Detailed justification:
(Continued)

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by different regulatory authorities including the EPA[4] and FDA, who considered the health aspects of carbon dioxide as a food additive[5]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

- Technical feasibility
  While it is possible to carry out an *in vitro* gene mutation study in bacteria for carbon dioxide, it will be technically very difficult, full of constraints and expensive. Some of the problems include the fact carbon dioxide is naturally produced by all aerobic cells as a by-product of respiration. This makes it impossible to remove carbon dioxide from the negative controls. Even if the natural atmospheric concentrations of carbon dioxide were taken into account when doing the test, the fact the test cells on both the treated and untreated plates are continually producing carbon dioxide as a by-product of respiration means that there will be variable concentrations of carbon dioxide at a cellular level. The design of the test could address the various complicating factors such as background carbon dioxide levels, possible pH effects and low oxygen before concluding whether the effect was due to the toxic effects of carbon dioxide, but given the other factors outlined in this data waiver, carrying out an *in vitro* gene mutation study on carbon dioxide is not going to provide any useful data for the risk assessment.

<u>Conclusion</u>
On the basis of exposure alone, it is not scientifically necessary to conduct an *in vitro* gene mutation study in bacteria 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.

A gene mutation study, while technically possible, will be very difficult, and given the points outlined in this data waiver, will not provide any useful data for the risk assessment.

(Continued....)

Detailed justification:
(Continued)

Section 6.6.1 Annex Point IIA, VI, 6.6.1	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.2 **Annex Point IIA, VI, 6.6.2**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* cytogenicity study in mammalian cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [4]	**Technically not feasible** [4] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	An *in vitro* cytogenicity study in mammalian cells is not considered necessary for a number of reasons, including: • 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. The use of carbon dioxide as a biocide is far less that that used in other industries such as brewing. [redacted] • Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [2]. 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 example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm [4]. The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[3]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of a laboratory test on bacteria is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value. (continued)	

Detailed justification:
(Continued)

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by different regulatory authorities including the EPA[4] and FDA, who considered the health aspects of carbon dioxide as a food additive[5]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

- Technical feasibility
  While it is possible to carry out an *in vitro* cytogenicity study in mammalian cells for carbon dioxide, it will be technically very difficult, full of constraints and expensive. Some of the problems include the fact carbon dioxide is naturally produced by all mammalian cells as a by-product of respiration. This makes it impossible to remove it from negative controls. Even if the natural atmospheric concentrations of carbon dioxide were taken into account when doing the test, the fact that the test cells in both the treated and untreated medium are continually producing carbon dioxide as a by-product of respiration means that there will be variable concentrations of carbon dioxide at a cellular level. The design of the test could address the various complicating factors such as background carbon dioxide levels, possible pH effects and low oxygen before concluding whether the effect was due to the toxic effects of carbon dioxide, but given the other factors outlined in this data waiver, carrying out an *in vitro* cytogenicity study in mammalian cells for carbon dioxide is not going to provide any useful data for the risk assessment.

<u>Conclusion</u>

On the basis of exposure alone, it is not scientifically necessary to conduct an *in vitro* cytogenicity study in mammalian cells 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.

A cytogenicity study, while technically possible, will be very difficult, and given the points outlined in this data waiver, will not provide any useful data for the risk assessment.

(Continued....)

Detailed justification:
(Continued)

Section 6.6.2 Annex Point IIA, VI, 6.6.2	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.3 **Annex Point IIA, VI, 6.6.3**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* mammalian cell gene mutation test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [4]	**Other justification** [ ]	
Detailed justification:	An *in vitro* mammalian cell gene mutation test on carbon dioxide is not considered necessary for a number of reasons including: • 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. The use of carbon dioxide as a biocide is far less that that used in other industries such as brewing. [redacted] • Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [2]. 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 example the US OSHA permissible exposure level (PEL)is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm [4]. The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[3]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of a laboratory test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value. (Continued)	

Detailed justification:
(Continued)

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by different regulatory authorities including the EPA[4] and FDA, who considered the health aspects of carbon dioxide as a food additive[5]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

- Technical feasibility
  While it is possible to carry out an *in vitro* mammalian cell gene mutation test for carbon dioxide, it will be technically very difficult, full of constraints and expensive. Some of the problems include the fact carbon dioxide is naturally produced by all mammalian cells as a by-product of respiration. This makes it impossible to remove it from negative controls. Even if the natural atmospheric concentrations of carbon dioxide were taken into account when doing the test, the fact that the test cells in both the treated and untreated medium are continually producing carbon dioxide as a by-product of respiration means that there will be variable concentrations of carbon dioxide at a cellular level. . The design of the test could address the various complicating factors such as background carbon dioxide levels, possible pH effects and low oxygen before concluding whether the effect was due to the toxic effects of carbon dioxide, but given the other factors outlined in this data waiver, carrying out an *in vitro* mammalian cell gene mutation test is not going to provide any useful data for the risk assessment.

<u>Conclusion</u>

On the basis of exposure alone, it is not scientifically necessary to conduct an *in vitro* mammalian cell gene mutation 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.
A gene mutation study, while technically possible, will be very difficult, and given the points outlined in this data waiver, will not provide any useful data for the risk assessment.

(Continued)

Detailed justification:
(Continued)

Section 6.6.3 Annex Point IIA, VI, 6.6.3	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* mammalian cell gene mutation test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.4 **Annex Point IIA,VI, 6.6.4**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian bone marrow cytogenetic test / micronucleus test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [4]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that an *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage, or a micronucleus test are only required when a positive result is obtained in either of the three following tests: Annex Point IIA6.6.1 *In vitro* gene mutation study in bacteria Annex Point IIA6.6.2 *In vitro* cytogenicity study in mammalian cells Annex Point IIA6.6.3 *In vitro* gene mutation assay in mammalian cells. No *in vitro* genotoxicity studies have been conducted for carbon dioxide because the tests are not technically possible. Therefore, the *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage, or a micronucleus test cannot be waived on the basis of a negative result in the *in vitro* genotoxicity studies. While it is technically possible to conduct an *in vivo* mammalian bone marrow cytogenetic test / micronucleus test, it is not considered necessary for a number of reasons including: • 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. [redacted]The use of carbon dioxide as a biocide is far less that that used in other industries such as brewing. [redacted] (Continued.....)	

Detailed justification:
(Continued)

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [2]. 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 example the US OSHA permissible exposure level (PEL)is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm [4]. The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[3]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of a laboratory test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

  There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by Different regulatory authorities including the EPA[4] and FDA, who considered the health aspects of carbon dioxide as a food additive[5]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

Conclusion

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.

Section 6.6.4 Annex Point IIA,VI, 6.6.4	Genotoxicity in vivo Section 6: Toxicological and Metabolic Studies *In vivo* mammalian bone marrow cytogenetic test / micronucleus test

Detailed justification:
(Continued)

[redacted]

Section 6.6.4 Annex Point IIA,VI, 6.6.4	Genotoxicity in vivo Section 6: Toxicological and Metabolic Studies *In vivo* mammalian bone marrow cytogenetic test / micronucleus test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.5 **Annex Point IIA, VI, 6.6.5**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian cytogenetic test in cells other than bone marrow	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible [4]** **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that an *in vivo* mammalian cytogenetic test in cells other than bone marrow is only required when a negative results is obtained in the following test: Annex Point IIA6.6.4 — *In vivo* mammalian bone-marrow cytogenetic test (chromosome analysis), or micronucleus test. but a positive result was obtained in one of the following tests: Annex Point IIA6.6.1 — *In vitro* gene mutation study in bacteria Annex Point IIA6.6.2 — *In vitro* cytogenicity study in mammalian cells Annex Point IIA6.6.3 — *In vitro* gene mutation assay in mammalian cells. No *in vitro* genotoxicity studies have been conducted for carbon dioxide because the tests are not technically possible. The *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage or micronucleus test (annex point IIA6.6.4) has been waived for a number of reasons. 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. In addition, there is no existing data available which suggests that carbon dioxide is a genotoxic compound. Refer to the data waiver submitted in Document IIIA, Section 6.6.4 for full details of the reasons why it is not considered necessary to conduct the *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage or micronucleus test. Further *in vivo* tests, including the *in vivo* mammalian cytogenetic test in cells other than bone marrow are not considered scientifically necessary for the same reasons as those detailed in the data waiver submitted in Document IIIA, Section 6.6.4.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.6.5 Annex Point IIA, VI, 6.6.5	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian cytogenetic test in cells other than bone marrow

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.6 **Annex Point IIA, VI, 6.6.6**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies Rodent dominant lethal test or *In vivo* mammalian germ cell cytogenetics	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that rodent dominant lethal test or *in vivo* mammalian germ cell cytogenetics is only required when a positive result is obtained in the following test: Annex Point IIA6.6.4 — *In vivo* mammalian bone-marrow cytogenetic test (chromosome analysis), or micronucleus test. The *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage or micronucleus test (annex point IIA6.6.4) has been waived for a number of reasons. 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. In addition, there is no existing data available which suggests that carbon dioxide is a genotoxic compound. Refer to the data waiver submitted in Document IIIA, Section 6.6.4 for full details of the reasons why it is not considered necessary to conduct the *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage or micronucleus test. Further *in vivo* tests to determine germ cell effects, including the rodent dominant lethal test or *in vivo* mammalian germ cell cytogenetics are not considered scientifically necessary for the same reasons as those detailed in the data waiver submitted in Document IIIA, Section 6.6.4.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.6.6 Annex Point IIA, VI, 6.6.6	Genotoxicity in vitro Section 6: Toxicological and Metabolic Studies Rodent dominant lethal test or *In vivo* mammalian germ cell cytogenetics

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.7	**Metabolites of Concern in Mammals** Section 6: Toxicological and Metabolic Studies Already submitted for carbon dioxide for Product Type 14.	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	It is not scientifically necessary to submit tests considering the metabolites of carbon dioxide because the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that these tests are only required if metabolites of concern are formed in mammals. From the data submitted under Annex Point IIA6.2 "Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study", it can be seen that the process of production, transport and excretion of carbon dioxide by the human body has been established for decades and is well understood. There are no metabolites of concern produced by the normal metabolism of carbon dioxide by the human body, and so it not necessary to submit any further tests on these metabolites.	

Section 6.6.7	**Metabolites of Concern in Mammals** Section 6: Toxicological and Metabolic Studies

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.7 **Annex Point IIA, VI, 6.7**	**Carcinogenicity** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data **[4]**	**Technically not feasible** **[4]** **Scientifically unjustified** **[ ]**	
Limited exposure **[4]**	**Other justification** **[ ]**	
Detailed justification:	**<u>Scientific necessity</u>** It is not considered scientifically necessary to determine the carcinogenic potential of carbon dioxide for a number of reasons, including: • 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. • In addition to the above, the potential for exposure to carbon dioxide is minimal [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture. • [redacted]The use of carbon dioxide as a biocide is far less than that used in other industries such as brewing [redacted] • Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers[7]. 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 example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm.[5] The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[8]. • As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value. (Continued.....)	

Detailed justification:
(Continued)

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available that consider carcinogenicity or genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. Given the large volume of data available for carbon dioxide, only the typical findings have been summarised below with regards to the carcinogenic potential of carbon dioxide. A number of reviews have been carried out by different regulatory authorities including the EPA[5] and FDA, who considered the health aspects of carbon dioxide as a food additive[6]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the carcinogenic or genotoxic potential of carbon dioxide.

Technical feasibility

While it is possible to carry out a carcinogenicity study on carbon dioxide, it will be technically very difficult, full of constraints and expensive. The data given below shows how the body's metabolism and physiology are extremely sensitive to carbon dioxide levels, and will adjust to any atmospheric changes. This effects the body 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 carbon dioxide levels. Because of this, even if the carcinogenicity study was carried out, it is not going to provide any useful data for the risk assessment.

Exposure to increasing concentrations of carbon dioxide: Effects and Observations

Carbon dioxide is a natural substance, produced by cellular breakdown of carbon-based materials. It is excreted by exhaling. Toxicity is acute, by cellular acidosis disrupting enzyme activities and reducing cellular respiration beyond the point where the organism as a whole can survive [1].

(Continued.....)

Detailed justification:
(Continued)

Carbon dioxide is naturally produced by the body, and is effectively regulated by a series of homeostatic mechanisms designed to maximise the carbon dioxide-carrying capacity of the blood. Cells produce carbon dioxide as part of the normal catabolic process. This carbon dioxide diffuses in solution from the cell to the blood plasma and thence to the red cells. Under normal circumstances, in the resting human, the dissolved concentration of carbon dioxide in the blood is between 48 (arterial) and 52 (venous) ml/100 ml blood. Very low levels of carbon dioxide may lead to failure to stimulate inspiration. Vigorous exercise increases the amount of carbon dioxide carried and exhaled (mainly by increased heart rate and respiratory rate), but as the excretion of the gas depends on a diffusion gradient across the alveolar wall, the amount of carbon dioxide already present in the air will govern the efficiency of excretion. Normal alveolar partial pressure of carbon dioxide is 5-6% carbon dioxide. Typically, normal air contains 0.03% carbon dioxide. If extra carbon dioxide is added such that alveolar concentration increases by just 0.2%, the resting pulmonary ventilation is doubled [2]. If the concentration of carbon dioxide is so high that the organism cannot cope by further increasing respiratory rate, death occurs when the diffusion gradient between the cells of the body and the blood no longer functions.

Exposure to increasing levels of carbon dioxide produces respiratory distress, as the animal attempts to exhale the increasing amounts accumulating in the body [2]. Breathing rate increases to a maximum, followed by loss of consciousness and death. When guinea pigs were exposed to 15% carbon dioxide in 21% oxygen continuously for seven days, blood pH initially fell after 1 hour of exposure, and then rose to 7.10 after 6 hours, and continued to rise back to the initial pH value. Blood corticosteriods rose markedly, and adrenal epinephrine fell. Levels of free fatty acids in the arterial blood rose, and lymphocytes and adrenal cholesterol decreased. These changes occurred only during the first three days of exposure. After this, corticosteriods, adrenal epinephrine, free fatty acids, lymphocytes and adrenal cholesterol content all returned to initial levels, as the body's metabolism compensated for the increase in carbon dioxide [3]. There is also data available to show this effect in man, when 23 subjects were exposed to 1.5% carbon dioxide in 21% oxygen for 42 days. The body began to compensate for the increased level of carbon dioxide after 23 days exposure [4]. The compensation effect does not appear to occur when animals are exposed to increased levels of carbon dioxide for intermittent periods [3]. An occupational exposure study on brewery workers, over five days where the time weighted average concentrations of carbon dioxide ranged from 0.5 to 1.95% (with a mean of 1.08 % but momentary concentrations reached 8%), concluded that there were no significant physiological effect of chronic intermittent exposure to these levels of carbon dioxide [7].

Conclusion

On the basis of exposure alone, it is not scientifically necessary to conduct a carcinogenicity study 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.

(Continued.....)

Detailed justification:
(Continued)

A carcinogenicity study is technically feasible, but difficult, and given the body's metabolic and physiological sensitivity to changes in carbon dioxide levels it is unlikely to provide any useful data for the risk assessment. The toxicological profile of carbon dioxide is well established with a substantial amount of data. Although this information has it's limitations and it does not address the issue of carcinogenicity or genotoxicity specifically, it is considered sufficient to address the toxicity of carbon dioxide particularly given the low level of exposure expected from it's use as a rodenticide.

[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.8.1 **Annex Point IIA, VI, 6.8.1**	**Teratogenicity Study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [4]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [**4**]	**Other justification** [ ]	
Detailed justification:	A teratogenicity test for carbon dioxide is not considered scientifically necessary for a number of reasons including: • 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. • In addition to the above, the potential for exposure to carbon dioxide is minimal [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture. [redacted]The use of carbon dioxide as a biocide is far less than that used in other industries such as brewing. [redacted] (Continued…..)	
[redacted]		

Detailed justification:

continued

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [2]. 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 example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm.[3] The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period)[4].

  As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

- There is a substantial volume of information available for carbon dioxide, including a number of studies that consider the teratogenic potential of carbon dioxide, and it's possible effects on fertility. While it is uncertain from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide) these data have been included.
  Refer to study summaries for details about the data available on the teratogenicity of carbon dioxide.

Conclusion
On the basis of exposure alone, it is not scientifically necessary to conduct a teratogenicity study 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.

Given this, it seems unnecessary to conduct a teratogenicity test for carbon dioxide given the need to minimise unnecessary vertebrate animal testing wherever possible.

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.8.1 Annex Point IIA, VI, 6.8.1	Teratogenicity Study

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.1
Annex Point IIA, VI, 6.8.1

Teratogenicity Study (1 of 4)

Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

Official use only

1. REFERENCE

1.1 Reference [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No.

Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC.

2.2 GLP No.

GLP was not compulsory at the time study was performed.

2.3 Deviations Yes.

No set guideline followed.

3. MATERIALS AND METHODS

3.1 Test material As given in section 2.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

[redacted]

Section A6.8.1 **Teratogenicity Study (1 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

3.2	**Test Animals**	
3.2.1	Species	Rats.
3.2.2	Strain	Sprague-Dawley.
3.2.3	Source	Not reported.
3.2.4	Sex	Female.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	6 to 12.
3.2.7	Control animals	21.
3.2.8	Mating period	Not reported.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of exposure	Other: day 5-21 of pregnancy.
3.3.2	Post-exposure period	Not reported.
		Inhalation
3.3.9	Concentrations	Nominal concentration 6% carbon dioxide. No analytical concentration reported.
3.3.10	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.11	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.12	Type of exposure	Whole body.
3.3.13	Vehicle	Gas.
3.3.14	Concentration in vehicle	Gas mixture contains 6% carbon dioxide with 20 % oxygen and 74% nitrogen.
3.3.15	Exposure period / day	Earliest day of exposure was the 5th day of pregnancy, the latest day was the 21st day of pregnancy. Rats were exposed for a single 24-hour period during this time.
3.3.16	Controls	21 control animals of the same breed, age and mating time were kept in identical test conditions, but in normal atmospheric conditions.
3.4.	**Examinations**	
3.4.1	Body weight	Yes.
3.4.2	Food consumption	No.
3.4.3	Clinical signs	No
3.4.4	Examination of uterine content	No
3.4.5	Examinations of foetuses	
3.4.5.1	General	Yes.
3.4.5.2	Skelet	Yes.
3.4.5.3	Soft tissue	Yes.
3.5	**Further remarks**	None reported.
		4. RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	None reported.
4.2	**Teratogenic /embryotoxic effects**	Table A6_8-1 at the end of this study summary gives details of mortality, sex distribution and weight of young born under conditions of 6% carbon dioxide. <u>Litter size and mortality rate.</u> 71 pregnant rats under test conditions yielded 530 young. 21 mothers in the control group gave birth to 159 infant rats. The average size of the litter was very similar for both groups: 7.57 in

(Continued....)

Section A6.8.1 **Teratogenicity Study (1 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

4.2 Teratogenic /embryotoxic effects

(Continued....)

the control and 7.47in the treated group. The size of the control litter was low because the mothers were young. The number of rats stillborn or dying shortly after birth was 41 out of 530 (7.7 %) among the test animals and 5 out of 159 (3.1%) in the untreated group. Of the 41 perinatal-death cases in the test group, 27 resulted after spontaneous delivery and 14 after Caesarean delivery. In 4 dams under the test conditions (on days marked with an asterisk in Table A6_8-2 which is at the end of this study summary) the entire litter was resorbed. Exposure of the mother to 6% carbon dioxide increased the mortality rate among the male foetuses (refer to Table A6_8-1 at the end of this study summary for further details). In the 159 control animals, 80 (50.3%) were males and 79 (49.7%) were females, while in the 530 test animals 238 (44.9%) were males and 292 (55.1%) were females. The difference is significant by statistical analysis ($p < 0.001$).

Body weight of offspring

The body weight of newborn offspring is given in Table A6_8-1 and Table A6_8-2 at the end of this study summary. The average weight of the newborn rat whose mother was exposed to increased carbon dioxide during pregnancy is higher than the average weight of the control group. The differences between the means were all statistically highly significant ($p < 0.001$). There was no correlation between the age and weight of the mean weight of the offspring. This was equally true of control and treated animals. In every size litter, the average weight of the carbon dioxide-treated young was higher than that of the control young in the same litter size. The average weight of the 159 control offspring was 5,275 mg, while the average weight of the 530 treated animals was 6,276 mg or 18.9% higher.

The length of the gestation period in both the test and control groups was an average of 22 days, 6 hours. Once parturition started, the entire litter was delivered within 1.5-2 hours. Only in 2 cases was delivery prolonged in the carbon dioxide treated animals to about 3 hours. It is not likely, however, that this fact would bear significant influence on the weight of the young.

Cardiovascular defects of offspring

435 hearts from the offspring born under test conditions, and 102 from offspring born under control conditions were studied. The incidence of cardiovascular defects was increased in the experimental animals when compared to the control group. 106 (24.3%) of animals had cardiac abnormalities among the treated group, compared to only 7 (6.8%) in the control group. The 106 young in the experimental group that had an abnormal heart could be divided into 5 groups.
High interventricular septal defects (isolated) (7 cases)
Low interventricular septal defects (isolated) (8 cases)
Overriding aorta (24 cases)
Partial transposition (24 cases)
Pulmonic or aortic stenosis with intact ventricular septum and myocardial hypertrophy (47 cases).

7 cases presented the abnormality of high interventricular septal defects (isolated). The defect was located only in the membranous part of the septum only. The days on which this type of defect was found were after exposure on the late days of pregnancy: the 17th, 18th, 19th and 20th days.

8 cases presented the abnormality of low interventricular septal defects (isolated). This abnormality presented itself as single or multiple

4.2 Teratogenic /embryotoxic effects

(Continued....)

septal defects situated in the mid-portion of the muscular interventricular septum. They occurred in rats exposed to high concentrations of carbon dioxide on the 11th, 12th 13th, 14th and 15th day of pregnancy.

24 cases presented the abnormality of riding aorta. In these cases, the aorta emerged from both ventricles and the pulmonary artery solely from the right ventricle. The interventricular septal defect was not large: however it involved not only the membranous septum but also the adjacent muscular portion (septum ventriculorum interaorticum). 5 of the 24 cases with this abnormality had a narrowing of the pulmonary outflow channel and hypoplasia of the pulmonary artery. In the other 19, the pulmonary trunk was normal. Riding aorta with pulmonary hypoplasia was found only in rats exposed to high carbon dioxide concentrations on the 7th, 8th and 10th days. Riding aorta with normal pulmonary artery occurred between the 5th and 18th day and on every day except the 8th and 10th day.

10 cases presented the abnormality of partial transposition. In these cases both the aorta and the pulmonary artery arose from the right ventricle. They all had an interventricular septal defect similar to the defect described above under "riding aorta". 6 of these cases were accompanied by pulmonary hypoplasia. In 4 others, the pulmonary artery was of normal calibre. These defects occurred after treatment on the following 5 days: 2 cases on the 8th day, 3 on the 9th, 2 on the 10th, 2 on the 12th and 1 on the 14th day. In this group were also included 10 cases of common trunk in which a single vessel emerged from the right ventricle. This vessel gave off the coronary and systemic arteries and the pulmonary branches. The pulmonary trunk and the ductus arteriosus were absent in 8 out of the 10 cases. In the 2 remaining cases, a partial septum separated the mouth of the markedly hypoplastic main pulmonary artery from the common trunk and the ductus was connected with a small left pulmonary artery. In every instance, a large ventricular septal defect was found. Atrial septal defects and common atrioventricular canal were associated 5 times. The 10 cases were found among offspring treated on the 8th, 11th, 12th 14th, 15th and 16th days.

47 cases presented the abnormality of pulmonic or aortic stenosis with intact interventricular septum. This condition had evidence of myocardial hypertrophy. 26 cases had a predominant pulmonic stenosis, in the other 21 the aortic stenosis was more marked. Some degree of dextroposition of the aorta or levoposition of the pulmonary artery was noticed in the majority of instances and the stenosis was almost subvalvular. The valves were normal in respect to the number of cusps and their structure. There was also some degree of hypoplasia of the affected vessel and in a certain number of cases besides the narrowing of the outflow tract, the inflow chamber was also reduced to a slit-like cavity surrounded by markedly hypertrophied walls and thickened endocardium. This type of malformation was not induced when rats were exposed to increased carbon dioxide after the 17th day of pregnancy. The highest incidence occurred on the 11th day.

Out of the 435 test subjects, the ductus arteriosus was functionally closed in 95 cases (21.8%), somewhat contracted but still open in 115 (26.4%), large and patent in 217 cases (49.9%) and absent 8 times (1.9%).

Section A6.8.1 **Teratogenicity Study (1 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

4.2	**Teratogenic /embryotoxic effects** (Continued.....)	Results from the control animals show that there was not a single case with a low interventricular septal defect, riding aorta, partial transposition or outflow tract-stenosis. The only type of cardiac abnormality presented in the control group was the isolated high interventricular septal defect in the membranous part (i.e. a persistent interventricular foramen). This abnormality was found in 7 of the 102 control offspring (6.8%) as a small slit-like defect. In 3 of these 7 cases, there was no real communication between the two ventricles. The defect presented itself as a pocket-like aneurysm of the membranous septum. The ductus arteriosus was functionally closed in 30 cases (29.4%), open in 55 (53.9%) and widely patent 17 times (16.7%). Noncardiovascular defects of offspring. Skeletal System 58 animals had a marked kyphosis, 25 a moderate hydrocephalus. Lungs Microscopic studies of sectioned lungs revealed abnormalities in 376 of the test animals (86.4%). The three types of abnormalities found were: Overgrowth of connective tissue around the pulmonary vessels was found in 283 test animals (65.1%), with the highest incidence among those exposed to excessive carbon dioxide between the 5th and 10th days. Cross-sections showed that the pulmonary arteries from the major branches down to the arterioles were surrounded by a thick cuff of loose connective tissue, edematous in some instances but more frequently fibrous. The second type of lung change that was found in 82 rats (18.8%) probably represents a more advanced stage of perivascular overgrowth mentioned above. In these rats there was an internal thickening, in addition to the perivascular overgrowth, which caused narrowing of the vessel lumen. All of these animals had a concomitant cardiac malformation. The third type of abnormality was a hypoplasia, which was found in 11 rats (2.5%). 8 of these had cardiac malformations too, which were accompanied by absence of the main trunk of the pulmonary artery. The other three animals had normal hearts.
4.3	**Other effects**	None reported.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC. A gas mixture of 6% carbon dioxide with 20% oxygen and 74% nitrogen was used as the teratogenic agent. Rats utilised in the study were pregnant young adults of the Sprague Dawley strain, purchased in groups of 6 to 12. Pregnancy was calculated from the time observed-copulation occurred (0 hour). The pregnant rats in groups of 2 were placed in plastic chamber for a single 24-hour period. Here, they were exposed to a gas mixture containing 6% carbon dioxide with 20% oxygen and 74% nitrogen. The earliest day of exposure was the fifth day of pregnancy, the latest day was the 21st day. Gas samples from the chamber were analysed at regular intervals for carbon dioxide and oxygen content. Laboratory pellets and water were placed in the cages in free amounts. As the

(Continued....)

Section A6.8.1 **Teratogenicity Study (1 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

5.1	**Materials and methods** (Continued)	plastic chamber was transparent, the rats could be observed during the experiments. With each group of exposed rats, control animals of the same breed, age and mating time were kept in separate cages maintained on the same food, but in normal atmospheric conditions. After removal from the chamber, the test rats, properly identified, continued under normal atmospheric conditions until delivery. The majority of rats were permitted to deliver spontaneously. A Caesarean section was performed in a certain number of cases where spontaneous delivery had already started. The Caesarean section was performed under light ether anaesthesia. After spontaneous or induced delivery was accomplished, the implantation sites counted and noted, and the mothers weighed. The newborn rats were measured and weighed immediately after birth and before they had suckled. In addition to recording the number of young per litter, the sex, weight and size of each, a note was made of the visible malformations, the condition of the young at birth, the method of delivery (spontaneous or Caesarean), and the way they were sacrificed and preserved for serial sectioning. After proper fixation, the chest organs were removed *in toto* and simultaneously embedded in paraffin. This method proved to the best for maintenance of proper topographic relationship. Heart and lungs were sectioned serially as a unit, stained with hematoxylin and eosin and microscopically studied.
5.2	**Results and discussion**	71 pregnant rats of the Sprague Dawley strain were exposed for a single 24-hour period to a gas mixture of 6% carbon dioxide, 20% oxygen and 74% nitrogen on different days during gestation. There were 530 offspring and these were compared with 159 newborn rats from 21 control dams. The incidence of cardiac malformations was 23.4% in the test group and 6.8% in the control group with the highest incidence in the test group occurring when the exposure was on the 10th day of gestation. Isolated ventricular septal defects, situated in the lower and posterior part of the muscle septum were found in 8 cases, ventricular septal defects with overriding aorta in 24 cases, and ventricular septal defects with partial transposition in 20 cases. 47 of the abnormal hearts had a myocardial thickening accompanied by narrowing of the pulmonic or aortic outflow channel with or without other abnormalities such as atresia of the mitral or tricuspid valves and hypoplasia of the aorta or pulmonary artery. There was a slight increase in perinatal mortality in the test group, and a lower frequency of male offspring. The average body weight was 18.9% higher in the test group. Note that it is uncertain from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide).
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	Not reported.
5.3.2	NO(A)EL maternal toxic effects	Not reported.
5.3.3	LOAEL embryotoxic/ teratogenic effects	Not reported.

Section A6.8.1 **Teratogenicity Study (1 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

5.3.4	NO(A)EL embryotoxic/ teratogenic effects	NO(A)EL has not been established. However, study indicates adverse effects to young born under conditions of 6% carbon dioxide.* *Note that it is uncertain from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide)
5.3.5	Reliability	3
5.3.6	Deficiencies (Continued)	Yes. It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effect of exposure to 6% carbon dioxide for single 24 hour periods during certain days of pregnancy on offspring of rats. While this study was not generated to modern, scientifically accepted protocols, and it is unclear from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide), it gives an indication about the possible teratogenic effects of carbon dioxide. This study, notwithstanding it's deficiencies, can be used to support the teratogenicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions. 3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide 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.

Section A6.8.1 **Teratogenicity Study (1 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

Table A6_8-1: Mortality, sex distribution and weight of young born under normal atmospheric conditions, and those exposed to 6% carbon dioxide for a single 24-hour period on different days during gestation.

	Control animals	Test animals
Number of rats bred	159	530
Number of male offspring	80 (50.3%)	238 (44.9%)
Number of female offspring	79 (49.7 %)	292 (55.1%)
Average weight of newborn rats in mg.	5,275	6,276 (18.9% higher than control)
Average number of rats recovered per litter	7.6	7.4
Number of rats stillborn or dead immediately after birth	5 (3.1%)	41 (7.7%)
Number of rats with skeletal malformations	1 (0.6%)	58 (10.9%)

Table A6_8-2: Number and weight of young whose mothers were exposed to 6% carbon dioxide for a single 24-hour period on different days during gestation.

Day of mother's exposure to carbon dioxide	Number of litters	Number of newborn	Mean weight of newborn in man
5th	5*	31	6,338 +/- 6.47
6	3	26	6,292 +/- 6.43
7	5*	40	6,277 +/- 6.37
8	5	40	6,255 +/- 6.31
9	5	45	5,748 +/- 6.39
10	4	23	6,480 +/- 5.88
11	6	44	6,013 +/- 7.45
12	7	29	6,129 +/- 6.27
13	4	36	6,237 +/- 6.34
14	3	30	5,861 +/- 5.83
15	6*	42	6,206 +/- 6.27
16	4	31	6,134 +/- 6.27
17	3*	23	6,215 +/- 6.52
18	3	22	6,158 +/- 6.31
19	3	22	6,331 +/- 6.49
20	3	27	6,331 +/- 6.46
21	2	19	5,862 +/- 6.04
Total for test animals	71	530	6,276
Controls	21	159	5,275

Key: * In one dam, the entire litter was resorbed.

Section A6.8.1 **Teratogenicity Study (1 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	COMMENTS FROM .....
Date	*Give date of comments submitted.*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion* *Discuss if deviating from view of rapporteur member state. .*
Results and discussion	*Discuss if deviating from view of rapporteur member state.*
Conclusion	*Discuss if deviating from view of rapporteur member state.*
Reliability	*Discuss if deviating from view of rapporteur member state.*

Acceptability	*Discuss if deviating from view of rapporteur member state.*
Remarks	

Section A6.8.1
Annex Point IIA, VI, 6.8.1

Teratogenicity Study (2 of 4)

Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

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1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.8.1 **Teratogenicity Study (2 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

3.2	**Test Animals**	
3.2.1	Species	Rats.
3.2.2	Strain	Wistar.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age of test animals not reported. Test animals weighed between 350-400g at study initiation.
3.2.6	Number of animals per group	Total test animals: 40. Number of animals per dose level not reported.
3.2.7	Control animals	Control animals were used in the test, but no details reported about the number of control animals used.
3.2.8	Mating period	Not applicable, as test investigated effect on testis tissue rather than conception and effects on the unborn foetus.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of exposure	1, 2, 4 or 8hr exposure.
3.3.2	Post-exposure period	Not reported.
		Inhalation
3.3.9	Concentrations	Nominal concentration 2.5%, 5.0% or 20.0% carbon dioxide. No analytical concentration reported.
3.3.10	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.11	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.12	Type of exposure	Whole body.
3.3.13	Vehicle	Gas.
3.3.14	Concentration in vehicle	Gas mixture contains dose level of carbon dioxide (as specified in 3.3.9) and 20% oxygen. The remainder of the test gas mixture was nitrogen.
3.3.15	Exposure period / day	1, 2, 4 or 8hr exposure.
3.3.16	Controls	Number of control animals used in the test has not been reported, however they were exposed to compressed air and air containing no carbon dioxide.
3.4.	**Examinations**	
3.4.1	Body weight	No.
3.4.2	Food consumption	No.
3.4.3	Clinical signs	No
3.4.4	Examination of uterine content	No
3.4.5	Examinations of foetuses	
3.4.5.1	General	No.
3.4.5.2	Skelet	No.
3.4.5.3	Soft tissue	No
3.5	**Further remarks**	Testis tissue of the male rats was examined after exposure to increased concentrations of carbon dioxide.
		4. RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	None reported.
4.2	**Teratogenic**	None reported.

4.3	**Other effects**	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. Photomicrographs show that cellular associations in normal seminiferous tubules are well defined. A distinct lumen is nearly always present, and the demarcation between the Sertoli cell cytoplasm and the spermatid is usually well characterised. After 4h of treatment with 2.5% carbon dioxide, however, intratubular relationships were observably disrupted. Luminal debris, consisting of spermatid and Sertoli cell fragments was consistently found in association with streaking or vacuolisation of the tubular components. Mature spermatids were not found in tubules that appeared otherwise to be in stage VII of the seminiferous epithelial cycle, or the stage just before sperm release from the Sertoli cell. Sloughing of tubular components and lack of luminal definition were in evidence following treatment with 5% carbon dioxide for the same length of time: all tubules appeared to be lacking attached spermatids in advanced stages of spermiogenesis. 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.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC. 40 mature, male Wistar rats (350-400g) were randomly assigned to groups for treatment by exposure to elevated atmospheric carbon dioxide. Rats were placed in a 9-litre desiccator with inlet and outlet valves to permit the continuous flow of gases. Rats were exposed to 1,2,4 or 8h to an atmosphere containing 0 (control), 2.5%, 5.0% or 10.0 % carbon dioxide. 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. At the end of the treatment period, animals were killed immediately. The testes and seminal vesicles were removed and weighed, and samples of testis tissue were taken for histological examination. These samples were fixed in Bouin's solution, embedded in paraffin wax and sectioned at a thickness of 5 µm. The sections were stained with PAS-haematoxylin and examined under the light microscope.

Section A6.8.1 **Teratogenicity Study (2 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

5.2	**Results and discussion**	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. The manner in which elevated carbon dioxide results in degenerative changes in the testis is unknown. The effect may be entirely indirect, resulting from changes in blood flow or from acidosis, both of which occur with elevated carbon dioxide in the blood. However, it is feasible that an increase in intratubular pCO_2 in the testes of treated animals could also result in direct effects on germinal cells.
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	Not reported.
5.3.2	NO(A)EL maternal toxic effects	Not reported.
5.3.3	LOAEL embryotoxic/ teratogenic effects	Not reported.
5.3.4	NO(A)EL embryotoxic/ teratogenic effects	NO(A)EL has not been established. However, study indicates adverse effects to male testis tissue in rats exposed to carbon dioxide levels between 2.5% and 10%.* *Note that it is uncertain from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide)
5.3.5	Reliability	3
5.3.6	Deficiencies	Yes. It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effect of exposure to carbon dioxide at levels between 2.5% and 10% on male testis tissues in rats. While this study was not generated to modern, scientifically accepted protocols, and it is unclear from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide), it gives an indication about the possible effects of carbon dioxide on testis tissue. This study, notwithstanding it's deficiencies, can be used to support the teratogenicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.

(Continued.....)

Section A6.8.1 **Teratogenicity Study (2 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

5.3.6	Deficiencies (Continued)	2.	The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.
		3.	The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide 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.

Section A6.8.1 **Teratogenicity Study (2 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rats.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	COMMENTS FROM .....
Date	*Give date of comments submitted.*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion* *Discuss if deviating from view of rapporteur member state. .*
Results and discussion	*Discuss if deviating from view of rapporteur member state.*
Conclusion	*Discuss if deviating from view of rapporteur member state.*
Reliability	*Discuss if deviating from view of rapporteur member state.*
Acceptability	*Discuss if deviating from view of rapporteur member state.*
Remarks	

Section A6.8.1
Annex Point IIA, VI, 6.8.1

Teratogenicity Study (3 of 4)
Section 6: Toxicological and Metabolic Studies
Inhalation, Mice.

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1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed.

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.8.1 **Teratogenicity Study (3 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Mice.

3.2	**Test Animals**	
3.2.1	Species	Mice.
3.2.2	Strain	Swiss.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age of test animals not reported. Weight of test animals at study initiation was between 32-37g.
3.2.6	Number of animals per group	¬뽤餞
3.2.7	Control animals	10.
3.2.8	Mating period	Test investigated effect on spermatozoa rather than conception and effects on the unborn foetus. In order to determine if the effects observed on the spermatozoa effected fertility, virgin females (of comparable body weight) were allocated to the control and test group, and litter sizes from the females were recorded. Further details are given in '5.1 Materials and Methods'.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of exposure	Total exposure period to increased carbon dioxide: 6h (intermittent exposure over 8 hour) 26.5 h (intermittent exposure over six days)
3.3.2	Post-exposure period	In order to determine if the effects observed on the spermatozoa had a delayed effect on fertility, males were mated with virgin females for 6 days, starting 15 days after the end of the treatment, and litter sizes from the females were recorded. Further details are given in '5.1 Materials and Methods'.
		Inhalation
3.3.9	Concentrations	Nominal concentration 1% carbon dioxide. No analytical concentration reported.
3.3.10	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.11	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.12	Type of exposure	Whole body.
3.3.13	Vehicle	Gas.
3.3.14	Concentration in vehicle	Air/carbon dioxide mixture in the proportion of 1.8/1.0 by volume. (equivalent to 65%/35% mixture).
3.3.15	Exposure period / day	Other: Total exposure period to increased carbon dioxide: 6h. During the winter months (air temperature 18°C) test animals were allowed to recuperate in normal air for 30 minutes after each 2h exposure to increased carbon dioxide. During the summer months (air temperature 30-32°C) test animals were allowed to recuperate in normal air for 15 minutes after each hour of exposure to increased carbon dioxide.
3.3.16	Controls	10 control animals were exposed to identical conditions to the test animals, except that they were exposed only to normal air.
3.4.	**Examinations**	
3.4.1	Body weight	No.
3.4.2	Food consumption	No.
3.4.3	Clinical signs	No
3.4.4	Examination of uterine content	No
3.4.5	Examinations of foetuses	
3.4.5.1	General	No.

3.4.5.2	Skelet	No.

3.4.5.3	Soft tissue	No.
3.5	**Further remarks**	Spermatozoan morphology and the effect on fertility was examined after exposure to an increased concentration of carbon dioxide.
4.2	**Teratogenic /embryotoxic effects**	None reported.
		4. RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	None reported.
4.3	**Other effects**	Table A6_8-1 at the end of this study summary shows that exposure to increased carbon dioxide caused a decrease in the average of all spermatozoan dimensions, statistically significant for all characteristics except mid-piece length and percentage of unstained spermatozoa, the mean square for mice within groups being used as error term. For the fertility study, the mean litter size per control and experimental male was calculated (refer to Table A6_8-2 at the end of this study summary for further details). It was found that in 82.3 +/- 9.3% of the 17 trials, the experimental group had a smaller litter size than did the control. This percentage differed from the 50% of a null hypothesis at a high level of significance ($P < 0.001$). It was concluded that males exposed to high levels of carbon dioxide give fewer offspring per mating than controls. When the zero class was excluded (litter size 0), the weighted mean difference (control experiment) in the early test was 0.07 +/- 0.60 offspring/litter, and 0.37 +/- 0.55 in the delayed test: clearly neither difference is significant. Hence the effect of exposure to high carbon dioxide levels was on the conception rate. This is evident in Table A6_8-2 given at the end of this study summary, where there are 12 occasions on which the number of fertile males was less in experimental than in control groups, 5 occasions when the numbers were equal and no occasions when experiment exceeded control. Analysis of a balanced factorial arrangement extracted from the original data showed significant variation ($0.05 > P > 0.025$) between the conception rate of experimental and control animals.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC. The object of the experiment was to increase carbon dioxide (and thereby reduce oxygen) in the inspired air of male mice and to study the effect on spermatozoan morphology and fertility. The apparatus shown in figure 1 at the end of this study summary supplied an air/carbon dioxide mixture to an experimental chamber whose inlet was connected to the top ends of two flow meters through one-way valves. One flow meter was connected to a carbon dioxide reservoir. The other, and a third flow meter were connected to an air pump. The top of the third flow meter was connected to the inlet of the control chamber through a one-way valve. The outlets of the two chambers were fitted with one-way valves to exclude extraneous air. Male mice (colony-bred, Swiss strain) weighing between 32 and 37g, were allocated at random to a control and an experimental group, each of 10 mice. The groups were placed in their chambers simultaneously. 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 the control chamber only air was supplied, at the same

flow rate as that of the mixture. In winter (air temperature 18°C) mice

(Continued....)

5.1	**Materials and methods** (Continued)	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. After a net exposure of 6h to the mixture, the treated mice (and controls) were killed by dislocating the neck. 5 permanent nigrosin-eosin slides were prepared from the mixed contents of two vasa deferentia of each male. The 100 slides were coded and examined in randomised order. Camera lucida drawings of four normal unstained spermatozoa per slide were made at a linear magnification of x 6560. The maximum breadth and projected area of the midpiece were measured as described by Beatty and Mukherjee[1]. In addition, the percentage of unstained spermatozoa was scored from 100 spermatozoa per slide. 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. [redacted]
5.2	**Results and discussion**	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. Note that it is uncertain from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide).
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	Not reported.
5.3.2	NO(A)EL maternal toxic effects	Not reported.
5.3.3	LOAEL embryotoxic/ teratogenic effects	Not reported.
5.3.4	NO(A)EL embryotoxic/ teratogenic effects	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.* *Note that it is uncertain from this data whether the observed effects

	were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide)

Section A6.8.1
Annex Point IIA, VI, 6.8.1

Teratogenicity Study (3 of 4)

Section 6: Toxicological and Metabolic Studies
Inhalation, Mice.

5.3.5	Reliability	3
5.3.6	Deficiencies	Yes.

It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effect of exposure to 35% carbon dioxide on the morphology of spermatozoa of mice, and their fertility. While this study was not generated to modern, scientifically accepted protocols, and it is unclear from this data whether the observed effects were due to carbon dioxide *per se* or a secondary effect such as acidosis, increased blood flow or increased oxygen tension (secondary to hyperventilation caused by increased carbon dioxide), it gives an indication about the possible teratogenic effects of carbon dioxide.

This study, notwithstanding it's deficiencies, can be used to support the teratogenicity of carbon dioxide because:

1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.

2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.

3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide 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.

Section A6.8.1 **Teratogenicity Study (3 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Mice.

Table A6_8-1: Group Means and Analysis of Variance of Spermatozoan Characteristics

Group Means	d.f.	Head area (μ^2)	Head breadth (μ)	Midpiece area (μ^2)	Midpiece length (μ)	Midpiece breadth (μ)	Angular percentage unstained
Control mean	-	22.97	3.63	14.84	22.80	0.640	49.30
Experimental mean	-	21.57	3.43	13.20	22.52	0.579	47.93
Analysis: Groups	1	9.11***	0.421***	12.42***	0.87	0.042***	47.65
Mice within groups	18	0.63*	0.034***	0.49	0.79**	0.003	176.44***
Slides within mice	80	0.32	0.008	5.50	0.356	0.002	33.23

Key: * $0.05 > P > 0.025$
** $0.01 > P > 0.005$
*** $0.005 > P$

Table A6_8-2: Summary of Fertility of Control and Carbon Dioxide-Treated Males

Trial No.	Number of males in each control and experimental group	Number of males giving a litter		Mean litter size (excluding litter size 0)	
		Control	Experiment	Control	Experiment
(a) Early tests of male fertility					
I	4	4	4	10.00	7.75
II	3	3	2	7.00	8.00
III	3	3	3	8.33	9.00
IV	5	5	3	7.60	9.33
Va	5	3	2	6.00	7.00
VIa	5	4	3	5.75	3.33
VII	5	3	1	7.00	8.00
VIIIa	5	3	2	6.00	8.00
IXa	5	4	3	7.25	9.00
Xa	5	3	3	8.00	3.33
XIa	5	3	2	7.00	8.50
(b) Delayed tests of male fertility					
Vb	5	3	1	7.33	5.00
VIb	5	4	3	6.75	7.33
VIIIb	5	4	4	7.75	6.25

IXb	5	5	4	7.20	9.25
Xb	5	5	4	7.80	5.50
XIb	5	5	5	8.00	8.00

Section A6.8.1 **Teratogenicity Study (3 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Mice.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	COMMENTS FROM .....
Date	*Give date of comments submitted.*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion* *Discuss if deviating from view of rapporteur member state. .*
Results and discussion	*Discuss if deviating from view of rapporteur member state.*
Conclusion	*Discuss if deviating from view of rapporteur member state.*
Reliability	*Discuss if deviating from view of rapporteur member state.*

Acceptability	*Discuss if deviating from view of rapporteur member state.*
Remarks	

Section A6.8.1
Annex Point IIA, VI, 6.8.1

Teratogenicity Study (4 of 4)

Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

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1. REFERENCE

1.1 Reference [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No.

Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC.

2.2 GLP No.

GLP was not compulsory at the time study was performed.

2.3 Deviations Yes.

No set guideline followed.

3. MATERIALS AND METHODS

3.1 Test material As given in section 2.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

[redacted]

Section A6.8.1 Teratogenicity Study (4 of 4)

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Bastard rabbits from external breeding.
3.2.3	Source	Not reported for rabbits.
3.2.4	Sex	Female.
3.2.5	Age/weight at study Initiation	Not reported, but rabbits were sexually mature.
3.2.6	Number of animals per group	Two (mother and father)
3.2.7	Control animals	Yes. 30 young animals from 3 litters served as a control.
3.2.8	Mating period	Rabbits were exposed to increased carbon dioxide on 8-10th day of covering. For full details, see table 6.8.1 at the end of this study summary.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of exposure	4-10 hours on 2 to 3 different days. For full details, see table 6.8.1 at the end of this study summary.
3.3.2	Post-exposure period	Not reported.
		Inhalation
3.3.9	Concentrations	Nominal concentration 10% carbon dioxide and 13% carbon dioxide. No analytical concentration reported. For full details, see table 6.8.1 at the end of this study summary.
3.3.10	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.11	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.12	Type of exposure	Whole body (Report states that test animals were placed in a climatic chamber. It is assumed from this description that exposure was whole body).
3.3.13	Vehicle	Gas.
3.3.14	Concentration in vehicle	Carbon dioxide content in exposure chamber was increased to 10-13%. Oxygen content in the chamber was held at the normal value for inhaled air by a constant oxygen feed. For full details, see table 6.8.1 at the end of this study summary.
3.3.15	Exposure period / day	Between 4 –10 hours. For full details, see table 6.8.1 at the end of this study summary.
3.3.16	Controls	30 young animals from 3 litters served as a control. Control animals were exposed to identical conditions to the test animals, except that they were exposed only to normal air.
3.4.	**Examinations**	
3.4.1	Body weight	Not reported.
3.4.2	Food consumption	Not reported.
3.4.3	Clinical signs	Not reported.
3.4.4	Examination of uterine content	Not reported.

Section A6.8.1 **Teratogenicity Study (4 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

3.4.5	Examinations of foetuses	For full details, see table A.6.8-2 at the end of this study summary. When examining the 67 young animals born under the effects of CO2, changes were found in the neck, chest or lumbar spine area in 16 animals. There were no abnormalities in the 3rd litter of doe number 3, the only one exposed to a CO2 increase up to 13% vol. – not on the 9th day, but on the 7th and 8th days, each for 10 hours. This confirms that in rabbits, the embryogenesis has particular susceptibility to malformations of the spine on the 9th day. In all cases of localised abnormalities in the cervical and chest spinal column only the vertebrae are affected. There was a hypoplastically deformed cervical vertebrae of a young animal from the 3rd litter of doe no. 1. Similar forms of vertebral dysplasia were found in another 13 animals from 6 litters. With the siblings from the 1st litter of doe number 3, vertebral arch as well as bodily defects have developed. In both cases the 4th lumbar vertebra only correctly placed on the right side, the left halves of the vertebrae and arches are missing. Apart from this the neighbouring vertebrae and arches are hypoplastically deformed on one side. The affected parts of the lumbar vertebra are also correspondingly twisted with scoliosis. In one of the two young animals the 1st lumbar vertebra is slightly hypoplastically deformed. The 30 young animals from the control litter show no changes to the spinal column. Only the dysplasia of the 3rd and 4th sternal sections is apparent in an animal from the control litter of doe number 2. The deformed fifth sternal section in a large number of test and control animals in all three series of tests, like the variability of the number of ribs and the length of the last ribs, are probably an expression of a development process that is still under way. 46 of the test and control animals examined were bucks and 50 were does. The gender could not be definitively identified in one animal. Deformities to the spinal column were found in 16 animals, of which 11 were bucks and 5 does. Whilst the proportion of each sex is more or less the same in the sample of animals examined, there are clearly more bucks amongst the animals affected by changes to the spinal column. Table A.6.8-2 at the end of this study summary, gives an overview of the localisation of the individual deformities across the genders of the animals affected.
3.4.5.1	General	Refer to "3.4.5 Examinations of foetuses" (above) for details
3.4.5.2	Skelet	Not reported.
3.4.5.3	Soft tissue	Not reported.
3.5	**Further remarks**	Not reported.
4.2	**Teratogenic /embryotoxic effects**	Skeletal abnormalities were observed when pregnant rabbits were exposed to 10-13% carbon dioxide on 8-10th day of covering. Refer to "3.4.5 Examinations of foetuses" (above) for details. Note that 10-13% carbon dioxide would induce unconsciousness in humans.
		4. RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	Not reported.

4.3	**Other effects**	Not reported.

Section A6.8.1 **Teratogenicity Study (4 of 4)**

Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Not carried out to Guideline B.31 in Annex V of Directive 67/548/EEC.

The influence of additional CO2 inhalation by carrier does on the development of their offspring was monitored by examining 97 young animals. The young animals originated from 3 bastard does from 11 litters. Examination of 30 young animals from 3 litters served as a control. 7 to 12 days after the covering date the mothers of the remaining 67 young animals were placed in a climatic chamber on 2 or 3 different days for 4 to 10 hours, where the CO2 content was increased to 10 to 13% volume. The oxygen content in the chamber was held at the normal value for inhaled air by a constant O2 feed.

The increase in CO2 was created in a climate chamber, which made it possible to monitor constantly not only the carbon dioxide but also the temperature and the relative humidity, and to adjust them to constant values. The temperature in the climate chamber was maintained during all the experiments at a value corresponding to the room temperature in each case.

Young animals from control litters from the corresponding pairs of parents were examined. The embryo development of the control animals was not disturbed by any experimental influences at all.

In total, 16 female sexually mature rabbits were used. Eleven of these were bastard rabbits from external breeding. The young were killed soon after birth and prepared. The skeleton revealed by the preparation was first dried, then, after maceration, dyed in a weak potassium solution with alizarin, and then lightened in an ethyl alcohol series and benzyl alcohol (following the Gruenberg method). The preparations were then examined and photographed under ten-fold magnification.

5.2 Results and discussion

Before the individual test results are discussed, a description is given to the state of development of a rabbit embryo during the experimental period, i.e. on the eighth to tenth day after covering. Implantation took place on the seventh to eighth day of embryonic development. On the eighth day the development of placentation on the mesodermic side of the uterus was initiated, on the ninth day trophoblasts grow around surface capillaries, and on the tenth day the villus formed. On the ninth day of its development, the embryo is some 4 mm long and in most cases exhibits 15 original segments on both sides of the chorda. The neural tube is closed.

The CO2 concentration in the test chamber was increased to a level which results in unconsciousness in humans (10-13%).

The comparative examination of all abnormalities arising in the different test series shows that pathological changes of the spinal column play a significant role in 37 animals. The most frequent malformations exist in the hypoplasia of whole vertebrae or individual vertebra.

Continued.....

5.2 Results and discussion

(continued..)

Hypoplastic malformed vertebrae did not only arise as individual findings, but also as concomitant results with all serious changes of the spinal column. In many cases they define morphologically damaged spinal column sections according to cranial and caudal with half-, split and fused vertebrae formations. Thus, in animals with several malformations, certain samples often develop in the damaged spinal column sections. A cranio-caudal transition from normally developed, to light and more serious hypoplastic distortion of the vertebrae, to severely damaged areas is evident in several animals. Caudally, this results in the reverse sequence to vertebrae that are arranged normally again. These flowing transitions, by which maximally damaged sections can be defined, possibly reflect the metabolic situation of the spinal column at the time of the damage.
Compared to the abnormalities within the area of the spinal column, pathological changes to the sternum arose in substantially smaller quantities and almost exclusively with malformations of the vertebrae.

The malformations of the skeletal system described after increased CO2 respiration comply with the abnormalities described by DEGENHARDT after the lack of oxygen at the time of the 9th day of embryonic development. Concerning the abnormalities of the spinal column discovered by DEGENHARDT, hypoplasia of the vertebral body is also a prominent subject, which occurs as an individual finding or combined with half, split and fused vertebrae formations. In many cases the malformations of the spinal column are also combined with the fusing of ribs.

The morphological comparison of abnormalities after the effect of different exogenous factors (lack of oxygen, increase in the level of CO2, low pressure) confirms the realisation of the experimental teratology, which can cause the same abnormalities through different exogenous agents if the embryonic development is interfered with in the same phase. According to the teratological effect, the time of damage is of more crucial importance than the type.

Even in spontaneous accumulation, DEGENHARDT found the malformations of the spinal column described above in Ermine rabbits from an in-breeding strain. Autosomal recessive hereditary could probably be made for the pathological factor that impaired the normal development of the spinal column. In this case, the pathological gene effect seems to intervene in the same metabolic processes as the effect of the exogenous agents of the tests described, because the same erroneous trends were found.

In the CO2 tests described, the maximum level tolerable for the animals did not seem to be reached with 13 vol % of CO2 in the inhalation air. Perhaps the development disturbances of the spinal column can be made clearer with increased levels of CO2.

The tests for rabbits show that damage can be caused with an increase in CO2 and a reduction in the pressure during certain phases of the embryonic development. The developmental disturbances that occurred correspond to the malformations of the spinal column, the ribs and sternum after oxygen depravation tests in rabbits, as described by DEGENHARDT. The enumeration of the spinal columns of 262

	rabbits confirms that the possibilities of regional differentiation of the spinal column are particularly diverse.

5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	Not reported.
5.3.2	NO(A)EL maternal toxic effects	Not reported.
5.3.3	LOAEL embryotoxic/ teratogenic effects	10% carbon dioxide on on 8-10th day of covering.
5.3.4	NO(A)EL embryotoxic/ teratogenic effects	Not reported.
5.3.5	Reliability	3
5.3.6	Deficiencies	Yes. It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effect of exposure to 10-13% carbon dioxide on the development of rabbit embryos. While this study was not generated to modern, scientifically accepted protocols, it gives an indication about the possible teratogenic effects of carbon dioxide. This study, notwithstanding it's deficiencies, can be used to support the teratogenicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions. 3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide 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.

Section A6.8.1 **Teratogenicity Study (4 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

Table A6_8-1: Test conditions and Details of Litters Born

Mother Father	Test period (relative to the covering day)	Duration of the test	CO2 content of the chamber	Number of young animals	Number of young animals with abnormalities
No. 1, 1st litter "Deutscher Riesenscheck"	8th day + 9th day	4 hours 4 hours	10% vol. 10% vol.	8	1
No. 1, 2nd litter "Deutscher Riesenscheck"	8th day + 9th day	5 hours 5 hours	12% vol. 13% vol.	9	4
No. 1, 3rd litter "Deutscher Riesenscheck"	9th day +10th day +11th day	10 hours 10 hours 10 hours	13% vol. 13% vol. 13% vol.	9	3
No. 2, 1st litter "Deutscher Riesenscheck"	8th day + 9th day	4 hours 4 hours	10% vol. 10% vol.	5	1
No. 2, 2nd litter "Deutscher Riesenscheck"	9th day +10th day	5 hours 5 hours	12% vol. 13% vol.	10	1
No. 2, 3rd litter "Deutscher Riesenscheck"	7th day + 8th day	10 hours 10 hours	13% vol. 13% vol.	8	
No. 3, 1st litter "Deutscher Riesenscheck"	9th day +10th day	5 hours 5 hours	13% vol. 13% vol.	10	2
No. 3, 2nd litter "Deutscher Riesenscheck"	9th day +10th day +12th day	10 hours 10 hours 10 hours	13% vol. 13% vol. 13% vol.	8	4
No. 1, control "Deutscher Riesenscheck"				10	
No. 2, control No. 912 725				11	1
No. 3, control "Deutscher Riesenscheck"				9	

Section A6.8.1 **Teratogenicity Study (4 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

Table A6_8-2: Examination of rabbit foetuses

Mother	Gender of the young animal	Localisation of deformities
No. 1, 1st litter	♂ (female)	3rd to 5th cervical vertebrae
No. 1, 2nd litter	♂ (female) ♂ (female) ♂ (female) ♂(female)	3rd cervical vertebra 3rd cervical vertebra 1st cervical vertebra 3rd cervical vertebra
No. 1, 3rd litter	♂(female) ♀ (male) ♂ (female)	3rd cervical vertebra 3rd cervical vertebra 3rd cervical vertebra
No. 2, 1st litter	♀ (male)	3rd cervical vertebra
No. 2, 2nd litter	♀ (male)	1st cervical vertebra
No. 3, 1st litter	♂ (female) ♀ (female)	2nd to 5th lumbar vertebrae 1st lumbar vertebra 3rd to 5th lumbar vertebrae
No. 3, 2nd litter	♂ (female) ♀ (male) ♂ (female) ♂ (female)	1st cervical vertebra 1st cervical vertebra 2nd cervical vertebra 3rd cervical vertebra 3rd cervical vertebra
No. 2, control	♂ (female)	Sternum

Section A6.8.1 **Teratogenicity Study (4 of 4)**
Annex Point IIA, VI, 6.8.1 Section 6: Toxicological and Metabolic Studies
Inhalation, Rabbit.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	COMMENTS FROM …..
Date	*Give date of comments submitted.*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion* *Discuss if deviating from view of rapporteur member state. .*
Results and discussion	*Discuss if deviating from view of rapporteur member state.*
Conclusion	*Discuss if deviating from view of rapporteur member state.*
Reliability	*Discuss if deviating from view of rapporteur member state.*
Acceptability	*Discuss if deviating from view of rapporteur member state.*

Remarks

Table 4-2: Standard form for justification of the non-submission of data

Section 6.8.2 **Annex Point IIA, VI, 6.8.2**	**Multigeneration Reproduction Toxicity Study** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data **[4]**	**Technically not feasible [4]** **Scientifically unjustified [4]**	
Limited exposure **[4]**	**Other justification [ ]**	
Detailed justification:	**Scientific necessity** It is not considered scientifically necessary to determine the reproductive effects of carbon dioxide for a number of reasons, including: • 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. • In addition to the above, the potential for exposure to carbon dioxide is minimal [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture. [redacted]The use of carbon dioxide as a biocide is far less than that used in other industries such as brewing [redacted] • Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers[7]. 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 example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm.[5] The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[8]. • As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value. (Continued...)	

Detailed justification:
(Continued)

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider carcinogenicity or genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. Given the large volume of data available for carbon dioxide, only the typical findings have been summarised below with regards to the carcinogenic potential of carbon dioxide. A number of reviews have been carried out by different regulatory authorities including the EPA[5] and FDA, who considered the health aspects of carbon dioxide as a food additive[6]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the carcinogenic or geneotoxic potential of carbon dioxide.

Technical feasibility

While it is possible to carry out a multigeneration study on carbon dioxide, it will be technically very difficult, full of constraints and expensive. The data given below shows how the body's metabolism and physiology are extremely sensitive to carbon dioxide levels, and will adjust to any atmospheric changes. This effects the body 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 carbon dioxide 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.

(Continued.....)

Detailed justification:
(Continued)

Exposure to increasing concentrations of carbon dioxide: Effects and Observations

Carbon dioxide is a natural substance, produced by cellular breakdown of carbon-based materials. It is excreted by exhaling. Toxicity is acute, by cellular acidosis disrupting enzyme activities and reducing cellular respiration beyond the point where the organism as a whole can survive [1].

Carbon dioxide is naturally produced by the body, and is effectively regulated by a series of homeostatic mechanisms designed to maximise the carbon dioxide-carrying capacity of the blood. Cells produce carbon dioxide as part of the normal catabolic process. This carbon dioxide diffuses in solution from the cell to the blood plasma and thence to the red cells. Under normal circumstances, in the resting human, the dissolved concentration of carbon dioxide in the blood is between 48 (arterial) and 52 (venous) ml/100 ml blood. Very low levels of carbon dioxide may lead to failure to stimulate inspiration. Vigorous exercise increases the amount of carbon dioxide carried and exhaled (mainly by increased heart rate and respiratory rate), but as the excretion of the gas depends on a diffusion gradient across the alveolar wall, the amount of carbon dioxide already present in the air will govern the efficiency of excretion. Normal alveolar partial pressure of carbon dioxide is approximately 5-6% carbon dioxide. Typically, normal air contains 0.03% carbon dioxide. If extra carbon dioxide is added such that alveolar concentration increases by 0.2%, the resting pulmonary ventilation is doubled [2]. If the concentration of carbon dioxide is so high that the organism cannot cope by further increasing respiratory rate, death occurs when the diffusion gradient between the cells of the body and the blood no longer functions.

Exposure to increasing levels of carbon dioxide produces respiratory distress, as the animal attempts to exhale the increasing amounts accumulating in the body [2]. Breathing rate increases to a maximum, followed by loss of consciousness and death. When guinea pigs were exposed to 15% carbon dioxide in 21% oxygen continuously for seven days, blood pH initially fell after 1 hour of exposure, and then rose to 7.10 after 6 hours, and continued to rise back to the initial pH value. Blood corticosteriods rose markedly, and adrenal epinephrine fell. Levels of free fatty acids in the arterial blood rose, and lymphocytes and adrenal cholesterol decreased. These changes occurred only during the first three days of exposure. After this, corticosteriods, adrenal epinephrine, free fatty acids, lymphocytes and adrenal cholesterol content all returned to initial levels, as the body's metabolism compensated for the increase in carbon dioxide [3]. There is also data available to show this effect in man, when 23 subjects were exposed to 1.5% carbon dioxide in 21% oxygen for 42 days. The body began to compensate for the increased level of carbon dioxide after 23 days exposure [4]. The compensation effect does not appear to occur when animals are exposed to increased levels of carbon dioxide for intermittent periods [3]. An occupational exposure study on brewery workers, over five days where the time weighted average concentrations of carbon dioxide ranged from 0.5 to 1.95% (with a mean of 1.08 % but momentary concentrations reached 8%), concluded that there were no significant physiological effect of chronic intermittent exposure to these levels of carbon dioxide [7]

(Continued.....)

Section 6.8.2 Annex Point IIA, VI, 6.8.2	Multigeneration Reproduction Toxicity Study Section 6: Toxicological and Metabolic Studies

Detailed justification:
(Continued)

Conclusion

On the basis of exposure alone, it is not scientifically necessary to conduct a multigeneration study 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.

A multigeneration study is technically feasible, but difficult, and given the body's metabolic and physiological sensitivity to changes in carbon dioxide levels it is unlikely to provide any useful data for the risk assessment. The toxicological profile of carbon dioxide is well established with a substantial amount of data. Although this information has it's limitations and it does not address the issue of fertility and reproduction specifically, it is considered sufficient to address the toxicity of carbon dioxide particularly given the low level of exposure expected from it's use as a rodenticide.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.9 **Annex Point IIIA , VI, 1**	**Delayed Neurotoxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [4]	**Other justification** [ ]	
Detailed justification:	Effects of excessive carbon dioxide exposure in man are well reported in the product literature. This data indicates that when present in sufficient quantities, carbon dioxide can cause adverse effects in man such as headaches, reduced hearing ability, loss of judgement and ultimately loss of consciousness. These studies have been summarised in Document IIIA Section 6.1.3 and Section 6.4.3, but the key results are as follows: Exposure to 3% carbon dioxide leads to deeper breathing, headache, reduced hearing ability, increased heart rate and acidosis. [1] At 5 – 10% carbon dioxide, in addition to the effects detailed for exposure to 3% carbon dioxide there is more laborious breathing and loss of judgement. [2,3] At 10% carbon dioxide, in addition to the symptoms detailed for 5 – 10% carbon dioxide, there is also loss of consciousness. [4] While the effects detailed above are acknowledged to be neurotoxic effects, a full study to consider the neurotoxic effects of carbon dioxide is not considered necessary for the following reasons. 1. It has been widely reported that the effects associated with carbon dioxide exposure are reversible once the carbon dioxide has been removed. [3,5 and others.] 2. 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. 3. 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. (Continued……)	

Section 6.9 Annex Point IIIA , VI, 1	Delayed Neurotoxicity

Official use only

Detailed justification:

[redacted]

(Continued....)

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.9 **Annex Point IIIA , VI, 1**	**Delayed Neurotoxicity**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.10 **Annex Point IIIA, VI, 7**	**Mechanistic Study** Already submitted for carbon dioxide dossier for Product Type 14.	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	It is not scientifically necessary to submit a mechanistic study for carbon dioxide, because the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that these tests are only required to clarify any effects reported in other toxicity studies. Such effects could include a possible non-genotoxic mechanism for carcinogenicity, species specific effects, adverse effects on reproduction, immunotoxicity or hormone related effects. There is a substantial volume of data available on the toxicity of carbon dioxide, and while this data gives an indication that carbon dioxide can have adverse effects at certain concentrations, none of the toxicity effects reported are of sufficient concern to justify further investigation by a mechanistic study. It is on this basis that it is not necessary to submit additional data about the mechanism of carbon dioxide toxicity.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.10 Annex Point IIIA, VI, 7	Mechanistic Study

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.11	**Studies on Other Routes of Administration (Parenteral Routes)** Already submitted for carbon dioxide dossier for Product Type 14.	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	It is not scientifically necessary to submit toxicity data on parenteral routes of exposure of carbon dioxide because the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that data on parenteral routes of exposure only needs to be submitted if it gives valuable supplemental information to the toxicokinetic studies (e.g. when the gastrointestinal absorption of the active ingredient is poor). If acute toxicity data is available on the intraperitoneal, intravenous subcutaneous and intramuscular routes, it should be submitted in this section. Carbon dioxide is a gas, which means that the significant route of exposure is by inhalation. There will be no delivery or uptake of carbon dioxide by any other route. It is on this basis that it is not scientifically necessary to submit additional data on the toxicity of carbon dioxide by parenteral routes of exposure.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.11	Studies on Other Routes of Administration (Parenteral Routes)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.12 **Annex Point IIA, VI, 6.9**	**Medical data in anonymous form**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [4] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	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. The key results for man include the following: Exposure to 1% carbon dioxide (time weighted average) during the working day has little effect on blood parameters, including bicarbonate and carbon dioxide. (It should be noted that the author of the study had great difficulty in monitoring the exposure of subjects to carbon dioxide because of their movements). [1] Exposure to 1.5% carbon dioxide led to lower heart rate, reduced tolerance to vigorous exercise. [2] There was no apparent changes in performance or basic physiological parameters when humans were exposed to 1.5% carbon dioxide for 42 days [2]. There was slight acidosis for 23 days, increased respiratory rate and increased systolic BP. Exposure to 3% carbon dioxide leads to deeper breathing, headache, reduced hearing ability, increased heart rate and acidosis. [4] At 5-10% carbon dioxide, in addition to the effects detailed for exposure to 3% carbon dioxide there is more laborious breathing and loss of judgement. [5, 6] At 10% carbon dioxide , in addition to the symptoms detailed for 5-10% carbon dioxide, there is also loss of consciousness. [7] It has been widely reported that the effects associated with carbon dioxide exposure are reversible once the carbon dioxide has been removed. [1, 6 and others.] 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. 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. (Continued….)	

Undertaking of intended data submission	[ ]	Not applicable.

Section 6.12	**Medical data in anonymous form**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.12.1 **Annex Point IIA, VI, 6.9.1**	**Medical surveillance data on manufacturing plant personnel if available.**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	There is no medical surveillance data on carbon dioxide manufacturing plant personnel available for submission. In addition, a full literature search was conducted in order to identify and obtain any human exposure data to carbon dioxide that is available in the public domain. No relevant data was found from this search. Note that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that medical surveillance data on manufacturing personnel should only be submitted if it is available.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.12.1	Medical surveillance data on manufacturing plant personnel if available.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.12.2 **Annex Point IIA, VI, 6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	There is no carbon dioxide poisoning data or data about clinical cases available for submission. In addition, a full literature search was conducted in order to identify and obtain any human exposure data to carbon dioxide that is available in the public domain. No relevant data was found from this search. Note that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that data about carbon dioxide poisoning and clinical cases should only be submitted if it is available.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.12.2	Direct observation, e.g. clinical cases, poisoning incidents if available

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.12.3 **Annex Point IIA, VI, 6.9.3**	**Health Records, Both from Industry and any other Available Sources**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	There is no data on health records following carbon dioxide exposure available for submission. In addition, a full literature search was conducted in order to identify and obtain any human exposure data to carbon dioxide that is available in the public domain. No relevant data was found from this search. Note that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that health records should only be submitted if it is available.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.12.3	Health Records, Both from Industry and any other Available Sources

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.12.4 **Annex Point IIA, VI, 6.9.4**	**Epidemiological studies on the general population, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [4]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	There is no epidemiological data on carbon dioxide exposures available for submission. In addition, a full literature search was conducted in order to identify and obtain any epidemiological data on carbon dioxide that is available in the public domain. No relevant data was found from this search. Note that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that epidemiological data should only be submitted if it is available.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.12.4	Epidemiological studies on the general population, if available

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.5
Annex Point IIA, VI, 6.9.5

Diagnosis of Poisoning, including specific signs of poisoning and clinical tests, if available

Already submitted for carbon dioxide dossier for Product Type 14.
[redacted]

1. **REFERENCE**

Official use only

1.1 Reference

[redacted]

1.2 Details

Contact with carbon dioxide liquid may cause cold burns/frost bite.

Concentrations of 10% carbon dioxide or more can produce unconsciousness or death and may cause asphyxiation in high concentrations.

Symptoms of asphyxiation may include loss of mobility and/or consciousness, and may come about so rapidly that victims may not be unaware and unable to protect themselves.