

Guidance Manual

Responsible & Safe Handling of C1 Gases

in Microbiology Laboratories



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Contents

Acknowledgement	3
Executive Summary.....	4
Chapter 1 - Factual Information of C1 Gaseous Feedstocks	8
1.1 Toxicity and Exposure Limit Thresholds of C1 Gaseous (CO, CO ₂ , CH ₄ and H ₂)	8
1.2 Factual Information on Gas/Vapour Flammability.....	10
1.3 Triangular Flammability Diagram	12
1.4 Mixed Gas Operating Range	13
1.5 Chapter 1 - Bibliography	14
Chapter 2 - Safety Considerations to Use C1 Gas Feedstocks	15
2.1 Introduction.....	15
2.2 Dangerous Substances and Explosive Atmospheres Regulations (DSEAR) ...	16
2.3 Dangerous Substances	18
2.4 Explosive Substances.....	19
2.5 DSEAR Risk Assessment.....	20
2.6 Chapter 2 - Bibliography	42
Chapter 3 - Guidance to Typical Microbiological Laboratories.....	44
Chapter 4 - Suggestions of Additional Safety Equipment in Typical Microbiological Laboratories	50
4.1 Gas Detectors and Alarms.....	50
4.2 Control Panels and Instrument Readouts	55
4.3 Local Exhaust Ventilation	56
4.4 Gas Cylinder Manifolds.....	58
4.5 Maintenance, Training and Risk Assessment.....	59
4.6 Chapter 4 - Bibliography	61
Appendix 1 - <i>DSEAR Template Example</i>	62
Appendix 2 – Industrial Gas Cylinder Information from Common Suppliers	68

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Executive Summary

Significant societal efforts are being made to curtail *Green House Gas* atmospheric emissions to address the adverse impact of climate change. Meanwhile, utilisation of these gases also offer significant potential for future manufacturing to achieve decarbonisation in several industrial sectors of products such as chemicals, fuels, and animal feedstocks. One key enabler to realise this potential is through microbiological conversion (by fermentation) of these gaseous feedstocks by efficient, economically viable, and scalable bioprocesses.

This guidance manual has been written to facilitate and encourage the growth of gaseous feedstock utilisation within the biotechnology development pipeline. In particular, this manual is intended to be used as guidance for the use and application of gases within typical microbiological and fermentation laboratory settings and includes use of hydrogen, carbon dioxide, carbon monoxide, methane, air, and mixtures thereof.

The barrier to entry, to make the change from typical microbiological (heterotrophic) feedstocks such as carbohydrates, glycerol, and others, towards gaseous alternatives poses challenges for most operational laboratories today. With this backdrop, this manual has been written to provide practical guidance and key safety considerations required to enable a greater uptake, adoption and deployment by the research and development community to facilitate the growth of this key enabling technology.

The document provides an instructional framework and guidance manual to facilitate operation and practical use of these C1 gaseous feedstocks in typical existing microbiological laboratories. We consider this publication as a “living document” and as such we welcome further contribution and input from the community who have particular expertise and experience, to further enhance the effectiveness of this manual for prospective users of gaseous feedstock in the biotechnology R&D community.

Whilst we cannot provide a single manual that is universally applicable to all specific R&D laboratory settings, this document aims to serve as a useful guide with respect to commonly encountered issues, providing practical guidelines and tips. This includes factual information relating to toxicity and flammability properties of hydrogen, carbon dioxide, carbon monoxide, methane, and air. Regulatory aspects related to *Health and Safety Executive* with reference to *Dangerous Substances and Explosive Atmospheres Regulations* (DSEAR) is also described. A *Decision Making Flowchart Template* has been provided to help users think about the preparatory steps they should consider when planning to work with these gases. Whilst every new laboratory method when introduced must conduct its own specific risk assessment, the authors have provided tools that can enable a wider assessment of safety. This includes how to conduct a site specific *Gap*

and Failure Mode Effect Analysis to enable better comprehension of risk and steps to mitigate where possible. Mitigation examples are provided along with gas monitoring options to provide a further level of safety control in handling certain gases.

NB this document has been prepared by the best efforts of the authors based on their own knowledge and experience but is for information purposes only. As such, none of the authors nor their employers can or will be held liable for any actions and outcomes arising from third parties in their respective implemented use of gas handling.

Table 1 Abbreviations

Abbreviation	Definition
ATEX	ATmospheres Explosible
BOC	Brin's Oxygen Company
BCGA	British Compressed Gas Association
BS EN	British Standard European Norm
CO ₂	Carbon Dioxide
CO	Carbon Monoxide
CAS	Chemical Abstracts Service
CLP	Classification, Labelling and Packaging
COSHH	Control of Substances Hazardous to Health
CSTR	Continuous Stirred Tank Reactor
DSEAR	Dangerous Substances and Explosive Atmospheres Regulations
EH40/2005	Workplace Exposure Limits
EPS	Equipment Protection System
ECHA	European Chemicals Agency
FMEA	Failure Mode Effect Analysis
H ₂	Hydrogen
HMSO	Hazardous Materials Storage Ordinance
HSE	Health and Safety Executive
IDLH	Immediate Danger to Life and Health
kg	kilo gram
L	Litre
LOC	Limiting oxygen concentration
LEV	Local Exhaust Ventilation
LEL	Lower Explosive Limit
LFL	Lower Flammable Limit
MESG	Maximum Experimental Safe Gaps
Methane	CH ₄
m ³	metres cubed

mg	milli grams
mL	milli litres
MIC	Minimum Igniting Currents
NB	Nota Bene (take note)
ppm	parts per million
PPE	Personal Protective Equipment
PSSR	Pressure System Safety Regulations
QMS	Quality Management System
R&D	Research and Development
RF	Radio Frequency
RA	Risk Assessment
SG	Specific Gravity
STP	Standard Temperature and Pressure
TRL	Technology Readiness Level
TWA	Time Weighted Average
UEL	Upper Explosive Limit
UFL	Upper Explosive Limit
v/v	Volume per volume
Water	H ₂ O
WEL	Workplace Exposure Limits
LEV	Local Exhaust Ventilation
SOP	Standard Operating Procedure

Chapter 1 - Factual Information of C1 Gaseous Feedstocks

1.1 Toxicity and Exposure Limit Thresholds of C1 Gaseous (CO, CO₂, CH₄ and H₂)

Carbon monoxide (CO; CAS number: 630-08-0) is a colourless and odourless gas at room temperature and atmospheric pressure, with a specific gravity of 0.97 [air = 1]. It is toxic and extremely flammable. It may react vigorously with oxygen, acetylene, chlorine, fluorine, or nitrous oxide. Carbon monoxide mixes well with air and explosive mixtures are easily formed. Inhalation of carbon monoxide can cause serious illness and kill: initially cause headache, nausea and vomiting, irritability, weakness, and tachypnoea, followed by dizziness, confusion, ataxia, agitation, syncope, hypotension, seizures, impairment of consciousness and respiratory failure. In serious cases, cerebral oedema and metabolic acidosis may develop ¹.

Carbon dioxide (CO₂; CAS number: 124-38-9) is a colourless and odourless gas at room temperature and atmospheric pressure, with a specific gravity of 1.52 [air=1]. It is not flammable and will not support combustion, indeed it is effective as a fire suppressant agent. It is commonly found in black labelled 'red' type fire extinguishers recommended for use on class B (oil, solvents, petrol) and electrical fires. It is naturally present in the air we breathe at a concentration of about 0.037% and is not harmful to health at low concentrations. However, it has been recognised as a workplace hazard at high concentrations. Inhalation of carbon dioxide can cause headaches, dizziness, confusion, and loss of consciousness ².

Methane (CH₄; CAS number: 74-82-8) is a colourless, odourless, and extremely flammable gas at room temperature and atmospheric pressure, with a specific gravity of 0.55 [air=1]. It is the primary component of natural gas, and may also be referred to as biogas, or marsh gas. It reacts violently with various substances including halogenated compounds, hydrogen, and oxygen. Low level exposure to methane in the environment or from the correct use of natural gas products appliances would not be expected to cause any adverse health effects. However, high levels of methane can cause mood changes, slurred speech, vision problems, memory loss, nausea, vomiting, facial flushing and headache. Skin or eye contact with liquefied methane released under pressure may cause frostbite ³.

Gaseous hydrogen (H₂; CAS number: 1333-74-0) is colourless, odourless, tasteless, and highly flammable at room temperature and atmospheric pressure, with a specific gravity of 0.07 [air=1]. Hydrogen poses a number of safety hazards to human beings, from potential detonations (a type of strong combustion) and spontaneous fires when leaking into external air. The low minimum ignition energy of hydrogen in air is one of the lowest (0.02 mJ) compared to other flammable gases. Ignition can occur when mixed even in small amounts with ordinary air due to the oxygen in the air and the simplicity and

chemical properties of the reaction. Because of this extremely flammable nature, any possible ignition source has to be considered in a safety risk assessment. Any electrical device, bond, or ground should meet Dangerous Substances and Explosive Atmospheres Regulations (See Chapter 2). Pure hydrogen-oxygen flames emit ultraviolet light and with high oxygen mix are nearly invisible to the naked eye, and thus can lead to accidental burns¹⁰. However, hydrogen has no rating for innate hazard for toxicity ¹¹.

Table 2 Exposure limit thresholds of C1 gaseous (CO, CO₂, CH₄ and H₂) ^{4, 5}

	Long-term exposure limit (8-hour TWA* reference period)		Short-term exposure limit (15-minute reference period)	
	ppm	mg/m3	ppm	mg/m3
Carbon monoxide	20	23	100	117
Carbon dioxide	5000	9150	15000	27400
Hydrogen	No guideline value specified ¹¹			
Methane	No guideline value specified ⁵			

* Time Weighted Average

1.2 Factual Information on Gas/Vapour Flammability

To produce an explosion, three key 'ingredients' are required simultaneously:

- **Flammable Substance** – fuel
- **Oxidizer** – oxygen, air or another oxidant (e.g., nitrous oxide)
- **Source of Ignition** - spark or high heat

Firstly, the fuel (gases or vapours) must be within its *Flammable Range*, also called *Explosive Range* (**Table 3**), i.e., at a concentration above the *Lower Explosive Limit* (LEL) or *Lower Flammable Limit* (LFL), but below the *Upper Explosive or Flammable Limit* (UEL/UFL). Below the flammable range, the mixture is too lean to burn (lacks fuel), and above the range, the mixture is too rich to burn (lacks oxidizer).

Secondly, the oxygen content of the gas mixture must be above a *Limiting Oxygen Concentration* (LOC), below which combustion is not possible, regardless of the concentration of fuel. The LOC varies with pressure and temperature. It is also dependent on the type of inert (non-flammable) gas used in the gas mixture. A scientific paper ⁷ presents LOC for the fuel-air mixture containing the inert gaseous additive i.e., nitrogen, assessed by a variety of methods (**Table 4**).

Table 3 The flammable range for flammable gas/vapour as listed in the *British Standards Publication* ^{6*}

	Carbon Monoxide	Methane	Hydrogen
Upper flam. Limit [vol.% in air]	74.0	17.0	77.0
Lower flam. Limit [vol.% in air]	10.9	4.4	4.0

* User of the data in this standard should be aware that all data are the result of experimental determinations and as such are influenced by variations in experimental apparatus and procedures, and in the accuracy of instrumentation.

Table 4 *Limiting Oxygen Concentration (LOC) for the fuel-air mixture containing the inert gaseous additive i.e., nitrogen, determined by a number of different methods ⁷. Unit: mol%*

Test methods	Carbon Monoxide (mol%)	Methane (mol%)	Hydrogen (mol%)
120 L closed vessel result ^a	5.1	11.1	4.6
20 L closed vessel result ^a	Not reported	10.7	4.7
12 L glass sphere result ^b	Not reported	11.3	Not reported
Flammability tube ^c	5.5	12.0	5.0

a. The data from 120 L or 20 L chambers (Cashdollar, Zlochower, Green, Thomas, & Hertzberg, 2000)

b. The data from a 12-L spherical flask by *Kondo et al.* (2006, 2008)

c. Flammability tube data summarized by *Kuchta* (1985)

Finally, ignition causes a release of flammable gas to become a fire (jet fire, flash fire, pool fire etc.) or explosion. There are many possible sources of ignition and those that are most likely will depend on the release scenario. Below are known ignition sources for gas/vapour mixes ⁸:

- Electric sparks and arcs (from electrical circuits, motors, switches etc.);
- Mechanical sparks (from friction and falling objects);
- Static electrical sparks;
- Lightning;
- Flame (including flaring, boilers, smoking);
- Hot surfaces (including hot work, hot processing equipment, electrical equipment);
- Heat of compression;
- Chemical reactions (e.g., auto-ignition of oil-soaked lagging on hot piping); and
- High energy radiation, microwaves, RF, etc.

The apparatus for the determination of minimum igniting current is defined in a *British Standards Document* ⁶ Equipment protection by intrinsic safety "i".

1.3 Triangular Flammability Diagram

The safest method to mitigate fires and explosions of flammable vapours is to prevent the presence of flammable mixtures occurring in the first place. To this end, a *Triangular Flammability Diagram* is the most useful tool to display the flammability region, and therefore to determine the way to avoid the existence of flammable mixtures during plant operations. The paper by *Machuga and Crowl* 1998 ⁹ describes how to draw and use a flammability diagram. The easiest way to understand them is to briefly go through three basic steps in their construction, using the flammability diagram of methane as an example (**Figure 1**).

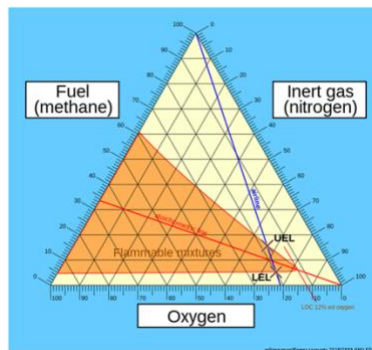


Figure 1 Flammability diagram representing a mixture of methane, oxygen, and nitrogen

- A typical *Triangular Flammability Diagram* can be used to represent all the possible mixtures of a three-component system e.g., oxygen, nitrogen, and fuel (methane). In particular, an airline represents all possible concentrations of fuel and air – this is shown in the diagram in blue.
- The flammability zone is a region on a flammability diagram in orange in which all mixtures are flammable. Those mixtures outside of the region in yellow are considered non-flammable. The actual envelope defining the flammability zone can only be determined based on experiments. The envelope would pass through the upper and lower flammability limits of fuel in air, as shown. The nose of the envelope defines the *Limiting Oxygen Concentration* (LOC).
- The stoichiometric line represents all possible concentrations of fuel and air. In this case, the combustion of methane is: $\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$. In mole terms the stoichiometric amount of methane to oxygen is therefore 1:2, which corresponds to 33 percent. Any stoichiometric mixture of methane and oxygen will lie on the straight line between pure nitrogen and 33 percent methane (and 67 percent oxygen) - this is shown as the red stoichiometric line.

1.4 Mixed Gas Operating Range¹

There are limited studies on the operating range for flammable gas mixtures. According to the *BS EN 60079-20-1: 2010* standard, the mixture of gas should be generally allocated to a group only after a special determination of the *Maximum Experimental Safe Gaps* (MESG) or *Minimum Igniting Currents* (MIC) ratio. The *BS EN 60079-20-1: 2010* standard also provides a method to determine the MESG of the mixture by applying a form of *Le Chatelier* relationship. This method should not be applied to mixtures and/or streams that have:

- Acetylene or its equivalent hazard;
- Oxygen or other strong oxidizer as one of the components;
- Large concentrations (over 5%) of carbon monoxide.

Because unrealistically high MESG values may result, caution should be exercised with two component mixtures where one of the components is an inert gas, such as nitrogen. For mixtures containing an inert gas such as nitrogen in concentrations less than 5% by volume, use an MESG of infinity. For mixtures containing an inert gas such as nitrogen in concentrations 5% and greater by volume, use an MESG of 2.

As a reference, a paper by *Zlochow and Green 2009*⁷ accessed experimental LOC values for a mixture of hydrocarbons, and compared them with calculated LOC values using modified *Le Chatelier* rule. The calculated results predicted the experimental values, and were barely outside the experimental error (**Table 5**).

Table 5 LOC for gas mixtures, as determined by a paper of *Zlochow and Green 2009*⁷

Fuel: % CH ₄	Methane-H ₂		Methane-CO		Methane-ethylene		CH ₄ -1:1 CO:H ₂	
	(LOC) _{exp}	(LOC) _{calc}	(LOC) _{exp}	(LOC) _{calc}	(LOC) _{exp}	(LOC) _{calc}	(LOC) _{exp}	(LOC) _{calc}
0	4.6	4.6	5.1	5.1	8.6	8.6	4.8	4.8
10	6.5	5.6	6.1	6.1	8.6	8.7		
20							7.3	6.7
25	7.7	6.9	7.8	7.4	8.9	9		
35	8.3	7.65						
40							8.7	8.2
50	9.1	8.64	9.5	9.0	9.3	9.45		
60							9.7	9.4
75	10.3	10	10.9	10.2	10.05	10.1		
80							10.5	10.3
90	10.9	10.7	11	10.8				
100	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1

¹ **Disclaimer:** The authors have limited knowledge on mixed gas operation. The above information is for reference only and end users are advised to perform specific safety assessment.

1.5 Chapter 1 - Bibliography

Further data on the properties of flammable materials may be found in the following references and database.

1. Carbon monoxide incident management, *UK Health Security Agency*, 2022
2. [https://www.hse.gov.uk/carboncapture/carbondioxide.htm#:~:text=Workplace%20exposure%20is%20calculated%20by,reference%20period\)%20of%2015000%20ppm](https://www.hse.gov.uk/carboncapture/carbondioxide.htm#:~:text=Workplace%20exposure%20is%20calculated%20by,reference%20period)%20of%2015000%20ppm)
3. Methane general information, *Public Health England*, 2019
4. *EH40/2005 Workplace Exposure Limits*
5. Methane incident management, *Public Health England*, 2016
6. *BS EN 60079-20:1 2010 Explosive Atmospheres. Material characteristics for gas and vapour classification*
7. I.A. Zlochow, G.M. Green, *Journal of Loss Prevention in the Process Industries*, 2009, **22**, 499–505
8. <https://www.hse.gov.uk/offshore/strategy/ignition.htm>
9. Mashuga, Chad V;Crowl, Daniel A, *Process Safety Progress*; Fall 1998; **17**, 3
10. Brown, WJ, *Safety Standard for Hydrogen and Hydrogen Systems*, NASA. NSS 1997
11. https://en.wikipedia.org/wiki/Hydrogen_safety

Chapter 2 - Safety Considerations to Use C1 Gas Feedstocks

2.1 Introduction

The key to successful gas safety management is the assessment and management of risks when using gases in enclosed workplaces such as microbiology laboratories. It is recommended that workers follow the guidance and recommendations of the relevant professional bodies for example the *British Compressed Gas Association* (BCGA) (<https://bcga.co.uk/>)¹.

BCGA provide a range of publications providing safety information and advice on best practice, as well as associated legal requirements within the UK. BCGA has a number of model risk assessments and reviewed publications, that are free to download, and are relevant to safe cylinder handling, the application of compressed gases in the workplace and the management of risk when they are used ([Publications - BCGA](#))².

It is recommended that guidance notes and principles of safe practices of handling and operation of gas cylinders described in these BCGA publications are read and understood by both employers and workers prior to commencing any risk assessments.

It is a requirement for employers to comply with the *Health and Safety at Work Act 1974* (<https://www.hse.gov.uk/legislation/hswa.htm>)³ and the *Pressure System Safety Regulations 2000* (PSSR2000) (<https://www.hse.gov.uk/pressure-systems/pssr.htm>)⁴.

In the context of microbial gas fermentation, it will often be necessary for operators to use pressurised gases such as CO₂, CO, CH₄, H₂ etc. Due to increased dangers associated with the use of stand-alone compressed gas cylinders located in buildings in the event of a fire, the preferred method of providing compressed gas is *via* piped systems attached to gas cylinders securely housed outside the building. This complies with BCGA recommendations and *Fire Service* regulations. However, the use of stand-alone cylinders within laboratories can be sanctioned (in accordance with the employer's *Health and Safety Policy*) in the following circumstances:

- Building design, layout or process prevents used of a piped gas system;
- Fire risk assessment has formally been written and reviewed, and deemed safe to do so.

This section of the manual aims to provide users and others guidance on the safe operation of pressure systems when compressed gases are required for lab scale biotransformations or fermentations from 250 mL up to 20 L scale.

For the purposes of this manual, the intention is to provide simple, practical guidelines on eliminating or lowering the risks associated with using stand-alone compressed gas cylinders and/or gas generators in the workplace. All gases under pressure, regardless

of their properties, are classified as dangerous substances and are in scope of *Dangerous Substances and Explosive Atmospheres Regulations* (DSEAR).

2.2 Dangerous Substances and Explosive Atmospheres Regulations (DSEAR)

This section outlines important *DSEAR* criteria that should be in place to protect employees and other workers from explosions associated with dangerous substances including compressed gases. In addition to providing general guidance on key aspects of working with dangerous substances, the workflow for carrying out a *DSEAR Risk Assessment* will be outlined and includes guidelines for risk mitigation measures that need to be considered and then appropriately implemented to ensure safe operation of gas fermentation in the workplace.

Explosive atmospheres can be formed in workplaces where dangerous substances (such as flammable and explosive materials) are manufactured, stored, moved, used, or transported and where dusts are formed or transferred. Materials that are capable of forming flammable or explosive atmospheres are classed as dangerous substances and the primary legislation applying to the control of substances that can cause fires and explosions in the workplace is the *Dangerous Substances and Explosive Atmospheres Regulations 2002* (DSEAR) (SI 2002 No.2776). From June 2015 *DSEAR* also covers the risk caused by gases under pressure and substances that are corrosive to metals. The text of the regulations can be found on the *HMSO* website as follows: [*Dangerous Substances and Explosive Atmospheres Regulations 2002 \(DSEAR\)*](#)⁵.

The regulations complement the requirement to manage risks under the *Management of Health and Safety at Work Regulations 1999* (SI 1999 No.3242): [*Management of Health and Safety at Work Regulations 1999*](#)⁶.

2.2.1 Employers' duties

Under *DSEAR*, employers are obliged to assess the risks of fires and explosions that may be caused by dangerous substances (including compressed gases) in the workplace.

Employers must:

- Ensure the *Health and Safety at Work* of employees and other people who may be at risk (such as visitors to the workplace or members of the public) where explosive atmospheres may occur, so far as reasonably practicable;
- Carry out *Risk Assessments* on areas where explosive atmospheres could occur;
- Eliminate or lower risks as far as reasonably practicable by implementation of control measures;
- Designate *Hazard Classification Zones* where explosive atmospheres could occur;
- Mark points of entry at designated hazard zones;

- Implement procedures to collect, contain and remove any releases of dangerous substances to a safe place, e.g., external ventilation;
- Ensure accident and emergency procedures are in place and the required equipment is available to deal with an explosion incident;
- Provide employees with appropriate information, instruction, and training;
- Provide employees with suitable *Personal Protective Equipment* (PPE), e.g., antistatic clothing;
- Ensure proper use of equipment designed for explosive atmospheres;
- Ensure procedures are in place for safe conditions and controls are maintained including testing/maintenance of equipment, control systems, protective systems and alarm systems if required;
- Compile and maintain records of actions and decisions taken in order to demonstrate compliance.

2.2.2 Employees' duties

Under the *Health and Safety at Work etc Act 1974* (<https://www.hse.gov.uk/legislation/hswa.htm>)⁷ employees are required to:

- Take reasonable care of their own health and safety and that of other people who may be affected by their activities at work;
- Co-operate with their employer to comply with health and safety duties;
- Make proper use of control measures, including *Personal Protective Equipment* (PPE);
- Report any shortcomings or deficiencies in working practices to senior management.

NB there are no specific duties on employees under the *DSEAR 2002*.

2.2.3 When to apply DSEAR:

DSEAR applies whenever:

- There is work being carried out by an employer;
- A dangerous substance is present (or liable to be present) at the workplace;
- The dangerous substance could be a risk to the safety of people as a result of fires, explosions, or similar energetic events or through corrosion of metal.

Fires and explosions create harmful physical effects – thermal radiation, overpressure effects and oxygen depletion. These effects can also be caused by other energetic events such as runaway exothermic reactions involving chemicals or decomposition of unstable substances such as peroxides. These events are also covered by *DSEAR*. Gases under pressure can also cause explosions creating harmful effects. Substances that are

corrosive to metal may cause damage to metal/metal containing structures which could result in reduced structural integrity if not suitably contained.

2.2.4 Where to apply DSEAR:

DSEAR applies to all workplaces where dangerous substances are present, used or produced and/or the potential for an explosive atmosphere to form. This includes workplaces such as academic labs, industrial and commercial premises.

2.3 Dangerous Substances

Dangerous substances are any substances or mixtures of substances used or present at work that if not properly controlled cause harm to people as a result of fire or explosion or corrosion of metal. Liquids, gases, vapours, and dusts that may be found in a workplace can all be dangerous substances.

Dangerous substances include:

- A substance or mixture which meets the criteria for classification as hazardous by the *European Chemicals Agency* (ECHA) under *Classification, Labelling and Packaging* (CLP) *Regulation* ((EC) No. 1272/2008) ([European Regulation \(EC\) No 1272/2008 on classification, labelling and packaging of substances 2009](#))⁸.
- Any kind of dust that when spread in air to form a cloud i.e., form an explosive atmosphere that can explode.
- Any other substances or mixtures of substances which due to their physical properties and the way they are present in the workplace, even transiently, create a risk to safety from fires and explosions but which may not be covered by *CLP Regulation* e.g., high flashpoint liquids present in the workplace at elevated temperatures.

Dangerous substances can be found in nearly all workplaces and include such things as petrol solvents, paints, varnishes, flammable gases, dusts from foodstuffs and machining/sanding operations, pressurised gases, and substances corrosive to metals.

Many of these substances can also create health risks, for example, they may be toxic or an irritant. These risks are usually covered under separate health law e.g., *Control of Substances Hazardous to Health Regulations 2002* (COSHH) and it is important to consider both safety and health criteria when understanding risks from substances in the workplace.

General information on COSHH provided by HSE is available at: <https://www.hse.gov.uk/coshh/index.htm>⁹. Further advice on how to complete a step by

step guide to COSHH Assessment is also available at: <https://www.hse.gov.uk/pubns/priced/hsg97.pdf>¹⁰.

2.4 Explosive Substances

Some requirements of *DSEAR* refer specifically to explosive atmospheres in the workplace caused by flammable gases, mists, or vapours or by combustible dusts. If there is enough of the substance, mixed with air, then a source of ignition can lead to an uncontrolled explosion. Explosions can cause loss of life and serious injuries as well as significant damage. Preventing release of dangerous substances and preventing sources of ignition are two widely used ways to reduce the risk of creating explosive atmospheres. Using the correct equipment can greatly help also. Atmospheric conditions are commonly referred to as ambient temperatures and pressures i.e., temperatures between -20 °C to 40 °C and pressures between 0.8 to 1.1 bar.

DSEAR places responsibility on employers to eliminate or control risks from explosive atmospheres in the workplace.

ATEX is the name commonly given to the two *European Directives* for controlling explosive atmospheres:

- **Directive 99/92/EC** (*ATEX 137* or *ATEX Workplace Directive*) on minimum requirements for improving health and safety protection of workers potentially at risk from explosive atmospheres ([Places where explosive atmospheres may occur](#))¹¹.
- **Directive 2014/34/EU** (*ATEX 114* or *ATEX Equipment Directive*) concerning equipment and protective systems intended for use in potentially explosive atmospheres ([ATEX equipment directive 2014](#))¹².

The requirements in *DSEAR* apply to most workplaces where a potentially explosive atmosphere may occur.

2.5 DSEAR Risk Assessment

This section provides guidance on the application of *DSEAR* and *Regulations for Compressed Gases* and can be used as a guide for the generation of *DSEAR Risk Assessments*. A DSEAR template example is provided as an Appendix.

Suitable and appropriate assessments of the risks associated with pressurised gases in the workplace must be carried out by trained personnel. The extent of the *DSEAR Risk Assessment* is dependent upon the flammability, oxidising, or corrosive properties of the gases and the risk of explosion, or the potential for structural damage if these gases are released to the atmosphere. When using gases under pressure and no flammable, oxidising or corrosive risks exist then the *DSEAR Risk Assessment* can record this and be completed. Assessments should take into account normal work activities, any non-routine work activities and also any risks associated with unplanned events such as power cuts, equipment failure, pipe rupture etc. Maintenance or repair work can often involve a high degree of risk of the release of a dangerous substance into the atmosphere. Normal work activities should only take place when a risk assessment of the current working status has been made and any control measures have been identified and implemented.

NB *DSEAR Risk Assessments* should be carried out and recorded in conjunction with other safety risk assessments that are appropriate for the experimental work to be undertaken in the workplace.

2.5.1 DSEAR requirement decision flow chart

A flow chart outlining the important stages and decision process of a DSEAR risk assessment is outlined in **Figure 2**.

This section will outline the key considerations when undertaking a *DSEAR Risk Assessment* and include:

- Identifying hazards and hazardous areas
- Hazardous work activities
- Evaluating the risk
- Control and mitigation measures
- Preparing emergency plans and procedures
- Instruction and training for employees

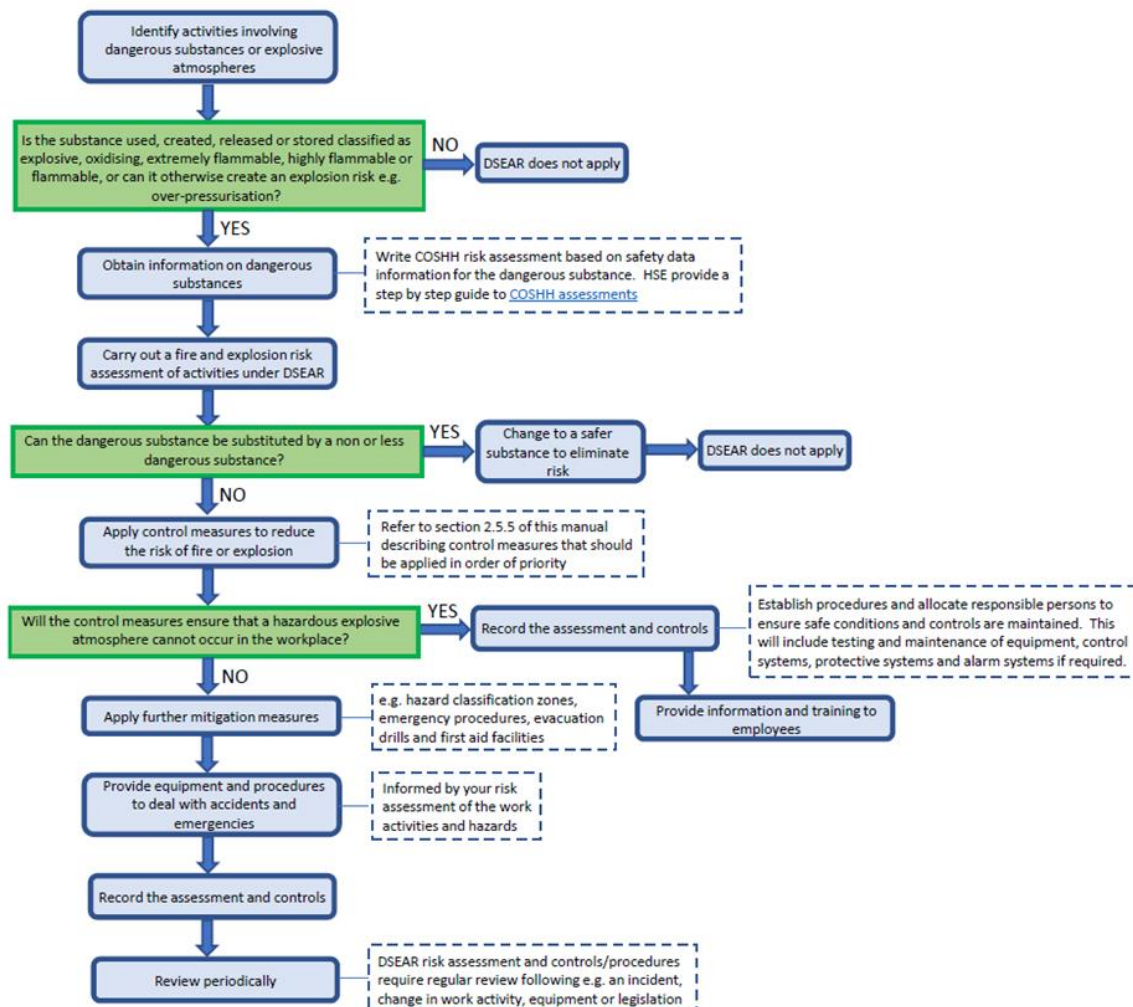


Figure 2 Stages of *DSEAR Risk Assessment* Flow Chart

For the purposes of this manual, and in the context of C1 gases typically used in microbiological laboratories, there is a focus on the hazards and risk mitigation measures for safe use of CO₂, CO, CH₄ and H₂ gases but can be generally applied to other common microbial laboratory gases such as Oxygen, Nitrogen, Argon, and Helium.

2.5.2 Identifying hazards and hazardous areas (zones)

Identifying hazardous or non-hazardous areas should be carried out in a systematic way.

DSEAR defines a place where an explosive atmosphere may occur in quantities that require special precautions to protect the health and safety of workers as **hazardous**. A place where an explosive atmosphere is not expected to occur in quantities that require such special precautions is deemed to be **non-hazardous**. For these purposes “special precautions” means precautions to control potential ignition sources within a hazardous area, particularly in relation to the construction, installation, and use of equipment.

Employers must classify areas where hazardous explosive atmospheres may occur into zones. The classification given to a particular zone, and its size and location, depends on the likelihood of an explosive atmosphere occurring and its persistence if it does. **Schedule 2** of DSEAR contains descriptions of the various classification zones for gases, vapours, and dusts ([Classification zones outlined in DSEAR 2002 Schedule 2](#))¹³.

Hazardous area classification should be carried out as an integral part of the risk assessment to identify places (or areas) where controls over ignition sources are needed (hazardous places), and also those places where they are not (non-hazardous places). Further information and guidance on the classification and zoning of areas where potentially explosive atmospheres may occur and the selection of equipment for use in those areas can be found at [Explosive atmospheres – Classification of hazardous areas \(zoning\) and selection of equipment](#)¹⁴.

For gases, this usually means Zone 0, Zone 1, or Zone 2:

- **Zone 0:** A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is present continuously or for long periods or frequently.
- **Zone 1:** A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is likely to occur in normal operation occasionally.
- **Zone 2:** A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is not likely to occur in normal operation but, if it does occur, will persist for a short period only.

2.5.2.1 General Hazards

The risk assessment should identify the hazards associated with the pressurised gases and their handling, storage and use in the workplace. When identifying hazards, it is necessary to consider:

- Where pressurised gases are used, stored or generated
- The way in which they are used stored or generated
- The potential for hazardous or explosive atmospheres occurring
- Potential ignition sources

For the general use of compressed gases, if handled properly, pressurised gas cylinders are safe, but if handled incorrectly, the same cylinders can present a severe hazard to individuals and the surrounding area that could lead to very serious injuries or even result in death.

There are a number of main hazards that need to be considered:

- Explosion of the cylinder due to mechanical damage, weakness, or over-pressurisation;
- Exposure to released gas or fluid, which may have harmful properties (asphyxiant, toxic, corrosive). For example, oxygen is particularly dangerous as it can promote fires/explosions and sustains combustion;
- Fire due to uncontrolled escape of flammable gas/fluid;
- Over-pressurisation in the event of fire;
- Impact from falling cylinders;
- Manual handling injuries.







For these reasons it is important users of gas cylinders are familiar with the principles of safe practice for the storage of gas cylinders and comply with the guidance and information produced by the *HSE* and other related bodies and trade associations (<https://www.hse.gov.uk/comah/sragtech/techmeascylinder.htm>¹⁵, <https://bcga.co.uk/wp-content/uploads/2022/06/BCGA-CP-44-Revision-1-17-06-2022.pdf>¹⁶).

2.5.2.2 Gas Identification and Primary Hazards

Commercially supplied gases are typically provided to the user either in bulk and stored in a tank or via a gas cylinder. Commercial gas suppliers will ensure that each container they supply will correctly identify the product it contains. On a gas cylinder the label identifies the contents and provides basic safety information on the hazards associated with the product. Gases are classified according to their hazardous properties with internationally recognised hazard pictograms to identify each class (**Table 6**).

Table 6 Class 2 Gases - Primary Hazards

Category	Carbon Monoxide	Methane	Hydrogen
Upper flam. Limit [vol.% in air]	74.0	17.0	77.0
Lower flam. Limit [vol.% in air]	10.9	4.4	4.0

Category	Hazard Pictogram	Example
Class 2 Division 2.1 Flammable	 	Methane Hydrogen
Class 2 Division 2.2 Non-flammable Non-toxic		Carbon Dioxide
Class 2 Division 2.3 Toxic Flammable	  	Carbon Monoxide

Each gas will have its own particular set of hazards based on its physical and chemical properties. Always refer to the *Material Safety Data Sheet* (MSDS) for specific information.

This sub-section covers the general hazards applicable to most gases including:

- Asphyxia
- Health Effects
- Density
- Cryogenic Gas

2.5.2.3 Asphyxia

Release of any gas will displace the existing atmosphere in an enclosed workplace, depleting the volume of oxygen available to breathe. If oxygen concentration in the workplace atmosphere decreases there is an increased risk of asphyxiation. Refer to **Table 7** for physiological effects of inhaling reduced concentrations of oxygen.

Table 7 O₂ Concentration – Effect and Symptoms (EIGA Safety Information 24 (23) – *Carbon dioxide physiological hazards “not just an asphyxiant”*) (https://www.eiga.eu/publications/?_sf_s=CARBON%20DIOXIDE)¹⁷

O ₂ concentration Volume %	Effects and symptoms
20.9	Normal level of oxygen in the atmosphere
19.5	Minimum safe level of oxygen
< 18	Potentially dangerous.
< 10	Risk of unconsciousness followed by brain damage or death due to asphyxia is greatly increased.
< 6	Immediate loss of consciousness occurs.
0	Inhalation of only 2 breaths causes immediate loss of consciousness and death within 2 minutes

2.5.2.4 Health Effects

Health effects can occur where people are exposed to gases. COSHH regulations require that exposure to any hazardous substance is managed <https://www.hse.gov.uk/nanotechnology/>¹⁸. The HSE provide guidance on workplace exposure limits in HSE EH 40 Workplace Exposure Limits <https://www.hse.gov.uk/pubns/books/eh40.htm>¹⁹.

2.5.2.5 Gas Density

Gas density values (specific gravity (SG)) relative to air (air specific gravity = 1 at STP) will indicate where the gas may preferentially accumulate. For example, a gas with density > 1 (e.g., CO₂ (SG)=1.52) will tend to fall in lower areas of the workplace. Or a gas with a density < 1 (e.g., H₂ (SG)=0.07) will collect under roofs and overhangs potentially creating an explosive atmosphere. Workplace conditions such as temperature can significantly influence the physical characteristics of gases and should also be considered

2.5.2.6 Flammable Gases (Class 2, Division 2.1)

Flammable gases when mixed with oxygen or air can combust and may explode if they are ignited. The primary hazard from a flammable gas is the risk of fire and explosion.

The likelihood that a flammable gas will ignite is determined by its flammability range. **Table 8** outlines flammability ranges of specific gases in air (determined at atmospheric pressure and 293 K):

Table 8 Flammability ranges for C1 gases and hydrogen as listed in the British Standards Publication ⁶

Gas	Flammable range in air (%v/v)
Methane	4.4 – 17.0
Hydrogen	4.0 – 77.0
Carbon monoxide	10.9 – 74.0

Some common examples of ignition sources are electrical (e.g., connections, switches, overheating or faults), friction, sparks, hot work, smoking, static electricity discharge and welding.

2.5.2.7 Non-Flammable, Non-Toxic Gases (Class 2, Division 2.2)

Non-flammable, non-toxic gases are typically referred to as non-reactive, inert gases. However inert gases still pose a serious asphyxiation risk given their potential to displace atmospheric oxygen in enclosed workplaces. Some examples include; Argon, CO₂, Helium and Nitrogen. In the case of CO₂, there are additional hazards that users of this gas need to be aware of. Atmospheric concentration of CO₂ in the air is around 400 ppm (0.04% v/v). If the CO₂ concentration increases, pulmonary gas exchange in the lungs can be compromised, meaning lower quantities of CO₂ leave the bloodstream leading to CO₂ intoxication. The typical physiological effects of inhaling various CO₂ concentrations are given in **Table 9**.

Table 9 Typical effects of inhaling carbon dioxide

CO ₂ concentration Volume %	Typical effects and symptoms
0.04	Normal level of carbon dioxide in the atmosphere
0.5	Minimum safe level of carbon dioxide. Maximum allowed exposure over an 8 hour period *
1 – 1.5	Slight and unnoticeable increase in breathing rate. 1.5 = maximum allowed exposure in a 15 minute period *
3	Breathing becomes laboured, rate increases. Hearing ability reduced, headache experienced with increase in blood pressure and pulse rate.
4 - 5	Breathing laboured at a greater rate. Symptoms as above, with signs of intoxication becoming more evident with longer exposure and a slight choking feeling.
5 - 10	Characteristic pungent odour noticeable. Breathing very laboured, leading to physical exhaustion. Headache, visual disturbance, ringing in the ears, confusion probably leading to loss of consciousness within minutes.
10 - 100	In concentrations above 10 %, unconsciousness will occur in under one minute and unless prompt action is taken, further exposure to these high levels will eventually result in death.

(<http://www.lehaneenvironmental.com/wp-content/uploads/2016/08/ElGA-Carbon-Dioxide-Hazards.pdf>)²¹

(<https://www.hse.gov.uk/pubns/priced/eh40.pdf>)²²

2.5.2.8 Toxic Gases (Class 2, Division 2.3)

The primary hazard from toxic gases is the potential for severe physiological adverse effects on health. In addition to health hazards, secondary hazards including asphyxia, flammability and/or oxidising hazards may exist.

In the context of C1 gases, special consideration is required for the use of carbon monoxide for microbiological applications due to its toxic nature. Exposure to toxic gases impact individuals to varying degrees, from mild irritation to severe reaction, including death, depending on the concentration of gas and the susceptibility of the individual. For carbon monoxide the long-term exposure limit (8 hour TWA reference period) is 30 ppm (0.003 %v/v in air) (<https://www.hse.gov.uk/pubns/priced/eh40.pdf>)²².

2.5.2.9 Oxidising Gases

Oxidising gases, such as oxygen, will support the combustion process in the event of a fire. In an enriched oxygen atmosphere, many substances which would not otherwise combust may ignite, or in the case of combustible substances, may ignite at concentrations below their normal *Lower Explosive Limit* (LEL). The primary hazards in this scenario are increased risk of combustion coupled to increased intensity of combustion. Oxygen itself is non-flammable and present in the air at 20.9 % (v/v). However, oxygen enrichment in the atmosphere, even by a few percent, considerably increases the risk of fire and rate of propagation of fire or explosion. Concentrations above 23.5 % (v/v) should be avoided where possible or specific precautions need to be implemented if this is unavoidable. It is advisable to consider the source of any potential oxygen release in addition to an external oxygen supplied *via* a compressed gas cylinder for example. For instance, oxygen can be produced as a by-product of biological or chemical reactions, particularly in microbiological systems (e.g., decomposition of hydrogen peroxide to molecular oxygen by native catalases).

For more detailed information on fire hazards of oxygen and oxygen enriched atmospheres refer to: <https://www.hse.gov.uk/pubns/indg459.htm>²³ and <https://www.eiga.eu/uploads/documents/DOC004.pdf>²⁴

2.5.3 Hazardous work activities

Employers should consider all work activities that involve dangerous substances such as dispensing and decanting, movement of dangerous substances and containment of spillages/leaks. Additional information can include:

- Skills, knowledge, and experience of employees;
- Training and supervision of employees;
- Activities in adjacent areas/labs/premises particularly where this could present an ignition risk;
- Possible misuse/incorrect handling of dangerous substances e.g., incompatible mixing of waste substances;
- Past accidents/incidents/near misses involving the dangerous substance.

When gases are in use, below is a list of areas which typically present an increased risk in the workplace and should be considered in the risk assessment:

- Confined spaces i.e., small labs or rooms with restricted space with poor ventilation;
- Internal gas storage areas due to the quantity of gas stored;
- A “safe” area adjoining a place of gas storage/use where released gas can enter *via* air ducting, ventilation or from exhausts;
- Poorly ventilated areas where air change rate is insufficient e.g., lifts.

The risk factor (likelihood) of an explosion or fire incident occurring and the degree of severity of harm to people or property resulting from such an incident should be evaluated. A preliminary risk assessment must be undertaken before any new work activity involving the use of compressed gases commences. A simple risk assessment model is shown in **Figure 3**.

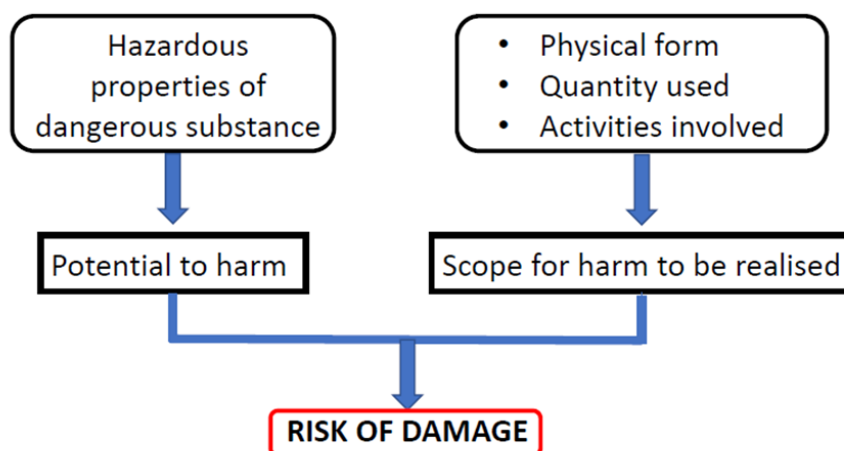


Figure 3 Simple risk assessment model

2.5.4 Evaluating the risk

Risk is a combination of the likelihood of harm occurring and the severity of that harm and special consideration should be given to people, the environment and property as part of the risk evaluation process.

As part of a preliminary assessment when using gases in an enclosed workplace it is essential to establish the likelihood of any potential change in the atmosphere taking place and if this presents a hazard to people in the vicinity. The following criteria should be considered:

- What is the largest volume of gas can be released into the workplace?
- What is the total volume of the workplace?
- Whether the estimated gas concentrations exceed safe threshold levels?

If the gas concentrations are within the limits in **Table 10** then the workplace can be considered low risk and a more detailed risk assessment is not necessary. As a guide for safe thresholds for common gases refer to **Table 10**.

If the preliminary assessment concludes that the gas concentration could exceed the threshold levels then a more detailed risk assessment is required.

Table 10 A guideline for limits for some common gases

Gas	Limit	Comments
Oxygen	Min: 19.5 %	
	Max: 23.5 %	
Carbon dioxide	Max: 0.5 %	
Inert gases, for example, nitrogen		Use oxygen minimum level
Flammable gases	Max: 25 % of the LEL	
Toxic		Use WEL for specific gas

2.5.4.1 Preliminary Risk Assessment Criteria

The *Preliminary Risk Assessment* should consider the worst case scenario in which the entire contents of a container(s) is released instantaneously into the workplace being assessed. The assessment should include:

1. Identify the gas container(s) from which the gas may be released

Where multiple cylinders are in use the combined total volume needs to be considered. The user needs to understand the relationship between container size (e.g., cylinder size) and the gas pressure inside the container. From this the maximum volume of released gas can be calculated.

2. Determine the volume of gas in the container(s)

- Compressed gas cylinder
 - Pressure (bar) x volume (litres)/1000 = m³
- Liquefied gas cylinder
 - Weight of product (kg) x specific volume at ambient temperature °C (m³/kg) = m³
- Cryogenic liquid container
 - Capacity of tank (litres) x expansion ratio at ambient temperature °C/1000 = m³

3. Determine the free air volume in the workplace

Workers should consider the approximate dimensions of the microbial lab (height x width x length) in metres to determine the total volume (m³). Deduct the volumes of any equipment, furniture, stock etc as these reduce the volume of free air in the workplace.

4. Calculate the percentage of gas concentration

Apply the following calculation:

$$C = 100 \times \frac{V_{gas}}{V_r}$$

Where:

C = percentage of gas concentration

V_{gas} = the volume of gas (m³)

V_r = the volume of free air in the workplace (m³)

Example scenario for release of an asphyxiant gas e.g., nitrogen

A compressed Nitrogen cylinder (size W) supplied by BOC gases which is charged to 230 bar and will be used in a workplace with a free air volume of 50 m³. In the worst case scenario, where all the nitrogen gas was released instantaneously into the workplace, what impact would this have on the atmospheric oxygen concentration for normal working?

Example calculation:

$$C = 100 \times \frac{V_{gas}}{V_r}$$

Dimensions of Size W cylinder (taken from BOC website): 146 cm height x 23 cm diameter

Volume (m³) = $\pi \times r^2 \times h = \pi \times 11.5\text{cm}^2 \times 146 \text{ cm} = 60,6059 \text{ cm}^3 = 60.6 \text{ L}$

Pressure = 230 bar

Volume of gas in the cylinder = 60.6 L x 230 bar /1000 = 13.9 m³

NB gas cylinder suppliers normally provide approximate nominal gas contents in m³ avoiding the need to calculate volumes based on cylinder dimensions and pressure.

V_r = 50 m³

Percentage of nitrogen gas = $100 \times (13.9 \text{ m}^3 / 50 \text{ m}^3) = 27.88\% \text{ (v/v)}$

To determine percentage concentration of oxygen V_{gas} can be substituted with V_o :

$$C = 100 \times \frac{V_o}{V_r}$$

Where **V_o** = volume of oxygen gas

= 0.21 (V_r - volume of gas in the cylinder)

= $0.21 \times (50 \text{ m}^3 - 13.9 \text{ m}^3) = 7.58 \text{ m}^3$

Percentage of oxygen gas = $100 \times (7.58 \text{ m}^3 / 50 \text{ m}^3) = 15.16 \% \text{ (v/v)}$

NB this oxygen concentration is below the minimum workplace concentration for normal working (refer to section 2.5.2.3 *Asphyxia*), however, instantaneous release of the whole contents of the compressed gas cylinder is worst case scenario and almost an

inconceivable event, therefore specific preventative measures should be assessed but are unlikely to be implemented in this case.

Often gas concentrations can be expressed in different units other than % v/v. Below is list of common units encountered when determining gas concentrations in the atmosphere and the conversion factors required to change between units using the % v/v oxygen gas value from the example calculation outlined above:

e.g. % oxygen gas = 15.16 % volume of oxygen/volume of air (%v/v)

= 0.1516 L oxygen gas in 1 litre of air (v/v)

= 151.6 L per cubic meter (L/m³)

= 151,600 parts per million (ppm)

oxygen density in air (at 20°C) = 1.314 kg/m³ (= g/L)

weight of oxygen = 0.1516 litres x 1.314 g/L = 0.1992 g

density of air (at 20°C) = 1.204 kg/m³ (= g/L)

Weight of air (at 20°C) = 1 L X 1.204 g/L = 1.204 g

oxygen gas in air = weight of oxygen / weight of air

= 0.1992 g / 1.204 g X 100% = 16.54% (%w/w)

2.5.4.2 Detailed Risk Assessment Criteria

If the outcome from the preliminary risk assessment deems it is necessary to carry out a more detailed risk assessment then many variables need to be considered to accurately establish the level of risk.

The further risk assessment should be carried out by competent staff and consider the following points for all gases to be used:

- Identify the specific gas(es) to be used and its properties:
 - Understand variation in effect due to temperature, density, pressure, physical state etc.
 - Understand implications for resulting atmosphere in event of accidental gas release
- Identify potential gas release points including rate of release, duration, volume, and likelihood of release due to:
 - Venting *via* safety relief valves

- Deliberate release e.g., venting
 - Natural boil off from cryogenic liquids
 - Process exhaust e.g., off gas output coming from fermentation
 - Unintended leakage from pipework/equipment e.g., leaking joints
 - Incorrect operation of equipment
 - Release during connection of hoses, pipework, fittings, and regulators
- Transfer of released gas into other spaces e.g., ventilation systems, air intakes, roof spaces, gulleys, trenches etc.;
 - The free air volume of the workplace and existing ventilation due to natural or forced air changes per hour;
 - Occupancy of the spaces where gas release may occur;
 - Potential sources of ignition for flammable gases, e.g., naked flames, sparks caused by mechanical friction from equipment, chemical processes, mobile phones, hot surfaces, and electrostatic charges from fabrics;
 - Increased fire risk or health risks from oxygen enriched atmosphere;
 - Acute and chronic health effects if intoxicating, toxic and/or corrosive gases are used.

Further risk assessment considerations for activities involving compressed gas cylinders within the workplace are provided by BCGA and can be found online (<https://bcga.co.uk/wp-content/uploads/2023/04/BCGA-TIS-49-Original-27-04-2023.pdf>)²⁵.

The following calculation can be used to approximate the resulting atmosphere where the gas release rate, the free air volume, ventilation rate and time duration are known. This method can be used to establish the hazard from a gradual release of gas into the workplace (under normal operating conditions) where an instantaneous release of gas(es) is unlikely to occur but where a build-up of an unsafe atmosphere could occur over a period of time.

The following equation gives the approximate resulting *Oxygen Percentage Concentration* (OC_t) after time (t) and should be used to establish the asphyxiation and oxygen enrichment risk:

$$OC_t = 100 \left(0.21 + \left(\left[\frac{0.21 \times n}{\left(\frac{L}{Vr} \right) + n} \right] - 0.21 \right) (1 - e^{-t/m}) \right)$$

And for long periods of time where t tends to infinity:

$$OC_{\infty} = 100 \times \left(\frac{Vr \times 0.21 \times n}{L + (Vr \times n)} \right) \text{ approximately}$$

The following equation gives the approximate resulting *Gas Percentage Concentration* (GCt) after time (t) and should be used to establish the intoxicating (CO₂), toxic or flammable risk:

$$GC_t = \left(100 - \left(100 \times \left[\frac{Vr \times n}{L + (Vr \times n)} \right] \right) \right) \left(1 - e^{-t/m} \right)$$

And for long periods of time where t tend to infinity:

$$GC_{\infty} = 100 - \left(100 \times \left[\frac{Vr \times n}{L + (Vr \times n)} \right] \right) \text{ approximately}$$

Where:

OCt	=	Oxygen percentage concentration after defined time
OC _∞	=	Oxygen percentage concentration after long periods (days)
GCt	=	Gas percentage concentration after defined time
GC _∞	=	Gas percentage concentration after long periods (days)
L	=	Gas release rate, m ³ /h
Vr	=	The volume of free air in the workplace, m ³
n	=	The number of workplace air changes per hour
t	=	Time, hours
e	=	2.72
m	=	$\frac{Vr \times n}{L + (Vr \times n)}$

Example scenario for continuous flow of carbon dioxide gas into a fermentor

Carbon dioxide gas from a compressed gas cylinder is being used to sparge a fermentation tank in a microbiology lab with a free air volume of 50 m³ with a gas flow rate

of 3 L min⁻¹. The clean air changes in the laboratory *via* general ventilation are 3 per hour and the time taken to complete the fermentation is 48 hours.

To establish the impact on the workplace atmosphere after 48 hours the following formula is used:

$$OC_t = 100 \left(0.21 + \left(\left[\frac{0.21 \times n}{\left(\frac{L}{Vr} \right) + n} \right] - 0.21 \right) \left(1 - e^{-t/m} \right) \right)$$

Where:

OC_t = oxygen percentage concentration after defined time

L = 3 L min⁻¹ = 180 L h⁻¹ = 0.18 m³ h⁻¹

Vr = 50 m³

n = 3 air changes per hour

t = 48 hours

e = 2.72

m = (Vr x n)/(L+(Vr x n)) = (50 x 3)/(0.18+(50 x 3)) = 0.9988

$$OC_t = 100 \left(0.21 + \left(\frac{\left(\frac{0.21 \times 3}{\left(\frac{0.18}{50} \right) + 3} \right) - 0.21}{1 - (2.72^{-48/0.9988})} \right) \right)$$

$$OC_t = 100 \left(0.21 + \left(\frac{0.63}{3.0036} - 0.21 \right) \left(1 - (1.34 \times 10^{-21}) \right) \right)$$

$$OC_t = 100 \left(0.20975 \right) \left(1 \right)$$

$$OC_t = 20.975 \% \text{ v/v}$$

The calculated concentration of oxygen in the air is 20.975%. In this scenario, oxygen concentration is very close to the normal levels in the atmosphere (see **Table 10**). It should be noted however, that if changes to air ventilation decreases or flow rate of CO₂ gas increases this could lower the oxygen concentration in the workplace atmosphere and the operator should be aware of threshold limits for safe working.

2.5.4.4 Risk Assessment Matrix

A *Risk Matrix* can often be helpful to determine the level of risk associated with particular activities. It is possible to categorise the likelihood of harm and the potential severity which is represented in a matrix. The risk level determines which risks should be mitigated by priority. Advice on risk matrices is available on the HSE website: <https://www.hse.gov.uk/simple-health-safety/risk/index.htm>²⁶

2.5.5 Identifying risk control measures

Employers must implement control measures to eliminate risks from dangerous substances, or reduce them as far as reasonably practicable. If it is not possible to completely eliminate risk, employers must take measures to control risks and reduce the severity and potential impact of any harmful event. Control measures reduce the probability of a hazard occurring, such as a hazardous atmosphere due to a gas release, and may include location of gas storage, control of gas usage and ensuring sufficient ventilation. It may be necessary to use more than one type of control measure as specified in the sections below:

2.5.5.1 Elimination

The best solution is to eliminate risk completely by removing the possibility of a hazardous atmosphere being created within an enclosed workplace. In practice where possible gas should be prevented from entering the workplace unintentionally. For example:

- Carry out operations that use gases outside in the open air
- Carry out operations in areas isolated from the general workplace where there is adequate ventilation
- Store gas cylinder and tanks outside where possible (see relevant BCGA documentation <https://bcga.co.uk/pubcat/codes-of-practice/>)²⁷.

2.5.5.2 Substitution

Use the most appropriate gas, with the lowest hazard, for any particular situation. For example, if carrying out leak testing using an inert gas e.g., nitrogen rather than a flammable gas e.g., hydrogen. Consider the properties of the gas and the environment in which it will be used

2.5.5.3 Engineering Controls

Engineering controls should be included depending on the types and specific properties of the gases that will be used. The quantities of gas, duration of use, location and their specific application in the workplace need to be considered. Engineering controls allow isolation of gas supplies from personnel and include early detection systems to alert workers of an accumulation of gas and/or the controlled release/removal of gas(es) through external extraction for example. It is normal for engineering controls to be designed, installed, and maintained by specialist companies or competent individuals.

Types of engineering controls include:

- **Workplace ventilation**

Achieved by increasing natural ventilation through the microbial lab ventilation system or by providing local exhaust ventilation systems.

- **Atmospheric monitoring equipment**

Monitors are used to alert workers to changes in the atmospheric composition. These may include fixed or portable detection devices or a combination of both as determined by a risk assessment.

In some cases, individual sensors for specific gases are required e.g., carbon dioxide enrichment or oxygen depletion sensors. Hydrogen gas sensors can be used to detect accumulation and eliminate potential for explosive atmospheres to occur.

- **Pipework**

Professionally installed pipework and gas tight fittings are recommended to deliver gas at the lowest pressure possible with restricted flow rates suitable for the intended application and in accordance with maximum ventilation capabilities.

- **Exhausts**

Exhaust lines from reactors, fermentation vessels, vent valves etc should be directed to a safe area. Ideally these should be located within externally vented fume cupboards if possible.

- **Exclusion**

Provide a dedicated area that separates personnel from places where gas might affect the atmosphere (e.g., fume cupboard, safety cabinet, isolated compartment).

2.5.5.4 Administrative Controls

- Suitable information, training and supervision should be provided to those who need it;
- Increasing hazard awareness through safety signage etc.;
- Monitoring and if necessary reducing the time workers are exposed to hazards (e.g., by job rotation);
- Restricting access to areas where gases are in use;
- Implementation of permit systems to control specific activities;
- Health surveillance of staff;
- Implementation of emergency procedures to manage and mitigate hazardous situations arising and escalating.

2.5.5.5 Personal Protective Equipment (PPE)

As part of the work activity risk assessment the requirement for the use of hazard controls including *Personal Protective Equipment* (PPE) should be established. *PPE* should be selected for particular tasks, personnel involved and location. Different *PPE* measures might be required for the use of specific gases or in certain situations. HSE provides guidance for selecting and using *PPE* at work (<https://www.hse.gov.uk/ppe/index.htm>)²⁸.

Some examples of PPE include:

- Personal atmospheric monitors
- Breathing apparatus for emergency activities
- Flame retardant clothes when using flammable gases
- Suitable clothing e.g., anti-static footwear for flammable gas environments

2.5.6 Emergency plans and procedures

Suitable arrangements must be made to deal with emergencies. These plans and procedures should cover safety drills and suitable communication and warning systems and should be proportionate to the risks. Some engineering controls (e.g. emergency gas shutoff buttons) should be considered and made accessible from well outside the working area for access by firefighters, etc. If an emergency occurs, workers tasked with carrying out repairs or other necessary work must be provided with the appropriate equipment to undertake the work safely. The information in the emergency plans and procedures must be made available to the emergency services to allow them to develop their own plans if necessary.

Instruction and Training for Employees

Employees must be provided with relevant information, instructions and training which includes:

- Identification of an emergency situation;
- Raising the alarm;
- Immediate actions for employees;
- The dangerous gases present in the workplace and the risks they present including access to any relevant safety data sheets and information on any other legislation that applies to the dangerous substance. The contents of containers, vessels, gas cylinders etc must be identifiable to alert employees and others to the presence of dangerous substances;
- How to prevent escalation of the emergency situation e.g., safe isolation of gases or shutdown procedures to cut off the gas supply;
- Safe evacuation to a safe assembly point and roll call;
- Assessment of potential escalation and who else might be affected;
- Communication to co-workers, management, neighbours, emergency services;
- Familiarisation with emergency evacuation procedures;
- Testing of the procedures including fire drills, including out of hours working.

Information, instruction, and training need only be provided to other people (non-employees) where it is required to ensure their safety. It should be in proportion to the level and type of risk.

NB most deaths in confined spaces are of those who attempt rescue. When working in or near areas where gases are in use or in a confined space, if a person collapses and no longer shows signs of life, assume the person may lack sufficient oxygen due to the presence of an asphyxiating atmosphere. Do not enter the confined space without adequate preparation and get proper assistance and support.

2.6 Chapter 2 - Bibliography

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Chapter 3 - Guidance to Typical Microbiological Laboratories

In microbial labs where fermentations are carried out these processes can be categorised into three different scales:

Small: Shake flask (0.25 L - 1 L)

Medium: Standard *Continuous Stirred Tank Reactor* (CSTR) (1 L – 5 L glass autoclavable, bench top)

Large: Standard CSTR (5 L - 30 L steel, sterilise *in-situ*)

For each of these processes if a C1 gas is being used as the feedstock they need to be individually risk assessed for:

- 1) The vessel being used (shake flask, glass, or steel fermentor);
- 2) If the vessel is a sealed or vented (open) system;
- 3) The scale of the process in terms of volume (small (0.25 L - 1 L), medium (1 L - 5 L) or large (5 L - 30 L));
- 4) The volume of the room in which the work is being carried out in;
- 5) The type of gas being used (CO₂, CH₄ etc.) as each gas poses different risks in terms of flammability, explosion, asphyxiation etc.;
- 6) The delivery of the gas (batch or continuous);
- 7) The likelihood of detection of an unwanted gas leak.

For example, typically in a shake flask the vessel is sparged with the C1 feedstock gas and then sealed. There is not a continual addition of the gas, therefore, the risk of a fire, explosion or asphyxiation is less. In a CSTR where the gas is constantly being added the risk is likely to be higher. Each lab where this work is being carried out should already have a *Quality Management System* (QMS) in place, which includes *Safety & Risk Management*, such as *COSHH Assessments*, *Risk Assessments*, and *DSEAR Assessments*. All of these should be considered and completed before any work with a C1 feedstock is carried out.

If after carrying out these risk assessments the risk status is deemed too high then any mitigation to lower the risk should be considered and implemented. This should lower the risk status to allow the work to be carried out. If this is not possible then it is not advised to proceed with the work if the risk to the operator is deemed to be too great.

Prior to commencing any fermentations involving C1 gases a full *Gap Analysis* for the particular type of work should be undertaken before any lab work starts. **Table 11** is to serve as an example of the *Gap Analysis* for an *in situ* steel CSTR. A similar sort of *Gap*

Analysis should be carried for any small or medium scale lab work. Please note that anything above TRL5 is out of scope of this document.

Table 11 Example as guidance on *Gap Analysis* for *in-situ* CSTR (5 L-30 L steel)

	Existing situation	Desired situation	Guidance on action plans
<i>In-situ</i> CSTR type	Standard CSTR, equipped with single gas sparger, standard temperature / pH /DO probes and O ₂ /CO ₂ off-gas monitor.	An ATEX-rated CSTR, equipped with double gas spargers and a gas overlay, EX-proof probes, O ₂ /CO ₂ /CO/CH ₄ off-gas monitor depending on gas type used, as well as auto shutdown cascaded with gas inputs.	It is relatively expensive to build an ATEX-rated CSTR. Alternatively, it can be designed to be close to an EX-rated system by working together with the manufacturer/fermentation engineers, with additional control measures in place. for example, the equipment can be located in a fume cupboard so there is active extraction of the gases during operation. Another consideration is to have the equipment located behind a protective screen to protect the operator further. Also, to minimise the possibility of any sparks from electrical equipment by regular servicing of the kit and location of any electrical power point supplying the equipment away from any of the gases.
Gas input	Standard gas pipes from cylinders/ air compressor located from within the lab space.	Gas cylinder external to the laboratory; Professionally fitted gas pipes with designated pressure relief valves and solenoid valves for auto shutdown purposes. Use of hydrogen gas generator (if required) opposed to hydrogen cylinders.	Consider pressure build-up in the gas pipe and fermenter. Working with the engineers to find out a local solution.

Gas output	Gas piping to the extraction hood or to the laboratory environment.	Gas piping to the outside atmosphere directly; alternatively, to a well-vented extraction hood.	Ideally run the gas output directly to the outside environment. If not possible, direct the gas pipe to a well-vented extraction hood or through external ducting - consider pressure build-up in the hood if the ventilation is accidentally shut or fails.
Local ventilations	Constant air exchange in the laboratory environment.	Consider the flammable gas build-up in the worst scenario – air supply is sufficient to ensure flammable gas remain below limits e.g., 10% of LEL.	If laboratory space is extended, it would be helpful to consider locating the fermenter within a walk-in enclosure, to ensure effective ventilation.
Local alarm	O ₂ CO ₂ , CO alarms	Additional flammable gas alarms, set at high/low level; ventilation alarm when air flow is failed below limits.	Installation of additional gas alarms and ventilation systems for fermenters

In a similar manner to the *Gap Analysis a Self-Assessment Questionnaire* should also be filled out prior to commencing any work with C1 gases. **Table 12** is to serve as an example of this type of self-assessment.

During C1 gas utilisation in typical microbiological laboratories there is a range of failure modes that can arise. Prior to setting up and conducting any laboratory work with C1 gases it can be helpful to perform a laboratory specific review of as many of the components, assemblies and systems that can create failure modes within the operations of using the respective gases. Each component should be treated in how a failure mode may arise and the resulting effect of this within a documented *Failure Mode Effect Analysis* (FMEA) *worksheet*. A *FMEA worksheet* output provides a record and documented process to capture information related to potential causes of failure, their respective impact and where possible, ways in which to reduce the possibility of the failure mode from occurring.

Table 13 is a representative *FMEA worksheet* for a small scale single 250 mL shake flask experiment to culture an aerobic methanotrophic bacteria in a platform shaker incubator operating under fuel light control. Fuel light, such that methane / air composition has an upper limit of methane that is beneath the flammability v/v% when mixed in air as defined by the *Triangular Flammability Diagram* (**Figure 1**). A similar *FMEA* should be carried out for medium and large scale work, specific for the type of reactor and gas as discussed earlier in this section.

Table 12 Self-assessment questionnaire to facilitate users to evaluate risk profile ahead of roll-out of C1 gaseous feedstock implementation within a typical microbiology laboratory

Please rate yourself in the following area by ticking one box along each line

#	Questions	Poor	Less than satisfactory	Satisfactory
1	Critical knowledge about the risk associated with C1 feedstock			
2	Risk mitigation for damage to gas cylinders/supplies			
3	Risk mitigation for damage to gas pipelines			
4	Risk mitigation for fermenter/incubator design			
5	Risk mitigation for off-gas streams			
6	LEL limit in the fermenter/incubator and associated setup			
7	LEL limit in the laboratory and associated setup			
8	Alarms internal to the laboratory			
9	Alarms external to the laboratory			
10	Emergency plan			
11	Plan for Out-Of-Hour's operation			
12	Plan in the event of power cut			
13	Documentation reviewed and updated (SOPs, risk assessments, COSHH)			
14	Device labels and local instructions			
15	The building management team has been satisfied with the modifications			
16	Trainings to lab teams			
17	Laboratory safety supervisor and deputy supervisor appointed			

Table 13 Example *FMEA worksheet* for a small scale single 250 mL shake flask.

NB this is not an exhaustive list but just a few examples of how you would go about doing *FMEA*

FEM A Ref	Item	Potential Failure mode	Potential cause / mechanism	Phase of work	Local effect of failure	Next high level effect	System level end effect	Probability (P)	Severity (S)	Detection (D)	Risk level P*S (+D) ¹	Actions for further investigation / evidence	Mitigation requirements
1.	Methane dispensing	Gas dispenser of methane cannot accurately deliver appropriate metered gas flow	Flow meter is either inappropriate for the task or not calibrated	Set up of nutrients in shake flask	Volume addition of methane into flask is not accurately provided. Either too much or too little is provided	Lack of methane is inadequate and microbe cannot grow (carbon limited). <u>Or</u> Excess methane poses risk of flammability, possibly explosive potential	In event of under delivery, growth rate of microbe will be retarded or fail due to lack of nutrient carbon In event of over delivery	Frequent	Low in event of under delivery (only loss of experimental time resource) Very high in event of over delivery of methane; potential for combustible composition of gases which in a sealed flask has scope to become explosive	Poor detectability since gas has no obvious sensory detection (i.e., not coloured or odorous)	Unacceptable without mitigation	Check certification and ensure minimum frequency manufacturer recommended calibration of gas flow meter used for methane dispensing is performed prior to use with an inert representative gas (e.g., N2 or air)	Ensure gas flow meter is regularly serviced and according to manufacturer's recommendations replace product
2.	Methane dispensing	Pipework of methane dispenser is compromised	Unsuitable product used or ill-fitting of pipework and/or pipe connections	Assembly of pipework between methane cylinder, gas regulator and end nozzle aperture of gas dispense	Inadvertent methane gas escapes from a failure in the gas pipework into the laboratory	Uncontrolled release poses risk of fire. In an inadequately vented microbiological laboratory this could create risk of explosive atmosphere being formed	Potential for methane contents of cylinder to uncontrollably be released in the laboratory. This represents a potential fire or explosion risk.	Moderate	Moderate in a well-ventilated laboratory or high in a laboratory with no active ventilation	Moderate. It may be possible to hear an audible 'hiss' if inadvertent gas is leaking from the fitted pipework.	Unacceptable without mitigation.	Use professional gas fitting engineer (e.g., Speck and Burke) to install fitted pipework. Ensure regular maintenance and inspections with competent provider is provided.	Prior to each use conduct a visual inspect of all pipework and a soapy water 'leak test' is provided to each inter-pipe union/connection points between cylinder and dispensing aperture.

FEM A Ref	Item	Potential Failure mode	Potential cause / mechanism	Phase of work	Local effect of failure	Next high level effect	System level end effect	Probability (P)	Severity (S)	Detection (D)	Risk level P*S (+D) ¹	Actions for further investigation / evidence	Mitigation requirements
3.	Cell growth	Platform shaker growth	Dislocation of shake flask from platform shaker	Post transfer of inoculated shake flask into shaker incubator	Departure of glass shake flask from platform results in flask being thrown from platform onto side of incubator and smashing.	Break in glass causes uncontrolled release of flask contents including fragments of glass, cell media, cells and methane/air mixture	Debris clean up creates added risk. Broken glass = scope for cuts. Media/cells to be cleaned out from mechanical shaker (problem compounded if microbe is >cat1). Gases contained in flask will be immediately discharged into laboratory space.	Low	Medium, rises though if microbe is greater than 1 in biosafety categorisation	High. It should be very obvious if this occurs.	Tolerable	Recommended appropriately sized flask clamp stands are utilised. Use of 'stick mat' or other non-manufacturer design clamps are not used.	Prior to each use, ensure clamp stands are checked and verified as being tightly secured. When necessary, tighten clamp stand to platform.

¹, It is advised to assign numbers (1-10) to Probability, Severity and Detection, and then having a matrix of responses based on the final score (e.g. risk judged to be unacceptable if score beyond a certain threshold).

Chapter 4 - Suggestions of Additional Safety Equipment in Typical Microbiological Laboratories

4.1 Gas Detectors and Alarms

4.1.1 General considerations

Flammable gas detectors can make a valuable contribution to the safety of these processes. They can be used to trigger alarms if a specified concentration of the gas or vapour is exceeded. This can provide an early warning of a problem and help to ensure people's safety. However, it should be noted that a detector does not prevent leaks occurring or indicate what action should be taken.

The following factors relating to setting an alarm should be considered when installing and using any type of gas detector ¹:

- 1) Whether detectors should be fixed and/or whether personnel should be issued with portable, which includes personal, detectors;
- 2) The location of the fixed detector and whether the work area is occupied or unoccupied;
- 3) Whether the fixed detector is a point or open-path (also known as beam or line of sight detectors) detector;
- 4) Whether the process control is difficult and/or time-consuming;
- 5) Whether *Workplace Exposure Limit* (WELs), other *Exposure Limit Values* or other health-based levels (e.g., *Immediate Danger to Life and Health*- IDLH) exist;
- 6) Instantaneous or *Time-Weighted Average* (TWA) alarm;
- 7) Background variations and events from the process, which may trigger "spurious" alarms;
- 8) Effects of interferents;
- 9) False alarms caused by instrumental effects;
- 10) The potential rate of gas build up (considering short-term peaks in point 7);
- 11) Time to alarm of the detection system;
- 12) Number of alarm levels (e.g., high, and low levels);
- 13) Mixture of gases/vapours.
- 14) Potential gaseous composition arising is likely to form with specific gravity greater or less than air such that detector should be located at either low or high level in the laboratory.

4.1.2 Selecting a suitable gas detection system

Firstly, the gas detection system should be protected from sources of ignition by selecting equipment certified as being suitable for use in hazardous areas. It should have the 'CE' and 'EX' markings to show conformity with the *Equipment and Protective Systems Intended for Use in Potentially Explosive Atmospheres Regulations* (EPS Regulations 1996).

Detectors can be fixed or portable.

A **Fixed Detector** is permanently installed in a chosen location to provide continuous monitoring of plant and equipment (**Figure 4**). It is used to give early warning of leaks from plants containing flammable gases or vapours, or for monitoring concentrations of such gases and vapours within a plant. *Fixed Detectors* are particularly useful where there is the possibility of a leak into an enclosed or partially enclosed space, where flammable gases could accumulate. The location of a *Fixed Detector* is important as this will affect the time for any gas release to reach the gas sensor and then, if the concentration is high enough, activate the alarm. Consideration of the likely sources and type of gas leaks should be undertaken in order to ensure the optimal position for the detector(s) which then minimises the time to alarm. Where proposed gas handling has a specific gravity greater than air (e.g., CO₂) it is preferable to locate detector in a relatively low position in the laboratory, whilst when gas has a specific gravity less than air (e.g., CH₄ and H₂) it is preferable to locate detector in a relatively elevated position such as ceiling attachment in the laboratory. It may also be worth considering the risk of gas build-up above ceiling tiles and implications for gas detector mounting positions.

A **Portable Detector** usually refers to a small, handheld device (**Figure 5**), that can be used for testing an atmosphere in a confined space before entry, for tracing leaks or to give an early warning of the presence of flammable gas or vapour when hot work is being carried out in a hazardous area. *Portable Detectors* are used for testing for gas free areas, particularly in confined spaces. The alarm limits for such detectors are therefore more directly related to exposure limits and are typically set at lower levels than instantaneous alarms for fixed detectors. These can also be attached to clothing or worn on person (e.g., breast pocket or clipped to belt), used for personal protection.



Figure 4 Examples of fixed Oxygen and Carbon dioxide detectors (left) and low oxygen alarm (right) (photo credit: Heriot-Watt University) (ranging from £100-500)



Figure 5 An example of a portable gas monitor (ranging from £100 to £500)

4.1.3 Selecting measurement types

There are point or point-path detectors ².

Point Detectors measure the concentration of the gas at the sampling point of the instrument. The unit of measurement can be: 1) % volume ratio; 2) % *Lower Explosion Limit* (LEL) for a flammable gas; 3) ppm or mg/m³ for low level concentrations (primarily used for toxic gases).

Open-path Detectors, also called *Beam Detectors*, typically consist of a radiation source and a physically separate, remote detector. The detector measures the average concentration of gas along the path of the beam. The unit of measurement is concentration multiplied by path length, % LEL x m or ppm x m. Systems can be designed with path lengths of 100 m or more. However, it is impossible to distinguish whether a reading is due to a high concentration along a small part of the beam or a lower concentration distributed over a longer length. Also, they are not specific to a particular gas, for example steam or water vapour can produce false readings and alarms.

4.1.4 Alarm settings

The *Gas Detector* should be set to alarm at a level low enough to ensure the health and safety of people but high enough to prevent false alarms. False alarms are most likely to be caused by fluctuations in sensor output due to environmental changes (e.g., ambient temperature, pressure, or humidity), sensitivity to other gases or vapours, or sensor drift.

In determining the required alarm levels for *Fixed Gas Detection Systems*, the following should be taken into account ²:

- Any industry standards and recommendations;
- The lower explosion limit of the gas or vapour;
- The size of the potential leak and the time to reach a hazardous situation;
- Whether the area is occupied;
- The time required to respond to the alarm;
- The actions to be taken following the alarm;
- The toxicity of the gas or vapour.

A suitable safety margin should also be incorporated to account for ventilation dead spots, where vapours could accumulate, and the variability of natural ventilation. One option is to set two alarm levels. The lower alarm could act as a warning of a potential problem requiring investigation. The higher alarm could trigger an emergency response such as evacuation or shutdown or both. For leak detection purposes (i.e., not process monitoring), the first alarm level should be set as low as practicable, preferably no higher

than 10% of the *Lower Explosion Limit* (LEL). The second alarm level should be no more than 25% LEL.

If gas detectors are installed as part of the process where a flammable atmosphere is expected (e.g., a solvent-evaporating oven) the alarm levels should take into account normal operating conditions and the maximum safe operating concentration of the equipment. The manufacturer should specify the alarm levels, which could be up to 50% LEL depending on the design of the equipment.

Setting alarm points should be a recurring event. Initial alarm set-points may be adjusted either up or down in the light of experience in the environment, i.e., process-related events and instrument characteristics. This requires analysis of the data over a suitable period. Minimising the risk by lowering the alarm levels has to be balanced by minimising spurious alarms, which lower efficiency. Nevertheless, it is good practice to try to drive down alarm levels. On the other hand, alarm levels may have to be increased from their initial values. In this case, the reasons need to be recorded and the risk assessment reviewed to ensure that the new *Risk Status* is tolerable.

4.1.5 Response time ²

Firstly, if there is a catastrophic failure leading to an extremely rapid build-up of gas, then it may not be possible for any detector to alarm in time.

Secondly, it is critical that alarm activation is designed to allow time for appropriate action to be taken. Consideration of gas build up rates and use of multiple alarms levels can allow sufficient time for action, i.e., evacuation, checking and possible rectification of the event which has led to a gas release.

The required *Response Time* will depend on the location, purpose of the system and speed of development of the expected problem. Protection of people from large leaks requires a fast *Response Time* but where the gas concentration is expected to build up slowly a slower *Response Time* may be acceptable. Setting a lower alarm threshold allows longer time to take executive action.

4.2 Control Panels and Instrument Readouts

Obtaining a reading of the gas concentration should be possible without entering a hazardous area. The control panel (**Figure 6**) should be sited so that readings can be taken in safety. There should also be access for calibration and maintenance. For example, if a building is being monitored then a suitable position could be on the outside wall of the building (possibly near an exit). This would allow the gas concentration to be checked before entry into the building. In addition, manual emergency stops for flammable or toxic gas flow could also be located here.

It may be necessary to have read-outs at more than one location. For example, one read-out panel could be located in a control room, however, a second read-out may be needed closer to the plant or equipment to inform personnel working locally.



Figure 6 An example of control panel and readouts (left) and instructions next to it (right) (photo credit: Heriot-Watt University) (ranging from £300-5000 excluding electrical engineering cost)

4.3 Local Exhaust Ventilation

4.3.1 Purpose and type

Traditionally, *Local Exhaust Ventilation* (LEV) is an extract ventilation system that takes airborne contaminants such as dusts, mists, gases, vapour or fumes out of the workplace air, so that they cannot be breathed in ³. As discussed previously, it could be employed to ensure effective ventilation taking place and lower the local explosive risk.

In the context of C1 gaseous feedstock handling, the *LEV* can be customised and present as a bench-top enclosure assisted with an extraction arm (**Figure 7**), or a walk-in enclosure equipped with an extraction hood (**Figure 8**).



Figure 7 Example of extraction arm for local ventilation (photo credit: Heriot-Watt University)



Figure 8 An example of LEV for bench-top equipment (left) and walk-in enclosure (right) (photo credit: Heriot-Watt University)

4.3.2 General guideline

The following factors should be considered when installing LEV for C1 gaseous feedstock handling:

- 1) In the first instance the *LEV Design Team* should establish an *LEV Specification* including the process and the source of C1 gases;
- 2) It is crucial that the *LEV System Designer* understands how the source behaves in its location at the workplace;
- 3) The design of the *LEV System* must match the process and how the operator carries out the process;
- 4) Maximum aeration flow rate required should be considered, in order to achieve adequate ventilation in the worst scenario;
- 5) Indicators should be fitted, such as pressure differential gauges or velocity meter, that show the system is working properly or giving alarms when the reading is below a set point;
- 6) The *LEV Supplier* should provide training on how to use, check and maintain the *LEV System*;
- 7) A logbook should be provided for the *LEV System* to record the results of checks and maintenance, after *LEV* installation and commissioning.

4.4 Gas Cylinder Manifolds

As discussed previously, placing gas cylinders external to the laboratory building (**Figure 9**) is a useful way to mitigate safety related risks in a gas fermentation laboratory.

NB standard gas cylinders handling procedure is required.



Figure 9 An example of gas cylinder set-up externally to the laboratory (photo credit: Heriot-Watt University)

4.5 Maintenance, Training and Risk Assessment

4.5.1 Maintenance

The maintenance of gas detectors, panels, LEV, as well as gas cylinder manifold, are extremely important, including regular function checks (*Bump Tests*) and calibration for both fixed and portable monitors, as is stressed in the *Toxic Gas Detector Standard* (BS EN 45544-4), *Flammable Gas Detector Standard* (BS EN 60079-29-2), and in the *HSE Guidance for Flammable Gas Detectors* (HSE, 2001a; HSE, 2001b; HSE, 2004). This will help to ensure that the alarm activates at the right concentration or TWA level and that spurious alarms are minimised.

Bump Tests are carried out in between calibrations; the periodicity of *Bump Tests* varies from days upwards while calibrations tend to be 6 monthly or annually. Both the *Bump Test* and calibration periods will, however, depend on the particular application.

Depending on the type of sensor, fault alarms can warn the user that the instrument is no longer functional. Ideally, the fault warning should provide adequate warning of an imminent failure, although, some failures occur suddenly. Obtaining a suitable and accurate diagnostic is, however, not always straight forward and typically depends on the environment the detector is working in.

On certain sites, there is a significant risk in sending personnel to an area where there may be potential for exposure to very high toxic gas concentrations arising from a major leak. The frequency of maintenance operations involving checking the operation of gas detectors should therefore be kept to a minimum commensurate with ensuring that the detectors are fit for purpose. The more reliable the detector the less frequent checks are necessary and the risk of exposure is minimised.

4.5.2 Training

Another important factor in risk management involving the use of gas detectors and alarms is that operators who use gas detectors or carry out maintenance on them are properly trained in their use and know what actions to take in the event of an alarm activation. The use of subcontractors or visiting researchers to a laboratory can make it harder to ensure that the necessary training has been undertaken. Reference to training is mentioned in *Gas Detector Guidance* previously cited (HSE, 2004; BS EN 45544-4; BS EN 60079-29-2). This guidance needs to be transmitted in a suitably effective form by the duty holder to the user. For instance, a *Standard Operating Procedure* (SOP) is useful to ensure the operation is carried out consistently every time while meeting the required guidelines and regulations.

4.5.3 Review and update of paperwork

All the above safety measures should be documented and reviewed periodically. Reasons behind any changes need to be recorded (*Change Control*) and reviewed, to ensure the changes of measures and/or new risk statuses are tolerable.

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Appendix 1 - DSEAR Template Example

DSEAR Assessment	Title		
DSEAR Code No.:	DSA-xxxx-xx	Effective Date:	
Replaces DSEAR No.:	First Issue	Department:	

APPROVAL / AUTHORISATIONS

Written By: _____

Print Name	Signature	Date
------------	-----------	------

Reviewed and Approved By:

Department	Print Name	Signature	Date

Authorised By

Head of Department: _____

Print Name	Signature	Date
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Authorised By

Health & Safety Manager: _____

Print Name	Signature	Date
------------	-----------	------

Authorised By


Quality Assurance: _____

Print Name	Signature	Date
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To the best of our knowledge, this DSEAR is true and correct using information available at the time of writing. By signing, authors/reviewers/authors do not accept liability for any procedure performed

TRAINING REQUIREMENTS

<input type="checkbox"/> Read and Understand Changes	<input type="checkbox"/> Training Required	[Author to Tick Relevant Box]
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DSEAR Assessment	Title		
DSEAR Code No.:	DSAxxxx-xx	Effective Date:	
Replaces DSEAR No.:	First Issue	Department:	

Section 1. Description of Process

Section 2. Classification of Substances

If the hazardous substances are commercially available, which of the following does the safety data sheet (SDS/MSDS) indicate are risks/hazards?

Flammable <input type="checkbox"/>	Highly Flammable <input type="checkbox"/>	Extremely Flammable <input type="checkbox"/> <small>(Flashpoint below 32 °C or releases a vapour gas that may produce an explosive atmosphere).</small>	Oxidising <input type="checkbox"/>	Explosive <input type="checkbox"/>
------------------------------------	---	---	---	------------------------------------

If the hazardous substance is produced during the reaction or is a material produced in-house or as a by-product which of the following apply:

Flammable <input type="checkbox"/>	Highly Flammable <input type="checkbox"/>	Extremely Flammable <input type="checkbox"/> <small>(Flashpoint below 32 °C or releases a vapour gas that may produce an explosive atmosphere).</small>	Oxidising <input type="checkbox"/>	Explosive <input type="checkbox"/>
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Could the process lead to the creation of an explosive atmosphere or an explosion?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
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DSEAR Assessment	Title		
DSEAR Code No.:	DSEAR-xxxx	Effective Date:	
Replaces DSEAR No.:	First Issue	Department:	

Section 3. Hazards

Detail the name of the hazardous substances used, the amount expected to be present/created/utilised in the reaction, the form of the substance (solid/liquid/gas) and the hazards associated with this material. Briefly detail the control measures that will be put in place to minimise the risk of this substance creating a dangerous environment.

Substance	Quantity	Form	Hazard	Control Measures

DSEAR Assessment	Title		
DSEAR Code No.:	DSEAR Code No.	Effective Date:	
Replaces DSEAR No.:	First Issue	Department:	

Section 4. Areas of Work

Identify the areas where the work will be carried out. Each area should be classified as hazardous or non-hazardous as detailed in the definition of terms. Areas identified as hazardous should also be assigned a zone label as detailed in the definition of terms.

Location	Hazardous/Non-Hazardous	Classification
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
	Choose an item	Choose an item.
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Definition of Terms

Hazardous	A place in which an explosive atmosphere may occur in such quantities as to require special precautions to protect the health and safety of the workers concerned.
Non-Hazardous	A place in which an explosive atmosphere is not expected to occur in such quantities as to require special precautions.
Zone 0	A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is present continuously or for long periods or frequently.
Zone 1	A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is likely to occur in normal operation occasionally.
Zone 2	A place in which an explosive atmosphere consisting of a mixture with air of dangerous substances in the form of gas, vapour or mist is not likely to occur in normal operation but, if it does occur, will persist for a short period only.
Zone 20	A place in which an explosive atmosphere in the form of a cloud of combustible dust in air is present continuously, or for long periods or frequently.
Zone 21	A place in which an explosive atmosphere in the form of a cloud of combustible dust in air is likely to occur in normal operation occasionally.

DSEAR Assessment	Title		
DSEAR Code No.:	DSA-xxxx-xx	Effective Date:	
Replaces DSEAR No.:	First Issue	Department:	

Section 5. Hazards associated with the process

Identify points where the process may fail or where it could result in a fire/explosion. Identify sources of ignition and explain any risk that may result from the process.

Section 6. Control Measures

Identify the control measures that are to be put in place to prevent an explosive/flammable atmosphere from forming. Identify any measures taken to prevent ignition of this explosive atmosphere. Detail any associated risk assessments/Standard Operating Procedures.

Section 7. Evaluation

Control measures sufficient to minimise risks.

Yes ☐

No ☐

If No, the process/control measures must be re-examined and additional safety protocols put in place to further minimise the risk.

Section 8. Document History

Version No.	Document History
01	First Issue

Appendix 2 – Industrial Gas Cylinder Information from Common Suppliers

BOC:

https://www.boconline.co.uk/en/images/Industrial-Gas-Cylinder-Identification-chart-BOC_tcm410-542206.pdf

Air Liquide:

https://www.airliquide.ca/file/general/Cylinder_Chart_ENG.pdf

Air Products:

<https://www.airproducts.expert/uk/maxxgases/article-how-to-choose-right-size-gas-bottle.php>

* It is advised to check the suppliers' updates before use.