Acute toxicity to invertebrates

Guideline/ Test method	Endpoint / Type of test	Exposure Design	Exposure Duration	Results LC_0 LC_{50} LC_{100}	Remarks	Reference
No set guideline followed. Refer to “Exposure Design” for summary of methodology followed.	Rather than looking at acute toxicity *per se*, this test investigates heart rate and pH changes at varying P_{CO2} /pH in *Daphnia magna*	A constant medium flow (8-10 ml min^{-1}) through the experimental chamber was generated by a peristaltic pump located after it. The test solution (1:14 diluted seawater) was sucked up from a thermostated glass vessel with a small opening (transport time from vessel to chamber 6s), where it was equilibrated with different gas concentrations using gas mixing pumps. Temperature and oxygen content of the medium leaving the chamber was continually checked using a needle-shaped thermoelement and a small oxygen electrode.	30 min	Rather than looking at acute toxicity *per se*, this test investigates heart rate and pH changes at varying P_{CO2} /pH in *Daphnia magna.* Heart rate changes could not be provoked by P_{CO2} changes during normoxia (normal levels of oxygen). However, during severe hypoxia (low oxygen concentration of 3.6%), the application of hypercapnia (2% carbon dioxide, pH 6) caused a decrease of heart rate of *Daphnia magna* by 20-50 beats per minute. The minimum was reached within a few minutes. After 20 minutes, the heart had returned back to a rate similar to the pre-hypercapnic value (the normocapnic value at pH 7.45). Switching then to normocapnia or acapnia (pCO2: 0%, pH 8.5) caused the heart rate either to non-transiently or transiently to increase. Concerning blood pH, the application of hypercapnia (2% carbon dioxide, pH 6) caused a transient decrease of pH even during normoxia. The minimum was reached within 10 minutes. After 20-30 minutes the pH had increased to a stable value which was a litlle lower than the pre-hypercapnic one.	This study gives an indication about the possible physiological effects exposure to 2% CO_2 may have on *Daphnia magna.* No mortality was observed. Note that *Daphnia magna* may be exposed to 2% carbon dioxide under normal natural conditions. This study, notwithstanding it’s deficiencies, can be used to support the acute toxicity of CO_2 to Daphnia because under normal conditions of use, the use of CO_2 as a fumigant insecticide will not cause any elevation in the level of CO_2 in water or air outside normal atmospheric ranges. Given this, it makes it unnecessary to conduct further studies on the toxicity of CO_2 to *Daphnia magna..* [1,2,3]	Document IIIA Section 7.4.1.2

Footnotes

1. Due to the results available on the acute toxicity of carbon dioxide to *Daphnia magna*, coupled with the fact that there is no exposure to the aquatic environment, it is not necessary to submit further studies on the effects of carbon dioxide to aquatic organisms (the data requirements detailed in Document III-A, 7.4.3). It is also not necessary to submit data on prolonged toxicity of carbon dioxide to fish (Document III-A, 7.4.3.1).

2. Due to the fact that there is no exposure to the aquatic environment, coupled with the fact that there is no data available which suggests that carbon dioxide will bioaccumulate in the environment, nor is there a risk of secondary poisoning through the use of carbon dioxide, it is not necessary to submit data on bioaccumulation in invertebrate species (the data requirements detailed in Document III-A 7.4.3.3.2).

3. Due to the results available in the core base set of environmental toxicity data for carbon dioxide, particularly that available on the acute toxicity to *Daphnia magna* and the fact that there is no exposure to the aquatic environment, it is not necessary to submit further studies on the effects of carbon dioxide on the reproduction and growth rate of invertebrates (the data requirements detailed in Document III-A, 7.4.3.4).

Growth inhibition on algae

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			Design	Duration	NOE_rC	E_bC_{50} [1]	E_rC_{50} [2]		
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	It is not scientifically necessary to calculate the growth inhibition of carbon dioxide to algae, because carbon dioxide is an essential substrate for photosynthesis. In addition, under normal conditions of use, the use CO_2 as a fumigant insecticide will not cause any elevation in the level of CO_2 in water or air outside normal atmospheric ranges. [1]	Document IIIA Section 7.4.1.3

Key

1. Calculated from the area under the growth curve
2. Calculated from growth rate

Footnotes

1. Due to the results available on the toxicity of carbon dioxide to algae, coupled with the fact that there is no exposure to the aquatic environment, it is not necessary to submit further studies on the effects of carbon dioxide to aquatic organisms (the data requirements detailed in Document III-A, 7.4.3). It is also not necessary to submit data on prolonged toxicity of carbon dioxide to fish (Document III-A, 7.4.3.1).

Inhibition of microbial activity (aquatic)

Guideline / Test method	Species / Inoculum	Endpoint/ Type of test	Exposure		Results			Remarks	Reference
			Design	Duration	EC_{20}	EC_{50}	EC_{80}		
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Whist elevated levels of carbon dioxide may affect environmental conditions for bacteria by reducing pH, there are a number of mitigating factors that would reduce any environmental impacts of such changes and make it unnecessary to generate new test data. (a) Most free living prokaryotic bacteria can tolerate a pH range of about 3 units (three orders of magnitude changes in pH). (b) There is a high level of functional redundancy amongst mixed communities of micro-organisms such that declines in population of some species e.g. due to unfavourable pH conditions, will be compensated for by increases in others. The effect of this biological diversity and different environmental optima for different species means that most bacteria can live in a wide range of pH conditions, from 0.5-9.0. In addition, it is not necessary to determine the effect of increased carbon dioxide levels on microbial activity because under normal conditions of use, the use of CO_2 as a fumigant insecticide will not cause any elevation in the level of CO_2 in water or air outside normal atmospheric ranges. [1]	Document IIIA Section 7.4.1.4

Footnotes

1. Due to the results available on the toxicity of carbon dioxide to aquatic microbes, coupled with the fact that there is no exposure to the aquatic environment, it is not necessary to submit further studies on the effects of carbon dioxide to aquatic organisms (the data requirements detailed in Document III-A, 7.4.3). It is also not necessary to submit data on prolonged toxicity of carbon dioxide to fish (Document III-A, 7.4.3.1).

Effects on sediment dwelling organisms

Remarks	Reference
This information is only required if the active substance partitions to, and persists in, aquatic sediments, such that sediment dwelling organisms are likely to be exposed to the active substance. Under normal conditions of use, the use of CO_2 as a fumigant insecticide will not cause any elevation in the level of CO_2 in water, outside normal atmospheric ranges thus sediment dwelling organisms will not be exposed to increased carbon dioxide. This makes it unnecessary to generate data on the effects of increased carbon dioxide to sediment dwelling organisms.	Document IIIA Section 7.4.3.5.1

Aquatic Plant Toxicity

Remarks	Reference
Under normal conditions of use, the use of CO_2 as a fumigant insecticide will not cause any elevation in the level of CO_2 in water, outside normal atmospheric ranges thus aquatic plants will not be exposed to increased carbon dioxide. This makes it unnecessary to generate data on the effects of increased carbon dioxide to aquatic plants. .	Document IIIA Section 7.4.3.5.2

4.2.2 Atmosphere

As a gas, under all environmental conditions that are likely to occur on earth, carbon dioxide will occur predominantly in air. Carbon dioxide occurs as a by-product of aerobic respiration. There is a natural "Carbon Cycle" whereby carbon dioxide is continuously added and removed from the environment though natural processes.

The report submitted under the data end point "7.1.1.1. Hydrolysis as a function of pH and identification of breakdown products" characterises the role, fate and behaviour of carbon dioxide in the environment.

4.2.3 Terrestrial compartment

Toxicity to terrestrial organisms, initial tests (1 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			Design	Duration	NOEC	LOEC	EC/LC_{50}		
N/A	Microbes, terrestrial	N/A	N/A	N/A	N/A	N/A	N/A	This data is only required if a concern for the terrestrial compartment is indicated by the risk assessment or if there is likely to be long-term exposure to the active substance. The use of CO_2 as a fumigant insecticide will not cause any elevation in the levels of carbon dioxide naturally found in terrestrial systems (outside normal atmospheric ranges). In addition, there is no mechanism for the carbon dioxide to be released directly into the terrestrial system. Consequently, there will be no increased carbon dioxide levels in the terrestrial system, so it is not necessary to determine the effect of increased carbon dioxide on microbial activity.	Document IIIA Section 7.5.1.1

Toxicity to terrestrial organisms, initial tests (2 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure Design	Exposure Duration	Results NOEC LOEC EC/LC_{50}	Remarks	Reference
No set guideline followed. Refer to "Exposure Design" for summary of methodology followed.	Earthworm (test species was a natural population of surface casting earthworm. Exact species not given)	Rather than investigating acute toxicity *per se*, this study investigated the effects of increased CO_2 on cast production.	Carbon dioxide was added to soil plots using a screen aided CO_2 control facility. Control plots contained 350µm CO_2 while test plots contained an increased level of CO_2 (610 µm) in natural soil	2 years.	NOEC, LOEC, EC_{50} or LC_{50} not given because test was not investigating acute toxicity *per se*. Exposure to increased levels of CO_2 caused rates of surface cast production to increase 6 fold. Cumulative surface cast production after 1 year was 35% greater in communities with elevated CO_2. CO_2 induced stimulation of earthworms which increased soil turnover and N and C cycling. Refer to the end of section 4, on page 77 to see how the information derived from this study has been used to establish PNEC sand PEC values.	This data is only required if a concern for the terrestrial compartment is indicated by the risk assessment or if there is likely to be long-term exposure to the active substance. The use of CO_2 as a fumigant insecticide will not cause any elevation in the levels of carbon dioxide naturally found in terrestrial systems (outside normal atmospheric ranges). In addition, there is no mechanism for the carbon dioxide to be released directly into the terrestrial system. Consequently, there will be no increased carbon dioxide levels in the terrestrial system, so it is not necessary to determine the effect of increased carbon dioxide on earthworms. Notwithstanding this, the study summarised here gives an indication about the possible effects increased CO_2 may have on cast production by earthworms. [1]	Document IIIA Section 7.5.1.2

Footnotes

1. Due to the results available in the core base set of environmental toxicity data for carbon dioxide, particularly that available on the toxicity to earthworms and the fact that there is no exposure to the terrestrial environment, it is not necessary to submit further studies on the effects of carbon dioxide on the reproduction of earthworms or other soil non-target macro-organisms (the data requirements detailed in Document III-A, 7.5.2.1).

Toxicity to terrestrial organisms, initial tests (3 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure Design	Exposure Duration	Results NOEC	Results LOEC	Results EC/LC_{50}	Remarks	Reference
N/A.	Plants	N/A	N/A	N/A	N/A	N/A	N/A	This data is only required if a concern for the terrestrial compartment is indicated by the risk assessment or if there is likely to be long-term exposure to the active substance. The use of CO_2 as a fumigant insecticide will not cause any elevation in the levels of carbon dioxide naturally found in terrestrial systems (outside normal atmospheric ranges). In addition, there is no mechanism for the carbon dioxide to be released directly into the terrestrial system. Consequently, there will be no increased carbon dioxide levels in the terrestrial system, so it is not necessary to determine the effect of increased carbon dioxide on plants. Notwithstanding this, it should be noted that carbon dioxide plays a vital role in the photosynthesis pathway of plants. It is widely accepted that commercial horticulturists, such as tomato growers, use carbon dioxide to enrich the atmospheres of their greenhouses to accelerate the growth of their crops. [1]	Document IIIA Section 7.5.1.3

Footnotes

1. Due to the results available in the core base set of environmental toxicity data for carbon dioxide, particularly that available on the toxicity to plants and the fact that there is no exposure to the terrestrial environment, it is not necessary to submit further studies on the long term effects of carbon dioxide on plants (the data requirements detailed in Document III-A, 7.5.2.2).

Toxicity to terrestrial organisms, initial tests (4 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure Design	Exposure Duration	Results NOEC	Results LOEC	Results EC/LC_{50}	Remarks	Reference
N/A.	Birds	N/A	N/A	N/A	N/A	N/A	N/A	An acute oral toxicity study for carbon dioxide cannot be submitted because it is not technically possible to determine the acute toxicity of carbon dioxide by the oral route. This is because there is no approved guideline for testing the acute toxicity of a gas by the oral route. An acute oral toxicity study for carbon dioxide cannot be submitted because it is not technically possible to determine the acute toxicity of carbon dioxide by the oral route. This is because there is no approved guideline for testing the acute toxicity of a gas by the oral route. Notwithstanding the above, it should be noted that carbon dioxide is constantly produced by all birds as a result of the numerous metabolic reactions involving carbon-containing compounds. Broiler chickens in a healthy barn environment can produce up to 60 litres CO_2 /bird/day without causing any toxic effects. (See attached study summary for details). Carbon dioxide is recognised as a humane method for the dispatch of birds and small mammals. As shown in the attached study summary, the UK Home Office recommends that a rising concentration of carbon dioxide be used to dispatch birds up to 1.5% in weight. In atmospheres containing 30% carbon dioxide, the bird will lose consciousness and at 70% death will occur.	Document IIIA Section 7.5.3.1.1

Toxicity to terrestrial organisms, initial tests (5 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure Design	Duration	Results NOEC	LOEC	EC/LC_{50}	Remarks	Reference
N/A.	Honeybees	N/A	N/A	N/A	N/A	N/A	N/A	It should be noted that carbon dioxide is constantly produced by arthropods as a result of the numerous metabolic reactions involving carbon-containing compounds, without causing any toxic effects	Document IIIA Section 7.5.4.1
N/A	Other terrestrial non-target organism	N/A	N/A	N/A	N/A	N/A	N/A	This data is only required if a concern for the terrestrial compartment is indicated by the risk assessment or if there is likely to be long-term exposure to the active substance. The use of CO_2 as a fumigant insecticide will not cause any elevation in the levels of carbon dioxide naturally found in terrestrial systems, outside normal atmospheric ranges. In addition, there is no mechanism for the carbon dioxide to be released directly into the terrestrial system. Consequently, there will be no increased carbon dioxide levels in the terrestrial system, so it is not necessary to determine the effect of increased carbon dioxide on terrestrial non-target organisms.	Document IIIA Section 7.5.6

Toxicity to terrestrial organisms, initial tests (6 of 6)

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			Design	Duration	NOEC	LOEC	EC/LC_{50}		
N/A.	Mammals	N/A	N/A	N/A	N/A	N/A	N/A	An acute oral toxicity study for carbon dioxide cannot be submitted because it is not technically possible to determine the acute toxicity of carbon dioxide by the oral route. This is because there is no approved guideline for testing the acute toxicity of a gas by the oral route.	Document IIIA Section 7.5.7.1.

4.2.4 Non compartment specific effects relevant to the food chain (secondary poisoning)

Result
Carbon dioxide does not have any intrinsic properties which suggest it will bioaccumulate in the environment. In addition, carbon dioxide is not classified as hazardous to health according to EC Directive 67/548/EEC, nor are there any indications of toxicity such as endocrine disruption. The toxicity profile of carbon dioxide, coupled with the fact that it is unlikely to accumulate in the environment, means that there is a low risk of secondary poisoning.

Full details of the environmental risk assessment can be found in Document III B, section 7.1.

Determination of the $PNEC_{water}$ for the aquatic compartment

There are no standard short or long term toxicity tests available on carbon dioxide to fish, algae, micro-organisms or any other aquatic organisms and therefore there are no $L(E)C_{50}$ or NOEC values that can be used to determine the $PNEC_{water}$ for carbon dioxide in the aquatic environment.

Data that is available on the toxicity of carbon dioxide to three species of fish shows that when they are exposed to 5.1% carbon dioxide, it caused non lethal, reversible physiological and behavioural. It is this data that has been used to determine the $PNEC_{water}$ for carbon dioxide in the aquatic environment, with an assessment factor of 1000 given the reliability of the data available.

(Note that because exposure to 5.1% carbon dioxide did not cause death of the fish tested, the lethal concentration of carbon dioxide to fish will be higher than the 5.1%. The $PNEC_{water}$ value determined from the calculation will therefore be lower than what is expected *in situ*. This gives an additional margin of safety to that which will be present in the risk assessment).

$PNEC_{water}$ for carbon dioxide (experimental): 0.0051 % [b]

The $PNEC_{water}$ value for carbon dioxide, experimentally determined to be 0.0051%, is actually significantly less than what is found naturally in the environment [c]. The normal atmospheric concentration of carbon dioxide is approximately 0.03%, which means that fish, and other aquatic organisms are being exposed to carbon dioxide levels significantly higher than the $PNEC_{water}$ value determined here, without adverse effects. In the absence of data suggesting a no-effect concentration higher than atmospheric concentrations of carbon dioxide, the $PNEC_{water}$ value used in the environmental risk assessment should be 0.03% carbon dioxide (i.e. atmospheric concentrations of carbon dioxide).

$PNEC_{water}$ for carbon dioxide (atmospheric concentrations)	0.03 %

Footnotes

a) Refer to Document II-A and Document III-A section 7 for full details of this study.

b) Determined by dividing the data available on the toxicity of carbon dioxide to fish (5.1%) by the assessment factor of 1000 (assigned given the reliability of the data).

c) It is accepted that this $PNEC_{water}$ value has limitations, given the quality of the data used.

Determination of the PEC_{water} for the aquatic compartment

Due to how and where a carbon dioxide fumigation takes place, there will be no exposure of carbon dioxide to the aquatic environment under normal conditions of use. Firstly, carbon dioxide fumigations take place indoors and secondly, if carbon dioxide, from a fumigation bubble were accidentally released under water the majority of the gas would rise up through the water and be released to atmosphere.

If, as a result of an accident, release into the aquatic environment occurs, localised increases are counteracted by an equilibrium that exists between the carbon dioxide found naturally in the water and the air.

From this information, we can conclude that the PEC_{water} for carbon dioxide in the aquatic environment as a result of the normal use of carbon dioxide as a fumigant insecticide is zero.

PEC_{water} for carbon dioxide	0 [a]

Footnotes

a) The $PNEC_{water}$ value determined for carbon dioxide is 0.03% (normal atmospheric concentrations of carbon dioxide). It is a moot point, but the PEC_{water} value for carbon dioxide from the use of carbon dioxide as a fumigant insecticide could also be reported as 0.03% (since no more is added to the aquatic environment). In order to be clear about the fact there is no exposure of carbon dioxide to the aquatic environment, a zero value has been used for the PEC_{water} value for carbon dioxide.

Determination of the $PNEC_{soil}$ for the terrestrial compartment

There are no standard short or long term toxicity tests available on carbon dioxide to primary producers (e.g. plants), consumers (e.g. soil invertebrates) or decomposers (e.g. micro- organisms) and as such, this means that there are no $L(E)C_{50}$ or NOEC values that can be used to determine the $PNEC_{soil}$ for carbon dioxide in the terrestrial environment.

The data that is available on the toxicity of carbon dioxide to earthworms shows when they were exposed to 610 µm carbon dioxide in natural soil (equivalent to 6.1% carbon dioxide) for two years, caused rates of surface cast production to increase 6 fold, but it was not lethal [a]. This data will be used to determine the $PNEC_{soil}$ for carbon dioxide in the terrestrial environment, with an assessment factor of 1000 given the reliability of the data available. (Note that because exposure to 610µm (6.1%) carbon dioxide did not cause death of the earthworms tested, the lethal concentration of carbon dioxide to earthworms will be higher than the 6.1% (610µm) value used. The $PNEC_{soil}$ value determined from the calculation will therefore be lower than what is expected *in situ*. This gives an additional margin of safety to that which will be present in the risk assessment).

$PNEC_{soil}$ for carbon dioxide (experimental): 0.0061% [b]

The $PNEC_{soil}$ value for carbon dioxide, experimentally determined to be 0.0061%, is actually significantly less than what is found naturally in the environment [c]. The normal atmospheric concentration of carbon dioxide is approximately 0.03% which means that earthworms, plants other terrestrial dwelling organisms are being exposed to carbon dioxide levels significantly higher than the $PNEC_{soil}$ value determined here, without adverse effects. In the absence of data suggesting a no-effect concentration higher than atmospheric concentrations of carbon dioxide, the $PNEC_{soil}$ value used in the environmental risk assessment should be 0.03% carbon dioxide (i.e. atmospheric concentrations of carbon dioxide).

$PNEC_{soil}$ for carbon dioxide (atmospheric concentrations)	0.03 %

Footnotes

a) Refer to Document II-A and Document III-A section 7 for full details of this study.
b) Determined by dividing the data available on the toxicity of carbon dioxide to earthworm 6.1% by the assessment factor of 1000 (assigned given the reliability of the data).
c) It is accepted that this $PNEC_{water}$ value has limitations, given the quality of the data used.

Determination of the PEC_{soil} for the terrestrial compartment

There is no mechanism for release of carbon dioxide directly to soil given that it is a gas.

We can therefore conclude that the PEC_{soil} for carbon dioxide in the terrestrial environment as a result of the normal use of carbon dioxide as a fumigant insecticide is zero.

PEC_{soil} for carbon dioxide	0 [a]

Footnotes

a) The $PNEC_{soil}$ value determined for carbon dioxide is 0.03% (normal atmospheric concentrations of carbon dioxide). It is a moot point, but the PEC_{soil} value for carbon dioxide from the use of carbon dioxide as a fumigant insecticide could also be reported as 0.03% (since no more is added to the terrestrial). In order to be clear about the fact there is no exposure of carbon dioxide to the terrestrial environment from its use as a fumigant insecticide, a zero value has been used for the PEC_{soil} value for carbon dioxide.

5. HAZARD IDENTIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

a. Thermal stability and identity of relevant breakdown products

At all pressures, there is a fairly wide range of temperatures in which carbon dioxide disassociates directly into CO and O_2 without precipitation of carbon. Refer to equation below. At higher temperatures C is also formed (in addition to CO and O_2).

$CO_2 \leftrightarrow CO + \frac{1}{2}O_2$.

For further details refer to Document III-A3 Section 3.10.

b. Flammability and flash point

Carbon dioxide is a non-flammable gas which does not support combustion. The flash-point of carbon dioxide cannot be determined because it is a gas at the normal temperatures and pressures which it will be used as a biocide. (Flash point data can only be determined for liquids).

For further details refer to Document III-A3 Section 3.11 and 3.12.

c. Explosive properties

Carbon dioxide is thermodynamically stable, so does not exhibit explosive properties.

For further details refer to Document III-A3 Section 3.15

d. Oxidising properties

Oxidising properties of carbon dioxide cannot be determined because it is a gas at the normal temperatures and pressures which it will be used as a biocide. (Oxidising properties can only be determined for solids).

For further details refer to Document III-A3 Section 3.16

e. Reactivity towards container material

Carbon dioxide is supplied in containers designed and [redacted] Containers manufactured to this specification will ensure that there is no reactivity between contents and containers.

For further details, refer to Document III-A3 Section 3.17

Section A8.1
Annex Point IIA, VIII, 8.1

Recommended Methods and Precautions concerning Handling, Use, Storage, Transport or Fire.

			Official use only
	1. **REFERENCE**		
	Already submitted for carbon dioxide dossier for Product Type 14. [redacted]		
1.1 References	[redacted]		
1.2 Details	Handling:	Heavy protective gloves e.g. textile or leather must be worn at all times when handling cylinders in order to minimise the risk of hand injury. The use of protective safety footwear should also be considered if handling a number of cylinders regularly. When moving cylinder, use a cart designed to transport cylinders.	
	Use:	Ensure that before use the carbon dioxide cylinder is stood in a vertical position with the valve uppermost and firmly secured against a wall or other suitable support. Cylinders must only be used with suitable valve attachments.	
	Storage:	Store in original container preferably in a purpose built compound which should be well ventilated and in the open air. Keep out of reach of children, and away from food, drink and animal feeding stuffs. Do not heat cylinders, and keep below 40°C. Cylinders should be stored in the vertical position and properly secured to prevent toppling.	
	Conditions to Avoid:	Do not heat container, and always keep below 40°C.	
	Materials to Avoid:	None known.	
	Hazardous Breakdown Products:	None known.	
	Transport:	Carbon dioxide aerosols should be carried as hazardous goods, UN 1013 Carbon dioxide.	
	Fire:	Carbon dioxide is used as a fire extinguisher, however, if unable to extinguish fire keep adjacent cylinders cool with water hosed from a safe distance. Self contained breathing apparatus, and suitable personal protective equipment should be worn, particularly in confined spaces. Exposure to fire may cause cylinders to rupture and/or explode.	
	Accidental Release:	If cylinders are in an enclosed area, evacuate the area. Arrange for the area to be ventilated and check the atmosphere for correct oxygen/carbon dioxide content before re-entry. Check the valve on the cylinder is closed and move to a safe area. DO NOT contaminate watercourses or ground.	

Section A8.2 **In case of fire, nature of reaction products, combustion gases, etc.**

Annex Point IIA, VIII, 8.2

		Official use only
	1. **REFERENCE**	
1.1 Reference	Already submitted for carbon dioxide dossier for Product Type 14. [redacted]	
1.2 Details	No hazardous breakdown products, reaction products or combustion gases are known for carbon dioxide, in case of fire.	

Section A8.3 **Emergency Measures in case of an accident**

Annex Point IIA, VIII, 8.3

		Official use only
	1. **REFERENCE**	
1.1 Reference	Already submitted for carbon dioxide dossier for Product Type 14. [redacted]	
1.2 Details	First Aid Advice: Inhalation: Cylinder: This route of exposure is not anticipated. Fumigation bubble: Remove patient to fresh air, keep warm and at rest. Apply supportive measures if necessary and seek medical attention. Eye contact: Cylinder: This route of exposure is not anticipated. Fumigation bubble: Rinse affected eye with clean running water, or eyewash solution, for at least 15 minutes holding eyelids well apart. Rinse entire surface and do not allow run-off to contaminate unaffected eye. Seek medical attention. Skin contact: Cylinder: This route of exposure is not anticipated. Fumigation bubble: Remove and wash contaminated clothing immediately. Wash affected area thoroughly with soap and water. If the patient feels unwell seek medical advice. Ingestion: Cylinder and fumigation bubble: This route of exposure is not anticipated. Accidental Release: If cylinders are in an enclosed area, evacuate the area. Arrange for the area to be ventilated and check the atmosphere for correct oxygen/carbon dioxide content before re-entry. Check the valve on the cylinder is closed and move to a safe area. DO NOT contaminate watercourses or ground.	

Section A8.4 **Annex Point IIA, VIII, 8.4**	**Possibility of destruction or decontamination following release in or on the following: (a) Air, (b) Water, including drinking water, and (c) Soil.**	
	1. **REFERENCE** Already submitted for carbon dioxide dossier for Product Type 14. [redacted]	Official use only
1.1 Reference	[redacted]	
1.2 Details	If large amounts of carbon dioxide are released to the environment the contaminated area should be well ventilated. DO NOT contaminate watercourses or ground with large undiluted quantities of carbon dioxide.	

Section A8.5 Annex Point IIA, VIII, 8.5	Procedures for waste management of the active substance for industry or professional users.	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	Carbon dioxide is a gas, which means that it will be completely used. If there is waste product it will be vented to atmosphere. Empty cylinders are returned for refilling. Any damaged cylinders will be repaired and re-used or disposed on if a safe way in accordance with local, State or National requirements.	

Section A8.5.1 Annex Point IIA, VIII, 8.5.1	Possibility of re-use or recycling	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	There is no scope for re-use or recycling of spilt material because carbon dioxide is a gas which vents to atmosphere during it's normal use or if it is accidentally spilt.	

Section A8.5.2 Annex Point IIA, VIII, 8.5.2	Possibility of neutralisation of effects	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	Neutralisation procedures for carbon dioxide is not feasible, given that it is occurs naturally in the atmosphere. The procedure for dealing with both a small and large spillage of carbon dioxide is described in section A8.3 and section A8.4.	

Section A8.5.3 Annex Point IIA, VIII, 8.5.3	Conditions of controlled discharge including leachate qualities on disposal.	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	It is not necessary to provide advice about conditions for controlled discharge to surface water etc, because the carbon dioxide is a gas. This means that it will be completely used in it's application as a biocide. If there is any waste product it will be vented to atmosphere which has a diluting effect. Contamination of watercourses, leachate etc is not a potential exposure route because carbon dioxide is a gas.	

Section A8.5.4 Annex Point IIA, VIII, 8.5.4	Conditions for controlled incineration.	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	Controlled incineration is not a specified method of waste disposal for carbon dioxide. Carbon dioxide is a gas, which means it will be completely used in its application as a biocide. If there is any waste product it will be vented to atmosphere which has a diluting effect. As controlled incineration is not a specified method of waste disposal for carbon dioxide it is not necessary to provide information on the conditions needed for its safe incineration.	

Section A8.6 Annex Point IIA, VIII 8.6	Observations on undesirable or unintended side-effects, for example, on beneficial and other non-target organisms	
		Official use only
1. **Details**	When used as an insecticide fumigant, carbon dioxide is used within a fully enclosed and sealed fumigation bubble. The only organisms exposed to the gas will be those present within the commodities to be fumigated. By the very nature of the commodities being treated, all insects present will be deemed to be pests and therefore require controlling.	

Section A8.7 Annex Point IIIA, VIII, 8.7	Identification of any substances falling within scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances.	
	Already submitted for carbon dioxide dossier for Product Type 14.	Official use only
1. Details	Carbon dioxide is listed in List II of the Annex to Directive 80/68/EC (OJ No. L20, 26/1/1980, p. 43). None of the impurities present in carbon dioxide are listed in either List I or II of the Annex to Directive 80/68/EC (OJ No. L20, 26/1/1980, p. 43).	

Section A9 **Classification and Labelling**

Annex Point IIAIX

				Official use only
	Already submitted for carbon dioxide dossier for Product Type 14.			
1. Details	Classification:	Non-hazardous according to EC Directive 67/548/EEC. Carbon dioxide is not listed in Annex I of Directive 67/548/EEC and there is no data available to suggest it is hazardous to health or the environment. It is on this basis that carbon dioxide has been classified as non-hazardous.		
	Labelling:	Category of danger:	Not required.	
		Risk Phrases:	Not required.	
		Safety Phrases:	Not required.	

Note that the following information is identical to that found in Document IIA

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

Already submitted for carbon dioxide dossier for Product Type 14.

CAS- No.	124-38-9
EINECS-No.	204-696-9
Other-No. (CIPAC, ELINCS)	None known.
IUPAC Name	Carbon dioxide.
Common name, synonyms	Carbonic acid gas, carbonic anhydride.
Molecular formula	CO2
Structural formula	O=C=O
Molecular weight (g/mol)	44.01

1.2 PURITY/IMPURITIES, ADDITIVES

[redacted]

1.3 PHYSICO-CHEMICAL PROPERTIES

Already submitted for carbon dioxide dossier for Product Type 14.

Melting point	Sublimation temperature: -78.48°C (at 760 mmHg).
Boiling point	Not relevant, due to sublimation properties.
Density	Bulk density: 1.976 g/l (at 760 mmHg). Relative density: 1.527 (where air = 1).
Vapour pressure	Not applicable, as carbon dioxide is a gas.
Henry's Law Constant	Not applicable, as carbon dioxide is a gas.
Appearance	Physical state : Gas at 20°C, 101.3 kPa. Colour: Colourless at 20°C, 101.3 kPa. Odour: Odourless at 20°C, 101.3 kPa.
Absorption Spectra	[redacted]
Mass Spectrum	[redacted]
Solubility in water	Sparingly soluble (88 ml carbon dioxide in 100 ml water).
Dissociation constant	Not applicable, as carbon dioxide is a gas.
Solubility in organic solvents	Soluble in isobutanol. Soluble in cyclohexanol (677 cm^3 CO_2 /litre cyclohexanol).
Stability in organic solvents	Not applicable, as no organic solvents are used in the manufacture of the active substance.
Partition coefficient (n-octanol/water)	n-octanol / water: 0.83 (calculated) Isobutanol/water: 2.26 Olive oil/water: 1.74
Surface tension	Not applicable, as carbon dioxide is a gas.
Viscosity	Not applicable, as carbon dioxide is a gas.

1.4 ANALYTICAL METHOD FOR DETECTION AND IDENTIFICATION

1.4.1 Analytical methods for the determination of residues of a.s. and relevant metabolites.

Already submitted for carbon dioxide dossier for Product Type 14.

Sample	Test substance	Analytical method	Fortification range/Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination	Reference
						Range	Mean	St. dev.		
Carbon dioxide.	Carbon dioxide	Infra-red analysis	4 samples of carbon dioxide, each analysed 5 times.	R^2 : 0.9912	No interfering substances.	Not reported.	Not reported.	Not reported.	The procedure described is suitable for concentrations within the range 99-100% carbon dioxide.	Document IIIA, Section 4.1
Carbon dioxide	Carbon dioxide	Asco method	3 samples of carbon dioxide, each analysed 5 times.	R^2 : 0.9999	No interfering substances.	Not reported.	Not reported.	Not reported.	The method described is suitable for measuring the concentration of residual gases in carbon dioxide in the range 50-1000 ppm v/v (0.005% v/v to 1.000 % v/v) in graduations of 50 ppm v/v.	Document IIIA, Section 4.1

- All impurities in carbon dioxide are < 1g/kg, so it is not necessary to provide an analytical method to detect them according to the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products".

1.4.2 Formulation analysis

CO_2 is intended for use as an insecticide fumigant. The same analytical methods as detailed in 1.4.1 would be used for the analysis of any CO_2 used in this way

1.4.3 Residue analysis

Residue analysis for the active ingredient in soil
When used as a biocide within its totally enclosed system, carbon dioxide does not enter the soil compartment because there is no mechanism for it to be released directly into the soil. This means that the use of carbon dioxide, when used as a biocide, does not affect levels of carbon dioxide found in the environment, outside normal atmospheric levels. It is for these reasons that an analytical method for detection of carbon dioxide in soil has not been submitted.

Residue analysis for the active ingredient in air

Already submitted for carbon dioxide dossier for Product Type 14.

Given that carbon dioxide is a gas, the analytical method specified in 1.4.1 of this document is suitable for detecting carbon dioxide in air.

Residue analysis for the active ingredient in water

When used as a biocide within its totally enclosed system, carbon dioxide does not enter the aquatic compartment because there is no mechanism for it to be released directly into water. This means that the use of carbon dioxide, when used as a biocide, does not affect levels of carbon dioxide found in the environment, outside normal atmospheric levels. It is for these reasons that an analytical method for detection of carbon dioxide in water has not been submitted.

Animal and human body fluids and tissues

Already submitted for carbon dioxide dossier for Product Type 14.

As carbon dioxide is not classified as hazardous for supply, it is not necessary to provide an analytical method for detection in body fluids and tissues according to the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products".

Residue analysis for the active ingredient in food, feeding stuffs and other products

Carbon dioxide, either as an active ingredient or a biocidal product is not intended for use on agricultural or horticultural soil.

Carbon dioxide fumigations are carried out on certain food stuffs and it should be noted that carbon dioxide is generally recognised as a safe food substance (GRAS).

In 1981, the Environmental Protection Agency in the USA waived the need for data requirements pertaining to toxicological studies, metabolism studies, analytical methods and residue data.

The final ruling was given as follows: The food additive carbon dioxide may be safely used after harvest in modified atmospheres for stored product insect control on all processed agricultural commodities.

As such, it is not necessary to submit an analytical method for the determination of carbon dioxide in / on food or feeding stuffs or other products.

1.5 CLASSIFICATION AND LABELLING

1.5.1 Current classification

Already submitted for carbon dioxide dossier for Product Type 14.

Current classification of a.s.

Classification	Non-hazardous according to EC Directive 67/548/EEC
Class of danger	Not required.
R phrases	Not required.
S phrases	Not required.

1.5.2 Proposed classification

Already submitted for carbon dioxide dossier for Product Type 14.

Not applicable. Carbon dioxide will be classified according to EC Directive 67/548/EEC (as detailed in 1.5.1).

2. EFFECTIVENESS AGAINST TARGET ORGANISMS

2.1 FUNCTION

Insecticide.

2.2 FIELD OF USE ENVISAGED

MG03: Pest Control.
Product types: 14 & 18.
For professional users only.

NB: PT14 dossier already submitted to French RMS.

2.3 EFFECTS ON TARGET ORGANISMS

Experimental data on the effectiveness of the active substance against target organisms

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Acarus siro* Flour mite	*A. siro* was reared in a medium of plain flour and dried yeast powder in a ratio of 12:1 at 22°C and 75% RH. Approx. 5ml of live insect culture was inoculated into existing mite cultures and into a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed at the end of exposure period and again, 48 hours later. Atmosphere = 60% CO_2: air Temperature = 35°C Relative humidity = 75% - maintained using open jars of saturated sodium chloride Exposure time:1, 2, 4, 7 & 14 days.	Effects: 100% mortality of mixed stages after 1 day exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Anastrepha suspensa* Carribean fruit fly	50 (2-3 day old) eggs were placed on laboratory agar diets in petri dishes. Three dishes were placed on wet sand in each test jar. Tests were conducted at 2 temperatures (10°C and 15.6°C) and in 9 different atmospheres for 4 exposure periods (3,5,7 and 10 days). After exposure, the dishes were held in air at 24-26° and 90% RH to allow insect development to proceed. Normally formed puparia were counted as survivors. Atmosphere = 50%CO_2: 2%O_2 : 48% N_2 Temperature = 15°C Relative humidity = 90% Exposure time: 3, 5, 7, & 10 days	Effects: 100% mortality of egg and larval stages after 7 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/09

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Anobium punctatum* Common furniture beetle	*A. punctatum* was reared in a hazel tree branch of 25-35mm diameter at 22°C and 75% RH. X-radiography was used to select suitable branches for placement into a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed by X-radiography at 2days and then 3 months after exposure period. Atmosphere = 60% CO_2: 40% air Temperature = 35°C Relative humidity = 75% - maintained using open jars of saturated sodium chloride Exposure time:1, 2, 4, 7 & 14 days.	Effects: 100% mortality of larval stages after 14 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	***Anthrenus verbasci*** Varied carpet beetle	Cultures of *A. verbasci* were inoculated with larvae 7-10 days before exposure in a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed at the end of exposure period and again, 48 hours later. Atmosphere = 60% CO_2: air Temperature = 35°C Relative humidity = 75% - maintained using open jars of saturated sodium chloride Exposure time: 1, 2, 4, 7 & 14 days.	Effects: 100% mortality of larval stages after 2 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Blatella germanica* German cockroach	Nymphal stage and adult cockroaches were placed in clear plastic petri dishes that also contained cotton and a dog food pellet. A hole was drilled in the side of each dish that would allow the use of a hypodermic syringe to moisten the cotton without removing the lid. Each dish was plugged with cotton and the edges of the hole for the syringe was lined with petroleum jelly to prevent cockroaches moving in or out of petri dish. Tests were conducted in an incubator and all temperatures were regulated to ± 0.5°C. Atmosphere: 93.8% CO_2 <u>Exposure times:</u> Nymphal stages and adults : 3 hours Egg bearing females:0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 & 24 hours. <u>Temperatures:</u> Nymphal stages and adults were tested at 32.2°C. Egg bearing females were tested at 16.1°C, 22.8°C, 26.7°C, 31.1°C and 33.3°C.	A 3 hour exposure at of CO_2 at 32°C to nymphal stages and adults resulted in 100% knockdown, followed by death in a few days. 100% efficacy against eggs was achieved after 6.5 hours following exposure to CO_2 at 26.7°C.	Document III-A5.3/26

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Callosobruchus chinensis* Cowpea weevil	Different stages of *C. chinensis* of known age were obtained by subjecting clean cowpea seeds to an artificial infestation for 6 hours and retaining the infested product at 27°C for the required periods. Eggs used for tests were aged 24 hours, young larvae 7 days and mature larvae 14 days. Test specimens were exposed in duplicate in small wire gauze cages each containing 25 individuals. Various gas mixtures were prepared using carbon dioxide and air. After exposure observations were made on the rate of adult emergence in both treated and untreated groups. Atmosphere = 55.5-61.0 % CO_2: air Temperature = 21-25°C Atmosphere = 55.5-64.0 % CO_2: air Temperature = 22-25°C Exposure time: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 19, 21 & 23 days.	Effects in both conditions: 100% mortality of egg stage after 5-9 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This report indicates that mature larvae and pupae are resistant to carbon dioxide and as such, higher concentrations of carbon dioxide should be used for longer periods of time. This does not follow the normal patterns of resistance; insects will not die due to resistance, but due to exposure of a sub-lethal dose. Provided that a lethal dose is administered, the effects of carbon dioxide fumigation are lethal to the insects treated. When using carbon dioxide as an insecticide fumigant (i.e. as a biocide), there is no mechanism for resistance to develop because within the fumigation bubble, exposure to sub-lethal concentrations will not occur.	Document III-A5.3/10

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	***Cimex lectularius*** Bed bug	Laboratory cultures of Cimex lectularius were collected 1 day after a routine blood meal and allowed to recover for 24 hours prior to exposure. Groups of 10 bed bugs were placed inside plastic rearing jars 37mm diameter x 60mm high). Each rearing pot also contained a Whatman No 1 filter paper as a test substrate. The opening of each rearing pot was sealed with fine nylon gauze held in place with a suitable adhesive tape. Each rearing pot was held within a sealable plastic bag into which the carbon dioxide was dispensed, also in which was placed a salt solution- moistened pad to maintain correct humidity levels. Groups of three were then placed into larger plastic containers with a lined screw cap (70mm diameter x 130mm high). Bed bugs were exposed to 60% carbon dioxide at two different temperatures for 6, 12 and 24 hours. At the end of exposure periods, all bed bugs were retained at 25°C and mortality recorded 24 hours after removal of carbon dioxide. Atmosphere = 60% CO_2: 40% air Temperature = 10°C and 2°C Relative humidity = 50-65%	Effects: After only 24 hour exposure to 60% carbon dioxide, 90% mortality was achieved at 10°C and 100% mortality at 20°C. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/27

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Dermestes maculatus* Leather beetle	*D. maculatus* was reared in a medium of fishmeal, dried yeast powder and minced bacon in a ratio of 16:4:1 at 25°C and 50% RH. Insects were introduced into a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed at the end of exposure period and again, 48 hours later. Atmosphere = 60% CO_2: air Temperature = 23-25°C Relative humidity = 75% Exposure time: 1, 2, 4, 8, 14, 28 & 56 days	Effects: 100% mortality of mixed stages after 4 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/11

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Ephestia cautella* Tropical warehouse moth	Eggs of known age were obtained by utilising the ovipositional response in light/dark cycles. Most eggs were laid in the first part of the dark period. Batches of 100 eggs aged 2, 20 or 44 hours were tested at 15°C and 25°C with various atmospheres (50ml min^{-1}) through the exposure chamber. Control eggs were exposed to a flow of air at 15°C and 25°C and RH of 70%. *E. cautella* was raised in pitted, organically grown dates at 25°C and 50% RH. Insects were introduced into a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed at the end of exposure period and again, 48 hours later. Atmosphere = 40% CO_2: 40% O_2 : 20% N_2 Temperature = 15°C Relative humidity = 75% Exposure time: up to 7 days Atmosphere = 60% CO_2: 20% O_2 : 20% N_2 Temperature = 25°C Relative humidity = 75% Exposure time: up to 3 days Atmosphere = 60% CO_2: air Temperature = 35°C Relative humidity = 75% Exposure time:1, 2, 4, 7 & 14 days.	Effects in 1st two test conditions: 100% mortality of egg stages after 18 hours exposure to modified atmosphere. Effects in 3rd test condition: 100% mortality of mixed stages after 2 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/12 Document III-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Ephestia keuhniella* Mediterranean flour moth	Eggs of known age were obtained by utilising the ovipositional response in light/dark cycles. Most eggs were laid in the first part of the dark period. Batches of 100 eggs aged 2, 20 or 44 hours were tested at 15°C and 25°C with various atmospheres (50ml min-1) through the exposure chamber. Control eggs were exposed to a flow of air at 15°C and 25°C and RH of 70%. Atmosphere = 40% CO_2: 40% O_2 : 20% N_2 Temperature = 15°C Relative humidity = 75% Exposure time: up to 7 days.	Effects: 100% mortality of egg stages after 18 hours exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/12

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Lasioderma serricorne* Cigarette beetle	The adult cigarette beetles were insectary reared on a blend of flue-cured tobacco. Batches of 75 insects of the same stage were tested in a fumatorium capable of being maintained at a consistent CO_2 concentration. Ages were as follow: eggs: 1-24 hours, larvae: early 4th instar, pupae: 48-72 hours in pupal cell, adults: 12-48hours after emergence. Control insects were treated in a similar way but under normal atmospheric conditions. Atmosphere = 65% CO_2: 8% O_2 : 27% N_2 Temperature = 27°C Relative humidity = 65% Exposure time: up to 6 days.	Effects: 100% mortality of all stages after 2-7 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/13

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Lasioderma serricorne* Cigarette beetle	Fumigation took place in a warehouse (12,706m^3) containing 1,005 metric tones of tobacco. Prior to sealing of the building, specimens of all stages of the cigarette beetle were placed, in cages, throughout the stored tobacco. 494 adults, 270 pupae, 288 larvae and 508 eggs were used. Controls were set up in a similar untreated warehouse. Liquid CO_2 was used for the fumigation and distribution was aided by fans. 60% v/v CO_2 was maintained by daily additions of the liquefied gas. 50% of insects were removed at 5 days and the remainder at 7 for mortality assessment. Atmosphere = > 60% CO_2: air Temperature = 23.3°C Relative humidity = 65.4% Exposure time: 7 days.	Effects: 100% mortality of all stages after 5 days exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/14

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Lasioderma serricorne* Cigarette beetle	*L. serricorne* was reared in a medium of wheatfeed and dried yeast powder in a ratio of 10:1 at 25°C and 50% RH. Insects were introduced into a 35-litre fumigation chamber (a high-density polyethylene drum) and test conditions maintained. Mortality was assessed at the end of exposure period and again, 48 hours later. Atmosphere = 60% CO_2: air Temperature = 35°C Relative humidity = 75% - maintained using open jars of saturated sodium chloride Exposure time: 1, 2, 4, 7 & 14 days.	Effects: 100% mortality of mixed stages after 1 day exposure to modified atmosphere. Mode of action: Most insects respire by means of a tracheal system. In this system, gas is directly transported to the tissues by air-filled tubules that bypass blood. The pores to the outside, called spiracles, deliver the gases of respiration. The drawback of this system is that the gases diffuse slowly in the long narrow tubules; as a result, these tubes need to be limited in size for adequate gas transfer. The advantage is that oxygen and carbon dioxide diffuse much faster, 10,000 times faster, from the air than in water, blood or tissues. Unlike in mammals however, there is uncertainty as to whether the actions of carbon dioxide on insects result from a specific effect of this agent, from anoxia (reduced oxygen levels), and/or pH variations. What is known, is that with increasing levels of carbon dioxide, narcosis will occur, followed ultimately by death. Resistance: This test does not give any indication of resistance. In fact, resistance would not be expected with the use of carbon dioxide as an insecticide as it is lethal to the target insects in a single dose. As such, there is no mechanism for resistance to develop because target organisms are never exposed to sub-lethal concentrations of carbon dioxide (as a biocide).	Document III-A5.3/08