Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Callosobruchus chinensis* Cowpea weevil	Different stages of *C. chinensis* of know 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	100% mortality of egg stage after 5-9 days exposure to modified atmosphere. 100% mortality of young larval stage after 5-9 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).	A5.3/10 A5.3/10

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	<u>Effects</u>: After only 24 hour exposure to 60% carbon dioxide, 90% mortality was achieved at 10°C and 100% mortality at 20°C. <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).	A5.3/26

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Cimex lectularius* Bed bug	Laboratory cultures of Cimex lectularius were collected 1 day after a routine blood meal and allowed to recover for at least 3 days prior to exposure. Eggs were collected by removing them after then had fallen to the bottom of the rearing containers. Groups of 10 bed bugs, adults and nymphs, were placed inside plastic specimen tubes (38x58mm) with a folded paper enclosure as a substrate. The opening of each tube was closed with close meshed nylon gauze. Groups of 10 eggs were also exposed to the same conditions as adults and nymphs. Each tube was held within mini fumigation bubbles which were checked for gas soundness and flushed through with three changes of carbon dioxide at the start of the test. Two sets of control conditions were established, one using dry air containers and another using ambient air for adults and nymphs, whilst for eggs, the only control was with the dry air. Bed bugs were exposed to 60% carbon dioxide at two different temperatures (10 °C and 20°C) for 12 and 24 hours. At the end of exposure periods, all were retained at ambient conditions and mortality recorded 12 hours after removal of carbon dioxide.	Atmosphere: Mixture containing 60% carbon dioxide and 40% air. 50-60% RH at 20°C 50-65% RH at 10°C	After only 24 hour exposure to 60% carbon dioxide, 100% mortality was achieved at 10 °C (adults) and 20°C (adults & nymphs). <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).	A5.3/31

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of mixed stages after 4 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).	A5.3/11

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Ephestia cautella* Tropical warehouse moth	A5.3/06 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%	100% mortality of egg stages after 18 hours exposure to modified atmosphere.	A5.3/12
					Atmosphere = 60% CO_2: 20% O_2 : 20% N_2 Temperature = 25°C Relative humidity = 75%	100% mortality of egg stages after 18 hours exposure to modified atmosphere.	A5.3/12
				A5.3/02 *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 = 60% CO_2: air Temperature = 35°C Relative humidity = 75%	100% mortality of mixed stages after 2 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of egg stages after 18 hours 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).	A5.3/12

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Lasioderma sericorne* 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%	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).	A5.3/13

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of all stages after 5 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).	A5.3/14

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Lasioderma sericorne* Cigarette beetle	*L. sericorne* 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	100% mortality of mixed stages after 1 day 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 2 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Liposcelis sp.* Booklouse	*Liposcelis sp.* was reared on a medium of skimmed milk powder, wheat germ, yeast powder and organic wholemeal flour in the ratio of 2:2:4:1 at 27 ±2°C and 50 ±10% RH. Cultures were inoculated 7 days before exposure with many insects of mixed life stages. Fumigation bubble was purpose built and gas introduced.. Mortality was assessed at the end of the 7 day exposure period and again 4 days later. Exposure time: 7 days.	Atmosphere = 60% CO_2: air Temperature= 27 ±2°C Relative humidity = 50 ±10%	100% mortality of mixed 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).	A5.3/32

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Liposcelis bostrycho-philus* 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% using open jars of saturated sodium chloride	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).	A5.3/11

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 1 day 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of all stages after 4-5 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).	A5.3/15

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 1 day 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of larval stages after 7 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).	A5.3/16

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of pupal 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).	A5.3/17

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of pupal stages after 1 day – 40 hours 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).	A5.3/02

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 4 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Ptinus tectus* Australian spider beetle	*Ptinus tectus* was reared on a medium of fish meal and yeast powder at the ratio of 4:1 at 25 ±2°C and 50 ±10% RH. Cultures were inoculated 7 days before exposure with 15 adults Fumigation bubble was purpose built and gas introduced.. Mortality was assessed at the end of the 7 day exposure period and again 4 days later. <u>Exposure time:</u> 7 days.	Atmosphere = 60% CO_2: air Temperature= 25 ±2°C Relative humidity = 50 ±10%	<u>Effects:</u> 100% mortality of adults after 7 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).	A5.3/32

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Sitophilus granarius* Grain weevil	Adults were reared on heat sterilised whole wheat and transferred, for testing into 5cm diameter Perspex cylinders. Gas mixtures of CO_2, O_2 and N_2 were automatically mixed to the desired levels and introduced into the exposure chambers via the gas distribution manifold of the gas blender. CO_2 levels were monitored using an infrared CO_2 analyser. Mortality was first observed 2 days after treatment and then again 5 days later. Insects were then placed at 25°C. 2 weeks after the first mortality check, they were examined for the presence of a 2nd generation. If no progeny were observed a weekly check then followed until the latest time in emergence of a 2nd generation was likely.	Atmosphere = 10-30 CO_2 : 0.5 – 2.6 O_2: balance N_2 Temperature = 20°C Relative humidity = 70%	At CO_2 concentrations of between 10-30%, 100% efficacy against S. granarius was observed after a maximum of 11 days. <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).	A5.3/28

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Sitophilus granarius* Grain weevil	15 adult *Sitophilus granarius* were placed into each of 12 glass jars with untreated wheat. 3 of these were sealed into each of 4 fumigation bubbles, of which 3 were filled to approximately 60% CO_2. The control bubble was left with air. Gas concentrations were measured at the start of the trial with an ADC PM3 InfraRed gas analyser, on day 9 with Dräger stain tubes and at the end with an ADC PM3 InfraRed gas analyser. Fumigation bubbles were kept in a room maintained at 25°C ±2°C for 30 days. The bubbles were vented on the 3oth day of exposure and insect mortality was assessed. The glass jars were then retained for 7 days at 25°C ±2°C, 50%RH ±5% to check for insect recovery.	Atmosphere = approximately 60% CO_2 Temperature = 25°C ±2°C Relative humidity = not given for test conditions	At CO_2 concentrations of 60%, 100% efficacy against S. granarius was observed after 30 days. <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).	A5.3/30

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of egg stages after 7 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).	A5.3/17

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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 = 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%	100% mortality of larval and adult stages after 3-7 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).	A5.3/17

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of egg and adult stages after 5 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).	A5.3/15

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 4 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).	A5.3/08

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	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. <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).	A5.3/24

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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	100% mortality of mixed stages after 1-2 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).	A5.3/02

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of mixed stages after 5 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).	A5.3/15

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Tribolium castaneum* Rust red flour beetle	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%	100% mortality of adult and larval stages after 5 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).	A5.3/16

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	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).	A5.3/18

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*Tribolium castaneum* Rust red flour beetle	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%	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).	A5.3/03

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	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).	A5.3/18

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of adult stages after 3-14 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).	A5.3/17

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of eggs and adults after 2-4 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).	A5.3/19

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference *)
Insecticide	Product type 18	Carbon dioxide (specification identical to that given in section 2 of application)	*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%	100% mortality of mixed stages after 4 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).	A5.3/08