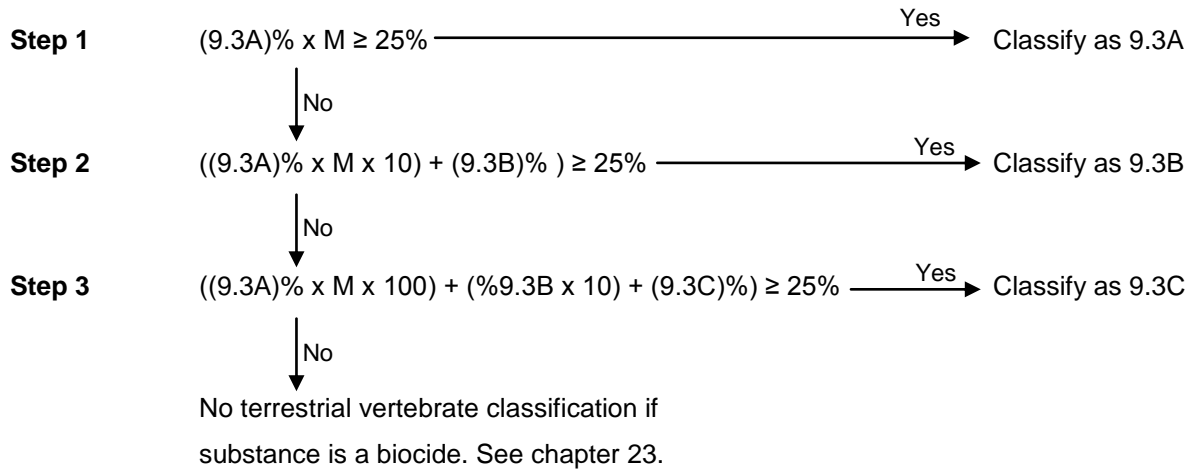
- Step 2: Consider components classified as 9.3A and 9.3B.

$$\Rightarrow 10((100 \times P) + Q) + B$$

$$\Rightarrow 10((100 \times 0.05\%) + 1\%) + 5\% = 60\% + 5\% = 65\% , \text{ which is } \geq 25\%$$

so the mixture is classified as 9.3B

Figure 21.2: Terrestrial vertebrate hazard classification of mixtures



References

EC 2002. Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC, SANCO/4145/2000 – final. European Commission.

http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc19_en.pdf

Appendix 21A: Classification notes for avian studies

Appendix contents

21A.1 Avian acute oral toxicity

Regurgitation can substantially reduce the dose absorbed by birds in acute oral toxicity tests. Therefore, evaluation of avian acute oral tests should include whether regurgitation or emesis has occurred. If so, it may be appropriate to repeat the study using birds that do not regurgitate, in particular if a high-risk use such as seed treatment is being assessed.

For example, if regurgitation is observed in an acute oral toxicity test at 500, 1,000, and 2,000 mg active substance/kg body weight (bw), but not at 200 mg a.s./kg bw, and if there is no mortality at 200 mg a.s./kg bw, then the conclusion is valid that the median lethal dose (LD_{50}) is > 200 mg/kg bw. Although this figure cannot be used for classification purposes it may be used in the initial risk assessment. If this assessment raises concern, then either an acute or a dietary study would be requested using a bird species that does not regurgitate. If the initial assessment does not raise concern, no further data will be requested. Sometimes regurgitation may occur in all doses while mortality occurs only in the top doses, that is, regurgitation is not sufficient to protect birds. Also, in this situation, a further study with a non-regurgitating species would be required.

21A.2 Avian short-term dietary toxicity

When the test diet has been analysed the results should be reported in the monograph. According to OECD guideline 205, a deviation up to 20% between measured feed concentrations and nominal values is considered acceptable. In the case of larger deviations, toxicity figures should be recalculated using effective concentrations.

21A.3 Avian reproduction

It should be noted that low acute and dietary avian toxicity are not sufficient to indicate a low reproductive toxicity. A reproductive toxicity study should always be conducted unless it can be demonstrated that the exposure of birds (adults and young) does not occur during the breeding season. When all relevant species are considered, the breeding season could be rather long and even short exposure periods may give rise to concern about potential reproductive effects. Thus, in the case of foliar applications during the breeding season, for example, the test should normally be required even if only one treatment per season is intended.

Reproductive data are always required for substances that are generally persistent or have a bio-accumulation potential.

Appendix 21B: Acceptable test methods for terrestrial vertebrate toxicity

21B.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The main references to international guidelines referred to in the tables in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1997. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 18 September 2007.
- International Organization for Standardization (ISO) guidelines:
Guidelines are available from the national standardisation organisations or the ISO website <http://www.iso.ch> Retrieved 18 September 2007.
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 18 September 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 18 September 2007.
- ASTM International (ASTM) guidelines are available from the ASTM homepage (<http://www.astm.org> search on 'standards').

21B.2 Terrestrial vertebrate toxicity test guidelines

The guidelines in Table 21B.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in the terrestrial environment.

For tests specific to the testing of microbial biopesticides, see:

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.
http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series Retrieved 18 September 2007.

See also Table 21B.2.

Table 21B.1: Terrestrial vertebrate toxicity test guidelines for chemicals, including mixtures

| Species | Test guideline number | | |
|--------------------------------------|--|--------------------|-------------|
| | OECD | EC | USEPA OPPTS |
| Mammalian acute | | | |
| Acute oral toxicity | 401: Acute Oral Toxicity* | None | 870.11001 |
| | 420: Acute Oral Toxicity – Fixed Dose Procedure | EC Method B.1 bis | None |
| | 423: Acute Oral Toxicity – Acute Toxic Class Method | EC Method B.1 tris | None |
| | 425: Acute Oral Toxicity – Up and Down Procedure | None | None |
| Acute dermal toxicity | 402: Acute Dermal Toxicity | EC Method B.3 | 870.1200 |
| Avian acute | | | |
| Acute oral toxicity | None | None | 850.2100 |
| Acute dietary toxicity | 205: Avian Dietary Toxicity Test | None | 850.2200 |
| Mammalian chronic | | | |
| Rodent sub-chronic oral toxicity | 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents | EC Method B.26 | 870.3100 |
| Non-rodent sub-chronic oral toxicity | 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents | EC Method B.27 | 870.3150 |
| Avian chronic | | | |
| Reproduction Test | 206: Avian Reproduction Test | None | 850.2300 |

Note

* OECD Test Guideline 401 was deleted from the OECD manual of internationally accepted test guidelines on 17 December 2002. Acute oral toxicity studies conducted after this date should now adhere to one of the three alternative methods (OECD Codes 420, 423 and 425).

Table 21B.2: Terrestrial vertebrate toxicity test guidelines for microbial biopesticides

| USEPA OPPTS guidelines |
|---|
| 885.0001 Overview for microbial pest control agents |
| 885.3050 Acute oral toxicity/pathogenicity |
| 885.3100 Acute dermal toxicity/pathology |
| 885.3150 Acute pulmonary toxicity/pathogenicity |

885.3550 Acute toxicology, Tier II

885.3600 Subchronic toxicity/pathogenicity

885.4000 Background for non-target organism testing of microbial pest control agents

885.4050 Avian oral, Tier I

885.4100 Avian inhalation test, Tier I

885.4150 Wild mammal testing, Tier I

885.4600 Avian chronic pathogenicity and reproduction test, Tier III

885.5000 Background for microbial pesticides testing

885.5200 Expression in a terrestrial environment

Appendix 21C: Maximum acceptable toxicant concentration conversion from milligrams per kilogram bodyweight to parts per million diet (milligrams per kilogram diet)

21C.1 Estimation of average daily food intake

For a species of a given weight, allometric equations can be used to predict its daily energy expenditure. (See Crocker et al, 2002.) Knowing the energy value and moisture content of the diet, and the efficiency with which the species digests the diet, we may calculate the average amount of food the organism is likely to eat in a day, using:

Daily food intake (wet g) =

$$\frac{\text{Daily energy expenditure (kJ)}}{\text{Energy in food (kJ/g dry)} \times (1 - \text{moisture in food}) \times \text{assimilation efficiency}}$$

where moisture and assimilation efficiency are proportions between 0 and 1.

The equation for daily energy expenditure (DEE) is:

$$\text{Log}_{10}(\text{DEE}) = \text{Log}_{10} a + (b \times (\text{Log}_{10} \text{body weight (g)})).$$

Where a and b are given in Table 21C.1 for birds and Table 21C.2 for mammals.

For both birds and mammals, a strong relationship exists between body weight and DEE. In addition, there are significant differences between taxonomic groups and between species occupying different habitats (Nagy, 1987; Nagy et al, 1999). Therefore, separate equations are calculated for passerines (perching birds), sea birds, desert birds, hummingbirds, and others. Placental mammals (eutherians) are similarly divided into non-eutherians, desert eutherians, sea eutherians, and terrestrial eutherians. These equations are presented in Table 21C.1 and Table 21C.2. Also shown are the standard errors (SE) for a and b, the number of species in each group (N), and the proportion of variation explained by each equation.

Table 21C.1: Relationship between body weight and daily energy expenditure in birds for five groups of avian species

| Group | Log ₁₀ a | SE Log ₁₀ a | b | SE b | N | r ² |
|--------------|---------------------|------------------------|--------|--------|----|----------------|
| Desert | 0.6107 | 0.1727 | 0.7299 | 0.0663 | 7 | 0.95 |
| Hummingbirds | 0.7495 | 0.0822 | 1.2064 | 0.1090 | 5 | 0.97 |
| Other | 0.6768 | 0.1896 | 0.7723 | 0.0861 | 11 | 0.89 |
| Passerine* | 1.0017 | 0.0647 | 0.7034 | 0.0503 | 38 | 0.84 |
| Seabird | 1.1482 | 0.1022 | 0.6521 | 0.0356 | 35 | 0.91 |
| All birds | 1.0220 | 0.0392 | 0.6745 | 0.0180 | 96 | 0.94 |

Notes: Variables a and b are given in Table 21C.1 (birds) and Table 21C.2 (mammals); N = number of species in each group; r^2 = proportion of variation explained by each equation; SE = standard error.

* Excluding marine and desert passerines.

Table 21C.2: Relationship between body weight and daily energy expenditure DEE in mammals for five groups of mammalian species

| Group | Log ₁₀ a | SE Log ₁₀ a | b | SE b | N | r ² |
|-------------------|---------------------|------------------------|--------|--------|----|----------------|
| Non-eutherians | 1.0232 | 0.0749 | 0.5814 | 0.0251 | 19 | 0.97 |
| All eutherians | 0.6794 | 0.0445 | 0.7646 | 0.0173 | 54 | 0.97 |
| Desert eutherians | 0.5120 | 0.0625 | 0.7843 | 0.0290 | 18 | 0.98 |
| Marine eutherians | 2.4203 | 0.7592 | 0.4266 | 0.1567 | 6 | 0.56 |
| Other eutherians* | 0.8459 | 0.0526 | 0.7050 | 0.0250 | 30 | 0.96 |
| All mammals | 0.7401 | 0.0467 | 0.0250 | 0.0174 | 73 | 0.96 |

Notes: N = number of species in each group; r² = proportion of variation explained by each equation; SE = standard errors.

* Excluding marine and desert eutherians.

21C.2 Moisture and energy content of foods

The means for 15 major groups of food types are in Table 21C.3.

Table 21C.3: Energy and moisture contents for 15 general categories of food type

| Food type | Energy content (kJ/g dry weight) n = 1,783 | Moisture content (%) n = 761 |
|----------------------------|--|---------------------------------|
| Dicotyledenous crop leaves | 11.2 | 88.6 |
| Grasses and cereal shoots | 18.0 | 76.4 |
| Non-grass herbs | 18.0 | 82.1 |
| Tree leaves | 20.7 | 51.4 |
| Orchard topfruit | 11.6 | 83.7 |
| Cereal seeds | 16.7 | 13.3 |
| Weed seeds | 21.0 | 11.9 |
| Small mammals | 21.7 | 68.6 |
| Bird and mammal carrion | 22.6 | 68.8 |
| Arthropods | 21.9 | 70.5 |

| | | |
|-----------------------|------|------|
| Caterpillars | 21.7 | 79.4 |
| Soil invertebrates | 19.3 | 84.6 |
| Fish | 20.7 | 71.1 |
| Aquatic invertebrates | 19.6 | 77.3 |
| Aquatic vegetation | 15.0 | 81.4 |

21C.3 Assimilation efficiency

The main categories used to calculate the daily food intake are listed in Table 21C.4 (for birds) and Table 21C.5 (for mammals).

Table 21C.4: Assimilation efficiencies for birds

| Order | Bird | No. of species | No. of cases | Food type | | | | | |
|-------------------|-------------------------------|----------------|--------------|-----------|--------|----------|-------|-------|-------------|
| | | | | Animal | Fruits | Herb-age | Seeds | Sugar | Arti-ficial |
| Struthioniformes | Ostriches | 2 | 6 | | | 36 | | | |
| Gruiformes | Cranes, coots, rails | 1 | 5 | 34 | | 59 | | | 69 |
| Ralliformes | Coots, rails | 1 | 1 | | | | | | |
| Charadriiformes | Gulls, waders | 7 | 19 | 69 | | | | | 74 |
| Lariformes | Gulls, terns | 1 | 3 | 79 | | | | | |
| Alciformes | Auks | 1 | 2 | 76 | | | | | |
| Sphenisciformes | Penguins | 7 | 26 | 75 | | | | | |
| Procellariiformes | Petrels | 2 | 3 | 87 | | | | | |
| Pelecaniformes | Pelicans, gannets, cormorants | 4 | 8 | 80 | 76 | | | | |
| Columbiformes | Pigeons | 4 | 36 | | | | | | 76 |
| Psittaciiformes | Parrots | 1 | 4 | | | | | 96 | |
| Strigiformes | Owls | 6 | 45 | 77 | | | | | |
| Falconiformes | Eagles, | 4 | 12 | 84 | | | | | |

| | falcons | | | | | | | | |
|------------------------|-------------------------|----|-----|----|----|----|----|----|----|
| Accipitriformes | Hawks | 11 | 22 | 82 | | | | | |
| Ciconiiformes | Hérons, storks | 4 | 8 | 80 | | | | | |
| Anseriformes | Ducks, geese | 22 | 98 | 87 | | 41 | 83 | | 74 |
| Galliformes | Fowl | 18 | 184 | 70 | 57 | 42 | 65 | | 67 |
| Opisthocomi- formes | Hoatzin (S. America) | 1 | 2 | | | | | | 74 |
| Trochiliformes | Hummingbirds | 7 | 16 | | | | | 98 | |
| Coliiformes | Mousebirds (Africa) | 4 | 15 | | 56 | | | | 73 |
| Piciformes | Woodpeckers | 1 | 14 | 64 | | 61 | | | 80 |
| Passeriformes | Passerines | 67 | 441 | 76 | 67 | 76 | 80 | 90 | 72 |

Source: Bairlein (1999).

Table 21C.5: Assimilation efficiencies for mammals, based on 91 published examples

| Mammal group | Food type | No. of studies | Mean | Standard deviation |
|-------------------|--------------------------------|----------------|------|--------------------|
| Shrews and bats | Insects | 8 | 88 | 5.9 |
| Carnivores | Vertebrates | 16 | 85 | 5.8 |
| Squirrels | Nuts | 10 | 85 | 7.5 |
| Small mammals | Nuts and seeds | 11 | 83 | 8.5 |
| Small mammals | Grasses | 15 | 46 | 10.7 |
| Small mammals | Crops, forbs, mixed vegetation | 17 | 74 | 12.3 |
| Lagomorphs | General vegetation | 4 | 74 | 13.5 |
| White tailed deer | Tree browse | 7 | 32 | 8.4 |
| Ruminants | Hay and browse | 3 | 80 | 2.8 |

Example

Conversion of a maximum acceptable toxicant concentration (MATC) of 40 mg a.i./kg body weight per day from OECD 408 (average rodent body weight taken as 0.250 kg) to parts per million (ppm) diet:

- Step 1: Convert milligrams per kilogram body weight per day (mg/kg bw/day) to exposure as milligrams per day (mg/day):
= mg chemical/kg bw/day × bw (kg)

$$= 40 \text{ mg a.i./kg bw/day} \times 0.250 \text{ kg}$$

$$= 10 \text{ mg/day}$$

- Step 2: Calculate rodent daily energy expenditure (DEE):

$$\text{Log}_{10} (\text{DEE}) = \text{Log}_{10} a + (b \times (\text{Log}_{10} \text{bw (g)}))$$

$$\text{Log}_{10} (\text{DEE}) = 0.6794 + (0.7646 \times (\text{Log}_{10} 250)^*)$$

$$\text{Log}_{10} (\text{DEE}) = 0.6794 + (0.7646 \times 2.398)$$

$$\text{Log}_{10} (\text{DEE}) = 2.513$$

$$\text{DEE} = 325.77 \text{ kJ}$$

* Values for $\text{Log}_{10} a$ and b taken from Table 21C.2, 'All eutherians'.

- Step 3: Calculate rodent daily food intake (kJ):

$$\text{Daily Food Intake (wet g)} =$$

$$\frac{\text{Daily energy expenditure (kJ)}}{\text{Energy in food (kJ/g dry)} \times (1 - \text{moisture in food}) \times \text{assimilation efficiency}}$$

Energy in food (kJ/g dry) \times (1 – moisture in food) \times assimilation efficiency

where moisture and assimilation efficiency are proportions between 0 and 1.

$$\text{Daily food intake} = 325.77 / (16.7 \times (1 - 0.133) \times 0.83)$$

$$\text{Daily food intake} = 325.77 / 12.02$$

$$\text{Daily food intake} = 27.10 \text{ wet g}$$

Where:

Energy in food = 16.7** (Table 21C.3, food type = cereal seeds)

Moisture in food = 13.3%** (Table 21C.3, food type = cereal seeds)

Assimilation efficiency = 83%** (Table 21C.5, food type: nuts and seeds)

** Assumption: Closest match to diet of laboratory animals.

- Step 3: Convert milligrams per day to ppm (as milligrams substance per kilogram diet (mg/kg diet)

$$= (\text{mg/day}) / (\text{kg diet/day})$$

$$= (10 \text{ mg/day}) / (0.0271 \text{ kg diet/day})$$

$$= 368.97 \text{ mg/kg diet}^{-1} \text{ (ppm)}.$$

Therefore, an MATC of 40 mg/kg body weight for 0.250 kg rodents can be considered equivalent to 369 ppm diet.

References

Bairlein, F 1999. Energy and nutrient utilization efficiencies in birds – a review. In: Adams, N, Slowtow, R (eds) *Proceedings of the 22nd International Ornithological Congress*. Durban Birdlife, South Africa.

Crocker, D, Hart, A, Gurney, J, McCoy, C 2002. *Methods for Estimating Daily Food Intake of Wild Birds and Mammals*. Central Science Laboratory, Department for Environment, Food and Rural Affairs, York, United Kingdom. http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Research_PN0908.pdf Retrieved 27 September 2007.

Nagy, KA 1987. Field metabolic rate and food requirement scaling in mammals and birds. *Ecological Monographs* 57: 111–128.

Nagy, KA, Girard, IA, Brown, TK 1999. Energetics of free-ranging mammals, reptiles and birds. *Annual Review of Nutrition* 19: 247–277.

22. Terrestrial Invertebrate Ecotoxicity – Subclass 9.4

22.1. Basic elements and general considerations

The basic element to consider in determining hazard classification under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) for effects on terrestrial invertebrates is acute toxicity to terrestrial invertebrates

While data from internationally harmonised test methods are preferred, in practice, data from national methods may also be used where they are considered equivalent. In general, test data are to be derived using Organisation for Economic Co-operation and Development (OECD) test guidelines or equivalent according to the principles of Good Laboratory Practice (GLP). Where such data are not available, classification should be based on the best available data.

See section 18.6 in chapter 18 [above](#) for definitions of the key terms used in this chapter.

See section 1.3 in chapter 1 [above](#) for information about assessing data quality.

See Appendix 22A [below](#) for a detailed list of acceptable test methods.

22.1.1. Acute toxicity to terrestrial invertebrates

The toxicity of substances to terrestrial invertebrates is assessed by oral and contact toxicity.

The usual acute tests for effects on terrestrial invertebrates used for HSNO Act classification are:

- 48-hour LD₅₀ for acute oral toxicity to honeybees (OECD 213 or equivalent); and
- 48-hour LD₅₀ for acute contact toxicity to honeybees (OECD 214 or equivalent).

The lowest value from these tests, with the results expressed in µg/ terrestrial invertebrate, is used to classify the substance.

Guidelines (Society of Environmental Toxicology and Chemistry and European and Mediterranean Plant Protection Organisation (Candolfi et al, 2000)) are available to assess the effects of plant protection products to non-target arthropods (other than honeybees). The approaches of these tests differ from the HSNO Act threshold as they are based on field application rates, which mean these test data cannot be readily compared with the threshold. The guidelines for honeybees use the same units of micrograms per bee as the in HSNO Act for its threshold, and can be readily used.

Conversion of data

The results of a feeding toxicity test can be expressed as a median lethal concentration (LC₅₀) (milligrams of substance in diet). These results can be converted to the threshold format by multiplying the average quantity of treated diet per bee (µL) by the concentration of substance in the diet per µL. The 'feeding test' guideline (SETAC) states that the treated diet should be prepared such that an average of 10–20 µL of diet is consumed by each bee.

Therefore, if the LC₅₀ for a substance is 1 µg/µL of diet and 10 µL of diet was consumed, the LD₅₀ would be:

$$1 \mu\text{g/L} \times 10 \mu\text{L/bee} = 10 \mu\text{g/bee}$$

This approach is valid only if:

- the average amount of diet consumed per bee is directly measured in the test;
- the average amount of diet can be predicted due to the feed being restricted to the quantity readily consumed within the exposure period (4 h for SETAC) test; and
- there are no obvious reductions in food palatability.

22.1.2. Metabolites

The substances may be transformed in the environment by abiotic or biotic processes. The potential hazards that these metabolites pose to terrestrial organisms must be evaluated when classifying the parent substance. An in-depth discussion of the classification of metabolites is in chapter 18.

22.2. Terrestrial invertebrate hazard threshold and classification criteria

22.2.1. Terrestrial invertebrate hazard threshold criteria

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—

...

(d) the substance is ecotoxic to terrestrial invertebrates because data for the substance indicates an acute oral or contact LD₅₀ of 25 micrograms or less of the substance per terrestrial invertebrate, as a result of exposure to the substance.

If data for the substance meet the above criteria, then the substance needs to be assigned a terrestrial invertebrate classification.

22.2.2. Classification

Schedule 6 to the Hazardous Substances (Classification) Regulations 2001 specifies three classification categories for substances that are ecotoxic to terrestrial invertebrates (subclass 9.4).

A subclass 9.4 classification and the subsequent category apply to any substance that meets the following criteria.

- Category 9.4A – substances that are very ecotoxic to terrestrial invertebrates:
A substance for which data indicate a contact or an oral LD₅₀ < 2 micrograms of substance per terrestrial invertebrate.
- Category 9.4B – substances that are ecotoxic to terrestrial invertebrates:
A substance for which data indicate a contact or an oral LD₅₀ ≥ 2 but < 11 micrograms of substance per terrestrial invertebrate.

- Category 9.4C – substances that are harmful to terrestrial invertebrates:

A substance for which data indicate a contact or an oral $LD_{50} \geq 11$ but ≤ 25 micrograms of substance per terrestrial invertebrate.

If the substance is used as a biocide and does not trigger classification under subclass 9.4, see chapter 23.

The classification criteria for single-component substances are summarised in Table 22.1 and Figure 22.1.

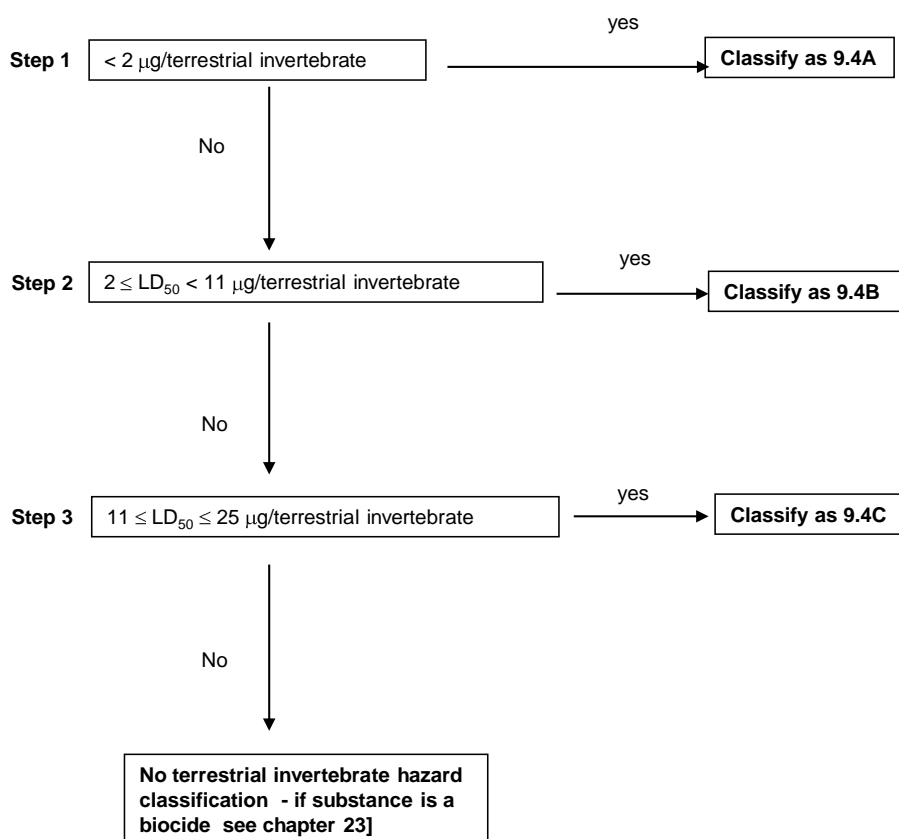
The application of the criteria to mixtures is set out in more detail in section 22.3.

Table 22.1: Terrestrial invertebrate classification of a single substance

| Acute LD_{50} of the tested mixture | Classification of substance |
|---|-----------------------------|
| $< 2 \mu\text{g}/\text{terrestrial invertebrate}$ | 9.4A |
| $2 \leq LD_{50} < 11 \mu\text{g}/\text{terrestrial invertebrate}$ | 9.4B |
| $11 \leq LD_{50} \leq 25 \mu\text{g}/\text{terrestrial invertebrate}$ | 9.4C |
| $> 25 \mu\text{g}/\text{terrestrial invertebrate}$ | Not classified as hazardous |

Note: LD_{50} = median lethal dose.

Figure 22.1: Terrestrial invertebrate hazard classification of a single component



22.3. Classification of substances

To make use of all available data for classifying the hazards of the mixture to terrestrial invertebrates, the following assumption has been made and is applied where appropriate.

The 'relevant components' of a mixture are those that are present in a concentration of 1% (by weight – w/w) or greater, unless there is a presumption (for example, in the case of highly toxic components) that a component present at less than 1% can still be relevant for classifying the mixture for hazards to terrestrial invertebrates.

The approach for classifying hazards to terrestrial invertebrates is tiered, and depends on the type of information available for the mixture itself and for its components. Elements of the tiered approach include classification based on:

- tested mixtures (see section 22.3.1);
- bridging principles (see section 22.3.2); and
- a summation approach using the classifications of components (see section 22.3.3).

22.3.1. Tested mixtures

For hazard classification to terrestrial invertebrates, the test data on the mixture can be used directly to assign a classification to a substance as indicated in the examples below (see Table 22.2).

Where components of the mixture are toxic, the concentrations of components with these properties are summed to determine the classification of the mixture. Where the sum of these components is $\geq 25\%$, then the more conservative classification applies.

Table 22.2: Classification of terrestrial invertebrates of tested mixtures

| Acute LD ₅₀ of the tested mixture | Classification of mixture |
|--|-----------------------------|
| < 2 µg/terrestrial invertebrate | 9.4A |
| 2 ≤ LD ₅₀ < 11 µg/terrestrial invertebrate | 9.4B |
| 11 ≤ LD ₅₀ ≤ 25 µg/terrestrial invertebrate | 9.4C |
| >25 µg/terrestrial invertebrate | Not classified as hazardous |

If the mixture is used as a biocide and does not trigger classification under subclass 9.4, see chapter 23.

22.3.2. Bridging principles

Guidance on the bridging principles for classifying mixtures without test data is in chapter 18.

22.3.3. Classification of a mixture based on the classifications of components: the summation approach

When test data on the mixture are not available and the bridging principles are not applicable, the summation approach is used to derive terrestrial invertebrate classification for the mixture.

Rationale

The toxicity criteria for the classification of terrestrial invertebrate categories differ by a factor of 10 in from one class to another. Substances with a classification in a high toxicity band may, therefore, contribute to the classification of a mixture in a lower band. The calculation of these classification categories, therefore, needs to consider the contribution of all substances that are classified for toxicity to terrestrial invertebrates.

When components are classified as 9.4A and their acute toxicity is well below the cut-off value (that is, $\ll 2 \mu\text{g}/\text{terrestrial invertebrate}$) they contribute to the toxicity of the mixture even at a low concentration. Under these circumstances the application of the normal cut-off values or concentration limits may lead to an 'under-classification' of the mixture. Therefore, multiplying factors are applied to account for highly toxic components, as described in 'Mixtures with highly toxic components' under 'Classification procedure' below.

Classification procedure

The steps to follow in applying the summation approach to terrestrial invertebrate hazard classification are set out below and summarised in Table 22.3 and Figure 22.2.

Mixtures with no highly toxic components

- Step 1: Consider all components classified as 9.4A.

If:

$$\sum(9.4A)\% \geq 25\%$$

then the mixture is classified as 9.4A and the classification process is complete.

- Step 2: Consider all components classified as 9.4A and 9.4B.

If:

$$(\sum(9.4A)\% \times 10) + \sum(9.4B)\% \geq 25\%$$

then the mixture is classified as 9.4B and the classification process is complete.

- Step 3: Consider all components classified as 9.4A, 9.4B and 9.4C.

If:

$$(\sum(9.4A)\% \times 100) + (\sum(9.4B)\% \times 10) + \sum(9.4C)\% \geq 25\%$$

then the mixture is classified as 9.4C and the classification process is complete.

If the sum is $< 25\%$ then the substance is not classified for hazards to terrestrial invertebrates. The exception to this is where the substance is used as a biocide. See chapter 23 for further guidance.

Mixtures with highly toxic components

Components with toxicities well below the cut-off for 9.4A classification ($\ll 2 \mu\text{g}/\text{terrestrial invertebrate}$) may influence the toxicity of the mixture and are given increased weight in applying the summation of classification approach.

The multiplying factors to be applied to these components are defined using the toxicity value, as summarised in Table 22.4. Therefore, to classify a mixture containing highly toxic components, the classifier needs to apply the multiplying factor (M) in assigning a terrestrial invertebrate hazard classification to the mixture.

See Table 22.5 and the worked example below.

Table 22.3: Classification of a mixture for terrestrial invertebrate hazards based on summation of classified components

| Process | Sum of % of components classified as | Cut-off | Mixture classified as |
|---------|--|-------------|-----------------------|
| Step 1 | $9.4A \times M$ | $\geq 25\%$ | 9.4A |
| Step 2 | $(9.4A \times M \times 10) + 9.4B$ | $\geq 25\%$ | 9.4B |
| Step 3 | $(9.4A \times M \times 100) + (9.4B \times 10) + 9.4C$ | $\geq 25\%$ | 9.4C |

Note: M = multiplying factor.

Table 22.4: Terrestrial invertebrates: multiplying factors

| LD ₅₀ value (µg/terrestrial invertebrate) | Multiplying factor (M) |
|--|------------------------|
| $0.2 < LD_{50} \leq 2$ | 1 |
| $0.02 < LD_{50} \leq 0.2$ | 10 |
| $0.002 < LD_{50} \leq 0.02$ | 100 |
| $0.0002 < LD_{50} \leq 0.002$ | 1000 |
| $0.00002 < LD_{50} \leq 0.0002$ | 10,000 |
| (continue in factor 10 intervals) | |

Note: LD₅₀ = median lethal dose.

Table 22.5: Example calculation for terrestrial invertebrate classification of Mixture Z

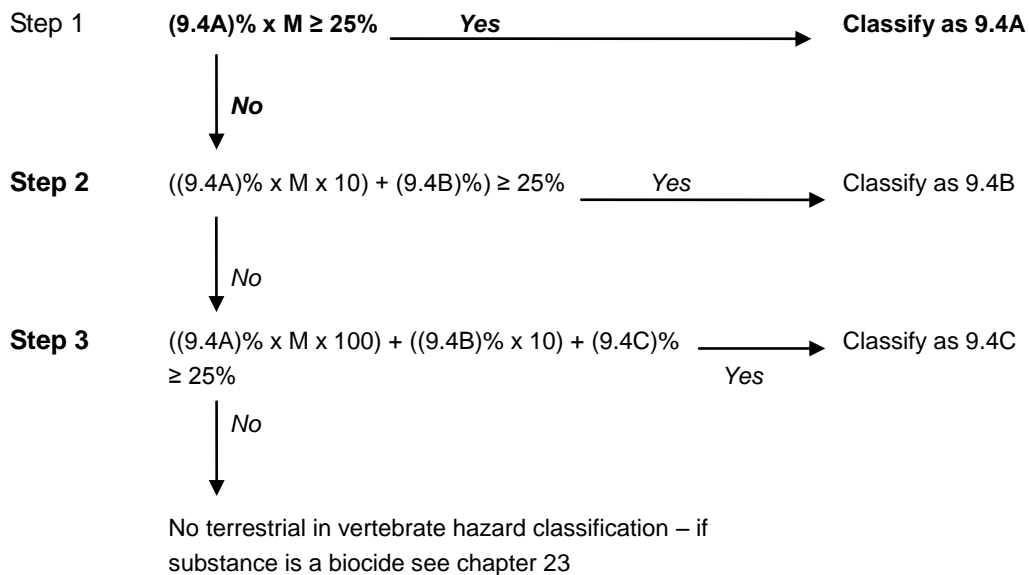
| Component | LD ₅₀ (µg/terrestrial invertebrate) | Classification of component | Concentration of component in mixture (%) | Multiplying factor (M) | Weighted concentration of component in mixture (M x %) |
|-----------|--|-----------------------------|---|------------------------|--|
| B | 5 | 9.4B | 5 | - | 5 |
| P | 0.01 | 9.4A | 0.05 | 100 | 5 |
| Q | 1 | 9.4A | 1 | - | 1 |
| T | 20 | 9.4C | 40 | - | 40 |
| U | 100 | Not classified | 53.95 | - | 53.95 |

Note: LD₅₀ = median lethal dose.

The steps to follow in applying the summation approach to terrestrial invertebrate hazard classification for mixtures with highly toxic components are set out below, using the information in Table 22.5.

- Step 1:**
 Component P is highly ecotoxic and attracts a multiplier of 100, resulting in weighted concentration of that component of 5%.
 Component Q although classified as 9.4A is not given additional weighting, that is:
 $\Rightarrow (100 \times P) + Q$
 $\Rightarrow (100 \times 0.05\%) + 1\% = 6\%$, which is $< 25\%$
 so the mixture Z is not classified as 9.4A.
- Step 2:** Consider components classified as 9.4A and 9.4B
 $\Rightarrow 10((100 \times P) + Q) + B$
 $\Rightarrow 10((100 \times 0.05\%) + 1\%) + 5\% = 60\% + 5\% = 65\%$, which is $\geq 25\%$
 so the mixture Z is classified as 9.4B.

Figure 22.2: Terrestrial invertebrate hazard classification of mixtures



Appendix 22A: Acceptable test methods for terrestrial invertebrates

22A.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The lists of guidelines provided below are not exclusive. If data have been generated using other valid international guidelines, then the results from those tests may also be applicable. The main references to international guidelines referred to in the tables in this appendix are as follows.

- European Commission (EC) guidelines:
European Commission 1997. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 14 August 2007.
- International Organization for Standardization (ISO) guidelines:
Guidelines are available from the national standardisation organisations or the ISO website (<http://www.iso.ch> Retrieved 14 August 2007).
- Organisation for Economic Co-operation and Development (OECD) guidelines:
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates.
http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html Retrieved 14 August 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency.
<http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 14 August 2007.
- ASTM International (ASTM) guidelines are available from the ASTM homepage (<http://www.astm.org>, search on 'standards').

22A.2 Terrestrial invertebrate toxicity test guidelines

The guidelines in Table 22A.1 are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides, which include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in the aquatic environment. For testing microbial biopesticides, see the USEPA website for specific tests.

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm> Retrieved 3 November 2011..

See also Table 22A.2.

Table 22A.1: Terrestrial invertebrate toxicity test guidelines for chemicals, including mixtures

| Species | Test guideline number | | | |
|---------------------------|---|--|---|--|
| | OECD | EC | USEPA OPPTS | Other |
| Honeybee | | | | |
| Acute oral | OECD 213 (1998), Honeybee acute oral toxicity test | EPPO PP 1/170(3) (2000), Side-effects on honeybees | | |
| Acute contact | OECD 214 (1998), Honeybee acute contact toxicity test | EPPO PP1/170(3) (2000), Side-effects on honeybees | OPPTS 850.3020 (1996), Honeybee acute contact toxicity | |
| Bee brood feeding test | | | | ICPBR method, Method for honeybee brood feeding test with insect growth- regulating insecticides, EPPO Bulletin volume 22, pp 613–616, 1992 |
| Higher tier | | | OPPTS 850.3030 (1996) Honeybee toxicity of residues on foliage | |
| Higher tier | | | OPPTS 850.3040 (1996) Field testing for pollinators | |

Protocols are available to evaluate the side-effects of plant protection products to non-target arthropods (see Guidelines to Evaluate Side-Effects of Plant Protection Products to Non-Target Arthropods: IOBC, BART and EPPO Joint Initiative (Candolfi et al, 2000)).

Acceptable test methods for biopesticides

Table 22A.2: Test methods for biopesticides

| 885.4000 Background for non target organism testing of microbial pest control agents |
|---|
| 885.4340 Non target insect testing, Tier I |
| 885.4380 Honey bee testing, Tier I |
| 885.5000 Background for microbial pesticides testing |
| 885.5200 Expression in a terrestrial environment |

References

Candolfi, MP, Blümel, S, Forster R, et al (eds.) 2000. *Guidelines to Evaluate Side-Effects of Plant Protection Products to Non-Target Arthropods: IOBC, BART and EPPO Joint Initiative*. IOBC/WPRS, Reinheim, Germany.

23. Biocidal Classification

23.1. Introduction

The biocidal threshold is intended to ensure that biocidal substances with a highly specific mode of action on a particular class of organism are assessed for possible environmental impacts prior to importation into, or manufacture in, New Zealand. This specificity means that when the substance is tested for any of the specific ecotoxicity thresholds using the species identified in the acceptable test methodologies, it may not trigger any of the thresholds for aquatic, soil, terrestrial vertebrate and terrestrial invertebrate ecotoxicity. However, there is still potential for these substances to pose a risk to organisms in the environment.

23.2. Threshold

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

2 *Minimum degrees of hazard*

(1) *A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—*

...

(e) the substance is designed for biocidal action.

(2) *A substance referred to in subclause (1)(e) is not hazardous for the purposes of this schedule if—*

(a) the substance is designed for biocidal action against a virus, protozoan, bacterium, or an internal organism in humans or in other vertebrates; and

(b) the substance does not meet any of the minimum degrees of hazard specified in subclause (1)(a) to (d).

Note that subclause (1)(a) to (d) contains the threshold requirements for effects on aquatic, soil, terrestrial vertebrate, and terrestrial invertebrate species.

23.3. Classification

Schedule 6 to the Hazardous Substances (Classification) Regulations 2001 classifies biocides under subclass 9.1 as 9.1D (substances that are slightly ecotoxic to the aquatic environment).

A 9.1D biocidal classification applies to any substance meets the following criteria.

- Subclass 9.1D – substances that are slightly harmful in the aquatic environment or are otherwise designed for biocidal action
A substance that is designed for biocidal action, other than a substance that is designed for biocidal action against a virus, a protozoan, a bacterium, or an internal organism in humans or in other vertebrates, but that does not meet the criteria for any hazard classification in class 9 other than 9.1D.

A substance is not assigned a 9.1D biocide classification if the substance is designed for biocidal action against:

- a virus, a protozoan, or a bacterium (in humans and other vertebrates) and it does not trigger any of the other aquatic, soil, terrestrial vertebrate, or terrestrial invertebrate thresholds; that is, the substance is specifically active against the virus, protozoan, and/or the bacterium with no other ecotoxic effects; and
- internal organisms in humans or other vertebrates and it does not trigger any of the other aquatic, soil, terrestrial vertebrate, or terrestrial invertebrate thresholds;

23.3.1. Example of a substance that triggers classification as only 9.1D (biocide)

A hypothetical formulated fungicide, Fuzzfree, has been tested for toxicity to aquatic and terrestrial organism as indicated in Table 23.1.

None of the test data trigger classification for a specific ecotoxicity subclass. However, Fuzzfree is intended to kill fungi, so is assigned the 9.1D (biocide) classification.

Table 23.1: Example of a hypothetical formulated fungicide Fuzzfree that triggers classification as 9.1D (biocide)

| Test species | Test result |
|--|---|
| Aquatic (subclass 9.1) | |
| Rainbow trout | 96-hour LC ₅₀ 150 mg/L |
| <i>Daphnia magna</i> | 48-hour LC ₅₀ 230 mg/L |
| Green alga, <i>Scenedesmus capricornutus</i> | 96-hour E _r C ₅₀ 330 mg/L |
| Soil (subclass 9.2) | |
| Earthworm, <i>Eisenia fetida</i> | 14-day LC ₅₀ 120 mg/kg soil |
| Soil microbial function | 28-day EC ₂₅ >250 mg/kg soil |
| Seedling emergence [range of species] | 14-day EC ₅₀ 110 mg/kg soil |
| Terrestrial vertebrates (subclass 9.3) | |
| Rat, acute oral toxicity | LD ₅₀ > 2,000 mg/kg bw |
| Rat, chronic toxicity (active ingredient) | NOEC 230 ppm diet; LOEC 500 ppm diet |
| Bobwhite quail acute oral toxicity | LD ₅₀ 2,500 mg/kw bw |
| Bobwhite quail acute dietary toxicity | LC ₅₀ 5,100 ppm diet |
| Bobwhite reproductive toxicity (active ingredient) | NOEC 150 ppm diet; LOEC 300 ppm diet |
| Terrestrial invertebrates (sub-class 9.4) | |
| Honeybee, acute oral | 48hr LD ₅₀ 30 µg/bee |
| Honeybee, acute contact | 48hr LD ₅₀ 30 µg/bee |

Note: E_rC₅₀ = median effective concentration based on growth rate; NOEC = no observed effect concentration; LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; LOEC = lowest observed effect concentration; ppm = parts per million.

23.4. Exemptions from the biocide classification

The EPA has specifically exempt mating disruptors from the biocide classification if they do not trigger any other hazard classification, as set out below (see EPA, 2011).

23.4.1. Mating disruptors

Insect pheromones and other chemical substances are sometimes used as mating disruptors, providing alternative strategies for managing insect pests such as the painted apple moth. Where pheromones and similarly used substances do not have specific inherent hazardous properties, they are not hazardous substances.

However, there is still the question of whether the substance is designed for biocidal action. Biocidal action triggers the HSNO Act class 9 threshold, and is defined in the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 as:

biocidal action, in relation to a substance, means the substance causes mortality, inhibited growth, or inhibited reproduction in an organism.

While the use of insect pheromones as mating disruptors may be considered to trigger the threshold for biocidal action, the EPA considers that this goes outside the intentions of the HSNO Act, since the substance is not directly acting on the reproductive function (it is simply confusing the male insects).

The EPA considers that since mating disruption (using pheromones or other substances) does not directly impact on the reproductive function, but simply alters the behaviour of the target organism, such substances do not trigger the biocidal action threshold under the HSNO Act.

References

EPA, 2011. *Interpretations and Explanations of Key Concepts for the HSNO Act*. EPA, Wellington.
<http://www.epa.govt.nz/Publications/ER-PR-03-22-Key-Concepts-Master-File.pdf>



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