Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Lepinotus patruelis* Booklouse	*L.patruelis* was reared on a medium of soya flour, wheatfeed, dried yeast powder, skimmed milk powder and plain white flour in the ratio of 1:1:1:1:1 at 22°C and 75% RH. Cultures were inoculated one generation time before exposure with at least 10 individuals of mixed stages. 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 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	*Liposcelis bostrychophilus* Booklouse	*L. bostrychophilus* was reared on a medium of soya flour, wheatfeed, dried yeast powder, skimmed milk powder and plain white flour in the ratio of 1:1:1:1:1 at 27°C and 60% RH. Cultures were inoculated one generation time before exposure with at least 10 individuals of mixed stages. 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% - maintained using open jars of saturated sodium chloride Exposure time: 1, 2, 4, 8, 14, 28 & 56 days.	Effects: 100% mortality of mixed stages after 8 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	*Oryzaephilus mercator* Merchant grain beetle	*O. mercator* was reared on a medium of wheatfeed, rolled oats and dried yeast powder in the ratio of 5:5:1 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. [illegible] his test does not give any indication of 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	*Oryzaephilus surinamensis* Saw-toothed grain beetle.	*O. surinamensis* was reared on oat meal. Test atmosphere was pre-mixed in chamber prior to passing into exposure chamber. 3-4 replicates of each test combination carried out. Controls were similarly treated in a normal atmosphere. Atmosphere = 60% CO_2: 8% O_2 : 32% N_2 Temperature = 15-32°C Relative humidity = 60-70% Exposure time: up to 5 days.	Effects: 100% mortality of all stages after 4-5 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-A5.3/15

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Periplaneta americana* American cockroach	*P americana* were reared on cereal pellets and wet cotton wool at 27°C and 60% RH. 10 each of the following were prepared during the week prior to exposure. Adult males, adult females without oothecae, medium nymphs and oothecae. 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

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Plodia interpunctella* Indian meal moth	Larvae were reared in a complex medium of cornmeal, whole-wheat, dog food, dried yeast, honey, glycerine, oatmeal and wheatgerm at 26.7± 1°C and 60± 5% RH. 2nd instar larvae were used. Insects were placed in cages of 10 individuals, which were then suspended in glass jars used as exposure chambers. Various atmospheres were used at 3 temperatures. Controls were exposed to compressed air under the same conditions. Atmosphere = 63.0-68.2% CO_2: air Temperature = 15.6-37.8°C Relative humidity = 61-64% Exposure time: 7 & 14 days.	Effects: 100% mortality of 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-A5.3/16

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Plodia interpunctella* Indian meal moth	Insects were reared in a mixture of cornmeal, whole wheat, Gaines dog food, dried yeast, honey, glycerine, oatmeal and wheat germ at 26.7± 3°C and 60± 5% RH. 10 pupae were placed in a cage and suspended centrally in the glass jar exposure chambers whilst fumigations were carried out. After fumigation, insects were held at rearing conditions for assessment at 1, 4 or 7 days following exposure. Control insects were fumigated under identical conditions with flowing air. Atmosphere = 62.5% CO_2: air Temperature = 27°C Relative humidity = 61% Exposure time: 3 & 7 days.	Effects: 100% mortality of pupal stages after 3 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-A5.3/17

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Plodia interpunctella* Indian meal Moth	Pupae were tested at different CO_2 concentrations and temperatures. Control insects were treated similarly. Atmosphere = 62.0% CO_2: air Temperature = 32-38°C Exposure time: up to 40 hours	Effects: 100% mortality of pupal stages after 1 day – 40 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-A5.3/02

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Ptinus tectus* Australian spider beetle	*P. tectus* was reared on a medium of fishmeal and dried yeast powder in the ratio of 4:1 on wet cotton wool 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 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-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Sitophilus oryzae* Lesser rice weevil	*S. oryzae* was reared in whole wheat kernels with 1% brewer's yeast at 26.7± 3°C and 60± 5% RH. 10 pupae were placed in a cage and suspended centrally in the glass jar exposure chambers whilst fumigations were carried out. After fumigation, insects were held at rearing conditions for assessment at 1, 4 or 7 days following exposure. Control insects were fumigated under identical conditions with flowing air. Atmosphere = 64.7% CO_2:7.0% O_2 : 28.3% N_2 Temperature = 26.7°C Relative humidity = 62% Atmosphere = 60.8-64.7% CO_2 : 6.9-8.0% O_2:28.3-31.9% N_2 Temperature = 15.6-37.8°C Relative humidity = 62-64% Exposure time: 3, 7 & 14 days.	Effects of 1st test conditions: 100% mortality of egg stages after 7 days exposure to modified atmosphere. Effects of 2nd test conditions: 100% mortality of larval and adult stages after 3-7 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-A5.3/17 Document III-A5.3/17

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Sitophilus oryzae* Lesser rice weevil	*S. oryzae* was reared on wheat feed mixed with 5% brewer's yeast. Test atmosphere was pre-mixed in chamber prior to passing into exposure chamber. 3-4 replicates of each test combination carried out. Controls were similarly treated in a normal atmosphere. Atmosphere = 60.0% CO_2:8.0% O_2 : 32.0% N_2 Temperature = 21-32°C Relative humidity = 60-70% Exposure time: up to 5 days	Effects: 100% mortality of egg and adult 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-A5.3/15

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Sitophilus oryzae* Lesser rice weevil	*S. oryzae* was reared on wheat 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 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-A5.3/08

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Sitotroga cerealella* Angoumois grain moth	Eggs were exposed to 4 test atmospheres at 2 different temperatures. After each exposure period, eggs were transferred to 5cm diam. Petri dishes in a culture room and examined regularly for adult emergence at 25°C until end point mortality was achieved. Atmospheres: *$\%CO_2$ $\%O_2$ $\%N_2$* *20 16 64* *40 12 48* *60 8 32* *90 2 8* Temperature =15°C & 25°C Relative humidity = 70% Exposure time: 1-7 days	Effects: At 15°C, 100% mortality was achieved after 7 days following exposure to 60% CO_2. *At 25°C, 100% mortality was achieved after 5 days following exposure to 60% CO_2.* 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).	A5.3/24

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Sitotroga cerealella* Angoumois grain moth	The effect of CO_2 on larvae and pupae was investigated as part of a larger study involving phosphine gas. Very few details are presented in report. Atmospheres: $\%CO_2$ $\%O_2$ $\%N_2$ 20 16 64 50 10 40 78 4.4 17.6 Temperature = 20°C & 28°C Exposure time: 2, 4, 8, 24 & 72 hours.	*Effects:* After just 72 hours at 20°C and 50% CO_2: 47.8±15% larval mortality and 61.9± 4.4% mortality at 78% CO_2. At 28°C, larval mortality was 61.2± 8.7% and 82.2± 11.2% at 50% and 78% CO_2 respectively. After just 72 hours at 20°C and 50% CO_2: 6.1± 13.6% pupal mortality and 54.8± 1.0% mortality at 78% CO_2. At 28°C, pupal mortality was 67.6± 16.7% and 73.2± 15.6% at 50% and 78% CO_2 respectively. 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-A5.3/25

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Tribolium castaneum* Rust red flour beetle	Four stages of *T. castaneum* were exposed to test atmospheres at different temps. Data for eggs was based on percentage reduction in emergence from three replicates of 75 eggs each. Larval, pupal and adult data was based on mortality in 3 replicates of 30 insects each. Atmosphere = 63% CO_2: air Temperature = 32-43°C Exposure time: up to 48 hours.	Effects: 100% mortality of mixed stages after 1-2 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-A5.3/02

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Tribolium castaneum* Rust red flour beetle	*T. castaneum* was reared on wheat feed mixed with 5% brewer's yeast. Test atmosphere was pre-mixed in chamber prior to passing into exposure chamber. 3-4 replicates of each test combination carried out. Controls were similarly treated in a normal atmosphere. Atmosphere = 60.0% CO_2:8.0% O_2 : 32.0% N_2 Temperature = 15-32°C Relative humidity = 60-70% Exposure time: Up to 5 days. Adults and larvae were reared in a 50:50 mixture of white flour and cornmeal at 26.7± 1°C and 60± 5% RH. Adults at 11-17 days old and 2nd instar larvae were used. Insects were placed in cages of 10 individuals, which were then suspended in glass jars used as exposure chambers. Various atmospheres were used at 3 temperatures. Controls were exposed to compressed air under the same conditions. Atmosphere = 63.0-68.2% CO_2 : 6.7-7.5% O_2 : 25.1-29.5% N_2 Temperature = 15.6-37.8°C Relative humidity = 61-64% Exposure time: 7 & 14 days	Effects from both test conditions: 100% mortality of mixed 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-A5.3/15 Document III-A5.3/16

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Tribolium castaneum* Rust red flour beetle	0-7 day old adults reared at 27°C and 60% RH on a 1:1mix of white wheat flour and cornmeal with 5% brewer's yeast. Commercially prepared gas mixture used and different RH obtained by passing the gas mix through water and glycerine solutions. Three cages, with 10 insects each were tested and combinations replicated three times. Control data obtained using compressed air in place of test gas mixture. Insects assessed for mortality 7 days after exposure. Atmosphere = 59.7% CO_2 : 9.8% O_2 : 30.5% N_2 Temperature = 26.3°C Relative humidity = 9-33% Exposure time: 7 days. Two replicates of 50 beetles were subjected to the test atmosphere for up to 7 days or until all insects died. Survivors were held at 25°C and 70% RH for comparison with controls after 2 and 7 days. Atmosphere = 60%CO_2: 8% O_2: 32% N_2 Temperature = 25°C Relative humidity = 70% Exposure time: up to 7 days.	Effects from both test conditions: 100% mortality of adult stages after 3 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-A5.3/18 Document III-A5.3/03

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Tribolium confusum* Confused flour beetle	0-7 day old adults reared at 27°C and 60% RH on a 1:1mix of white wheat flour and cornmeal with 5% brewer's yeast. Commercially prepared gas mixture used and different RH obtained by passing the gas mix through water and glycerine solutions. Three cages, with 10 insects each were tested and combinations replicated three times. Control data obtained using compressed air in place of test gas mixture. Insects assessed for mortality 7 days after exposure. Atmosphere = 59.7% CO_2 : 9.8% O_2 : 30.5% N_2 Temperature = 26.3°C Relative humidity = 9-33% <u>Exposure time:</u> 7 days.	100% mortality of adult stages after 3 days exposure to modified atmosphere. <u>Mode of action:</u> 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. <u>Resistance:</u> 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-A5.3/18

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Trogoderma glabrum* Warehouse beetle	Insects reared in Purina laboratory chow with 5% brewer's yeast at 26.7± 3°C and 60± 5% RH. 10 pupae were placed in a cage and suspended centrally in the glass jar exposure chambers whilst fumigations were carried out. After fumigation, insects were held at rearing conditions for assessment at 1, 4 or 7 days following exposure. Control insects were fumigated under identical conditions with flowing air. Atmosphere = 60.8-63.6% CO_2 : 6.9-7.3% O_2: 29.5-31.9% N_2 Temperature = 15.6-37.8°C Relative humidity = 64% Exposure time: 3 & 7 days.	Effects: 100% mortality of adult stages after 3-14 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-A5.3/17

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Trogoderma granarium* Khapra beetle	Laboratory cultures were reared on a mixture of wheat and wheatfeed (1:1) at 30°C and 60% RH. All stages subjected to test atmosphere and temperatures. Eggs for testing were obtained by placing adults on pre-sieved wholemeal flour and re-sieving the flour after 2 days, After exposure to the gas mixture for various periods, the eggs were placed with controls in the same chamber at 30°C and 70% RH. %hatch was determined 14 days after commencing the experiment. Adults between 0 and 4 days old were fumigated on about 10g of food. A group of 30 adults was removed from the chamber and placed at 30°C and 7-% RH with a control group each day. Insects were examined immediately after removal and again after 24 hours. Atmosphere = 60% CO_2: air Temperature = 30°C Relative humidity = 70% Exposure time: up to 18 days	Effects: 100% mortality of eggs and adults after 2-4 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-A5.3/19

Test substance	Test organism(s)	Test system/ concentrations applied/exposure time	Test results: Effects, mode of action, resistance	Reference
Carbon dioxide	*Tyrophagus putrescentiae* Mite	Mites were reared on fishmeal and dried yeast powder in a ration of 1:1 at 22°C and 75% RH. Mite cultures were inoculated during the week before exposure with approximately 5ml of live culture in which it was assumed large numbers of all stages were present. 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% Exposure time:1, 2, 4, 7 & 14 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-A5.3/08

3 HUMAN HEALTH EFFECTS ASSESSMENT

3.1 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

Result	Reference
Carbon dioxide is carried in the blood in three principle forms: • Dissolved in solution • As bicarbonate ions in red blood cells and blood plasma • Combined in the red blood cell, in the form of carboaminohaemoglobin. The body produces large volumes of carbon dioxide as a result of normal metabolic processes and is able to excrete it, while keeping the pH of the blood constant within a few hundredths of a pH unit and the tension of the blood is kept within a few millimetres of mercury without major dislocations of water or electrolytes. Due to the engineering controls in place, the normal use of carbon dioxide as an insecticide fumigant does not result in the exposure of operators or bystanders (of which there should be none) to elevated levels. The process of production, transport and excretion of carbon dioxide in humans is well understood, as its toxicity profile. There are no metabolites of concern, which are formed in mammals. It is on this basis that it is not scientifically necessary to submit additional data on metabolites of concern from carbon dioxide (the data requirements detailed in Document III-A 6.6.7).	Document III-A6 Section 6.2

3.2 ACUTE TOXICITY

Refer to page 8 for details of acute toxicity of carbon dioxide.

3.3 IRRITATION AND CORROSIVITY

Refer to page 9 for details of irritation and corrosivity potential of carbon dioxide.

3.4 SKIN SENSITISATION

Species	Method	Number of animals sensitised / total number of animals	Result	Remarks	Reference
Not applicable.	Not applicable.	Not applicable.	Not applicable.	It is not technically possible to determine the skin sensitisation potential of CO_2, using conventional assays because it is a gas.	Document III-A6 Section 6.1.5

3.2 ACUTE TOXICITY

Route	Method Guideline	Method	Species Strain Sex No/group	Dose levels Duration of exposure	Value LD_{50}/LC_{50}	Remarks	Reference
Oral	Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	It is not technically possible to determine the toxicity of carbon dioxide by the oral route, because carbon dioxide is a gas. Principle route of exposure will be by inhalation.	Document III-A6 Section 6.1.1
Dermal	Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	It is not technically possible to determine the toxicity of carbon dioxide by the dermal route using conventional test methods, because carbon dioxide is a gas. Principle route of exposure will be by inhalation.	Document III-A6 Section 6.1.2
Inhalation	No set guideline followed. Refer to "Method" for summary of methodology followed.	Refer to notes under "Remarks".	Human	Refer to notes under "Remarks".	10% CO_2 * *See note under "remarks" for details about how this figure was derived.	Effects of excessive carbon dioxide exposure in man are well reported in the product literature. These studies have been summarised in Document IIIA Section 6.1.3, 6.4.3, 6.5 and 6.12. Generally, these studies were carried out for purposes other than just determining the LC_{50} or acute toxicity, but information on fatal and non-fatal concentrations and major non-clinical effects can be used from them. Full details of the effects of carbon dioxide exposure in man, at concentrations up to 10% have been summarised in Document IIA, 3.10. As exposure to 10% carbon dioxide was not fatal to humans (although the effects experienced were very unpleasant), a value of 10% carbon dioxide has been used for the risk assessment for acute exposures to carbon dioxide. Due to the engineering controls in place, the normal use of carbon dioxide as an insecticide fumigant does not result in the exposure of operators or bystanders (of which there should be none) to elevated levels.	Document III-A6 Section A6.1.3

3.3 IRRITATION AND CORROSIVITY

Skin irritation

Species	Method	Average score 24, 48, 72h		Reversibility yes/no	Result	Remarks	Reference
Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	It is not technically possible to determine the skin irritation potential of CO_2 using conventional techniques because it is a gas.	Document III-A6 Section 6.1.4

Eye Irritation

Species	Method	Average score				Result	Reversibility Yes/no	Remarks	Reference
		Cornea		Iris	Redness Conjunctiva	Chemosis			
Not applicable.	Not applicable.	Not applicable.		Not applicable.	Not applicable.	Not applicable.	Not Applicable.	It is not technically possible to determine the eye irritation potential of CO_2 using conventional techniques because it is a gas.	Document III-A6 Section 6.1.4

3.5 REPEATED DOSE TOXICITY

Route	Duration of study	Species Strain Sex no/group	Dose levels Frequency of application	Results	LO(A)EL	NOAEL	Remarks	Reference
Inhalation	Refer to notes under "Remarks"	Refer to notes under "Remarks"	Refer to notes under "Remarks"	Refer to notes under "Remarks"	Refer to notes under "Remarks"	The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm / 0.5% (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm / 1.5% (15 minutes reference period)* *Refer to notes under "remarks" for details about why the workplace exposure limit for safe working conditions for carbon dioxide has been used.	Existing data on the subchronic toxicity of carbon dioxide are available, including data on man. However, it is acknowledged that this data, (which is summarised in Document IIIA Section 6.4.3) was carried out some time ago, and was therefore not carried out to current protocols or with current laboratory techniques. Given that this data is unavoidably weak, the current short-term workplace exposure limit of 1.5% has been used in the risk assessment. This is because: In the work that was carried out to monitor operator exposure to carbon dioxide whilst using a fumigation bubble it was seen that in all activities, except immediately after venting, the CO_2 levels did not exceed 15,000ppm (1.5%). At the moment the fumigation bubble is opened to remove the commodities after venting, high levels (25.1%) of CO_2 were measured. It should be noted however that these levels are only found at ground level, due to CO_2 being heavier than air. Operators retreating immediately are not affected by this as they are not working at ground level. They leave the risk area and do not return until the in-situ alarm monitor records a level of <0.5% CO_2 (5,000ppm). It should also be noted that after 5 minutes these CO_2 levels had dropped to<1,000ppm (below the short-term workplace exposure limit). The CO_2 levels observed during various stages of a fumigation alongside the 0.5% monitoring system in place confirm the suitability of using the short-term workplace exposure limit of 15,000ppm in the risk assessment. Continued…	Document III-A6 Section 6.4.3

Route	Duration of study	Species Strain Sex no/group	Dose levels Frequency of application	Results	LO(A)EL	NOAEL	Remarks	Reference
							… continued: Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers. Such data have been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For the same reasons, a conventional 90-day subchronic oral toxicity test for carbon dioxide has not been conducted.	

Footnotes

1. A 28-day repeated dose toxicity study (the data requirements detailed in Document III-A, 6.3.1, 6.3.2 and 6.3.3) is not required for carbon dioxide when an adequate 90 day study is available in a rodent.
2. A 90-day subchronic toxicity study by the oral and dermal route (the data requirements detailed in Document III-A 6.4.1 and 6.4.2) has not been submitted because it is not practicable to determine the oral or dermal toxicity of a gas using conventional techniques. In addition, the gaseous nature of carbon dioxide means that the most significant route of exposure is by inhalation, making this the most appropriate route for determining subchronic toxicity.

3.6 GENOTOXICITY

3.6.1 In vitro

Test system Method Guideline	Organism/ strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
			+/-/±	+/-/±		
Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	It is not technically possible to carry out an *in vitro* gene mutation study for CO_2 in bacteria or mammalian cells, because it is present naturally in the environment and it is also naturally produced by all aerobic cells as a by-product of respiration. This makes it impossible to remove it from negative controls. Even if the test conditions were adjusted to account for this, the fact that test cells are continually producing CO_2 as a by-product of respiration means that there will be variable concentrations at a cellular level, making it impossible to interpret any observations made in the test. The same problems would also apply to an *in vitro* cytogenicity study in mammalian cells. In addition, due to the engineering controls in place, the normal use of carbon dioxide as an insecticide fumigant does not result in the exposure of operators or bystanders (of which there should be none) to elevated levels. It is not scientifically necessary, on the basis of the genotoxicity data available, to submit additional *in vivo* genotoxicity tests (the data requirements detailed in Document III-A 6.6.5)	Document III-A6 Section 6.6.1 Document III-A6 Section 6.6.2 Document III-A6 Section 6.6.3

Footnotes

1. It is not scientifically necessary, on the basis of the genotoxicity data available, to submit additional *in vivo* genotoxicity tests (the data requirements detailed in Document III-A 6.6.5)

3.6.2 In vivo

Type of test Method / Guideline	Species Strain Sex no/group	Frequency of application	Sampling times	Dose levels	Results	Remarks	Reference
Not applicable	Not applicable.	Not applicable.	Not applicable.	Not applicable.	Not applicable.	On the basis of exposure alone, it is not scientifically necessary to conduct an *in vivo* mammalian bone marrow cytogenetic test or micronucleus test for carbon dioxide. As under normal working practices, the use of carbon dioxide as an insecticide fumigant is within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected. In addition, there is no existing data available which suggests that carbon dioxide is a genotoxic compound.	Document III-A6 Section 6.6.4

Footnotes

1. It is not scientifically necessary, on the basis of the genotoxicity data available, to submit additional *in vivo* genotoxicity tests (the data requirements detailed in Document III-A 6.6.6).

3.7 CARCINOGENICITY

Route	Species Strain Sex no/group	Dose levels Frequency of application	Tumours	Remarks	Reference
N/A.	N/A	N/A	N/A	It is not considered scientifically necessary to determine the carcinogenic potential of CO_2[1] for a number of reasons including: 1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected. 2. In addition to the above, the potential for exposure to carbon dioxide is minimal as it is manufactured [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture. 3. The maximum exposure limits for safe working conditions are well established for CO_2, and all of these exposure limits are in general agreement. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure limit based on human data takes precedence over animal data generated for the approximation of a theoretical safe value. 4. While it is possible to carry out a carcinogenicity study on CO_2, it will be technically very difficult, full of constraints and expensive. The body's metabolism and physiology are extremely sensitive to CO_2 levels and will adjust to any atmospheric changes. This effects the body's metabolism making it difficult to differentiate any observations on the test animal as a toxic effect of carbon dioxide itself, or as a secondary effect of the body's change in metabolism. Because of this, even if the carcinogenicity study was carried out, it is going to provide little useful data for the risk assessment.	Document III-A6 Section 6.7

Footnotes

1. For the same reasons detailed in the table above, it is not considered scientifically necessary to determine the chronic toxicity of carbon dioxide (the data requirements detailed in Document III-A 6.5).

3.8 REPRODUCTIVE TOXICITY

3.8.1 Teratogenicity (1 of 3)

Route of exposure	Test type Method guideline	Method	Species Strain Sex No/group	Exposure Period	Doses	Critical effects dams Foetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
Inhalation Study 1 of 3	No set guideline followed. Refer to "method" for summary of methodology followed.	Pregnancy was calculated from the time observed-copulation occurred. The pregnant rats in groups of 2 were placed in a plastic chamber for a single 24-hour period, where they were exposed to a gas mixture containing 6% CO_2 with 20% O_2 and 74% N_2 (the teratogenic agent). The earliest day of exposure was the 5th day of pregnancy and the latest day was the 21st day.	Rats Sprague-Dawley Female 6-12 per group	Single 24 hour periods.	6% CO_2	See footnote #	Not reported.	NO(A)EL has not been established. However, study indicates adverse effects to young born under conditions of 6% CO_2	See footnote*	Document III-A6 Section 6.8.1

Footnotes

\# No maternal toxic effects reported. There were increased abnormalities (intraventricular septal changes). Note there was also an increase in skeletal abnormalities. There was a slight increase in perinatal mortality in the test group, and a lower frequency of male offspring. The average pup weight was 18.9% higher in the test litters. Whist the effects could have been attributable to carbon dioxide they might also be a response to low pH or to increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide).

* This study determines the effect of exposure to 6% CO_2 for single 24-hour periods during certain days of pregnancy on offspring of rats. While this study was not generated to modern, scientifically acceptable protocols, it gives an indication about the possible teratogenic effects of CO_2. This study, not withstanding it's deficiencies, as be used to support the teratogenic assessment of CO_2 because:

1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected.
2. In addition to the above, the potential for exposure to carbon dioxide is minimal as it is manufactured [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture.
3. Objectives of toxicity testing include the prediction of possible toxicological effects in humans, the exposures at which these effects might occur and the mechanisms of action. However, as a maximum occupational exposure limit is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

3.8.1 Teratogenicity (2 of 3)

Route of exposure	Test type Method guideline	Method	Species Strain Sex No/group	Exposure Period	Doses	Critical effects dams Foetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
Inhalation 2 of 3	No set guideline followed. Refer to "method" for summary of methodology followed.	Rats were placed in a 9-litre desiccator with inlet and outlet valves to permit the continuous flow of gases. All gas mixtures contained 20% oxygen and were made up to 100% with nitrogen. Food and water were available in the treatment chamber and a granular desiccant was used to maintain low humidity.	Rats Wistar Male Total of 40 animals.	1,2,4 or 8h	0 (control), 2.5%, 5.0% or 10.0 % carbon dioxide.	See footnote #	Not reported.	NO(A)EL has not been established. However, study indicates adverse effects to male tesis tissue of rats exposed to 2.5% -10% carbon dioxide. The changes were positively associated with the concentration of carbon dioxide and the duration of treatment.	See footnote*	Document III-A6 Section 6.8.1

Footnotes

No maternal toxic effects reported. Treatment of rats with carbon dioxide at all levels employed (2.5% to 10%) caused a doubling of respiration rate, compared to controls exposed either to compressed air or to a gas mixture containing no carbon dioxide, but no other gross effects were noted. Neither the testis weight nor the weight of accessory glands was effected by the treatment. Histologically, testis tissue from treated rats exhibited changes that were positively associated with both the concentration of atmospheric carbon dioxide and the duration of treatment. After 4h of treatment with 2.5% carbon dioxide, however, intratubular relationships were observably disrupted. Sloughing of tubular components and lack of luminal definition were in evidence following treatment with 5% carbon dioxide for the same length of time. There was a progressive streaking and vacuolisation toward the basal membrane that occurred following exposure to 10% carbon dioxide, for 4h. These degenerative changes were typical of treated animals, and they occurred consistently. The most readily observable changes occurred with higher levels of carbon dioxide, as exposures were increased. However, further dramatic changes were not seen when exposure time was extended from 4 to 8h. Whist the effects could have been attributable to carbon dioxide they might also be a response to low pH or to increased oxygen tension (secondary to hyperventilation cause by increased carbon dioxide).

* This study determines the effect of exposure to 0 (control), 2.5%, 5.0% or 10.0 % carbon dioxide for 1,2,4 or 8h periods on the male testis tissue of rats. While this study was not generated to modern, scientifically acceptable protocols, it gives an indication about the possible teratogenic effects of CO_2. This study, not withstanding it's deficiencies, as be used to support the teratogenic assessment of CO_2 because:

1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected.
2. In addition to the above, the potential for exposure to carbon dioxide is minimal as it is manufactured [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture.
3. Objectives of toxicity testing include the prediction of possible toxicological effects in humans, the exposures at which these effects might occur and the mechanisms of action. However, as a maximum occupational exposure limit is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

3.8.1 Teratogenicity (3 of 3)

Route of exposure	Test type Method guideline	Method	Species Strain Sex No/group	Exposure Period	Doses	Critical effects dams Foetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
Inhalation 3 of 3	No set guideline followed. Refer to "method" for summary of methodology followed.	See footnote **	Mice Swiss Male 10 mice /group.	Total: 6h (intermittent exposure over 8h) Total: 26.5 h (intermittent exposure over 6 d)	65%/35% mixture air/carbon dioxide.	See footnote #	Not reported.	NO(A)EL has not been established. However, study indicates adverse effects to the morphology of spermatozoa of mice, and their fertility when they were exposed to 35% carbon dioxide.	See footnote*	Document III-A6 Section 6.8.1

Footnotes

**In the experimental chamber, an air/carbon dioxide mixture in the proportion of 1.8/1.0 by volume (equivalent to 65%/35% mixture) was supplied. In winter (air temperature 18°C) mice survived if allowed to recuperate in air for 30 minutes after each 2h exposure to the mixture. In summer (air temperature 30 to 32°C) a recuperation period of 15 minutes was necessary after each hour of exposure. To test male fertility, males and virgin females, all of comparable body weights were allotted in equal numbers to a control and an experimental group. On the first day males were treated for 4h and kept away from the females. On each of the subsequent 5 days, they were treated for 4.5h before rejoining their mates at night. The pairs were separated each morning. There were 11 repetitions of the experiment ('trials') with fresh animals for each trial. To study the delayed effect of the treatment, the same males of the 5th, 6th and 8th to 11th trials were paired again with virgin females for 6 days starting 15 days after the end of the treatment. Litter size was recorded in 17 trials. Whist the effects could have been attributable to carbon dioxide they might also be a response to low pH or to increased oxygen tension (secondary to hyperventilation cause by increased carbon dioxide).

Exposure of male mice to a 1.8/1.0 mixture of air/carbon dioxide (equivalent to 65%/35% mixture) for a total of 6h reduced the area and breadth of the head and of the mid-piece of live spermatozoa in the vasa deferentia. During a total of 26.5 h exposure spread over six days, males when test-mated, had a low conception rate but the numbers of offspring in the litters produced were normal. The low conception rate appeared to persist even 15 days after the end of the treatment.

* This study determines the effect of exposure to 0 (control), 2.5%, 5.0% or 10.0 % carbon dioxide for 1,2,4 or 8h periods on the male testis tissue of rats. While this study was not generated to modern, scientifically acceptable protocols, it gives an indication about the possible teratogenic effects of CO_2. This study, not withstanding it's deficiencies, as be used to support the teratogenic assessment of CO_2 because:

1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected.
2. In addition to the above, the potential for exposure to carbon dioxide is minimal as it is manufactured [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture.
3. Objectives of toxicity testing include the prediction of possible toxicological effects in humans, the exposures at which these effects might occur and the mechanisms of action. However, as a maximum occupational exposure limit is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

3.8.2 Fertility

Route of exposure	Test type Method guideline	Species Strain Sex No/group	Exposure Period	Doses	Critical effect	NO(A)EL Parental		NO(A)EL FI		NO(A)EL F2		Remarks	Reference
						m	f	m	f	m	F		
Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable		Not applicable		Not applicable		See footnote*	Document III-A6 Section 6.8.2

Footnote

* It is not considered necessary to determine the reproductive effects of CO_2 for a number of reasons including:

1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected.

2. In addition to the above, the potential for exposure to carbon dioxide is minimal as it is manufactured [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture.

3. The maximum exposure limits for safe working conditions are well established for CO_2, and all of these exposure limits are in general agreement. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure limit based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

4. While it is possible to carry out a multigeneration study on CO_2, it will be technically very difficult, full of constraints and expensive. The body's metabolism and physiology are extremely sensitive to CO_2, levels and will adjust to any atmospheric changes. This affects the body's metabolism making it difficult to differentiate any observations on the test animal as a toxic effect of carbon dioxide itself, or as a secondary effect of the body's change in metabolism as it adjusts to the change in atmospheric CO_2 levels. Because of this, even if the multigeneration study was carried out, it is not going to provide any useful data for the risk assessment.