Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

3-iodo-2-propynyl butylcarbamate (IPBC)

EC Number: 259-627-5

CAS Number: 55406-53-6

Index Number: 616-212-00-7

Contact details for dossier submitter: Danish EPA

Version number: 1.1 **Date:** 11 November 2022

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3.3.1.1 Acute inhalation toxicity, rat, Limit Test

Section A6.1.3/01	**Acute Toxicity**	
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, Limit test**	
	1 REFERENCE	**Official use only**
1.1 Reference	A6.1.3/01: [redacted] (1985): Acute Inhalation Limit Test in Rats 3-Iodo-2-propynyl butyl carbamate Revised Final Report; [redacted]; Doc. No. 523-001 (unpublished)	TROY
1.2 Data protection	Yes	
1.2.1 Data owner	Troy Corporation	
1.2.2 Companies with letter of access	Arch Chemicals, Bayer Chemicals, Sostram Corporation	
1.2.3 Criteria for data protection	Data on existing a.s submitted for the first time for entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	

Section A6.1.3/01		**Acute Toxicity**	
Annex Point IIA, VI.6.1.3		**Inhalation, Rat, Limit test**	
2.1	**Guideline study**	Yes, OECD 403 guideline (adopted 1981)	Current OECD TG 403 version adopted Septem-ber 2009 *
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Yes The actual test substance concentration was calculated and not determined by analysis.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Technical active substance IPBC (3-iodo-2-propynyl butyl carbamate)	
3.1.1	Lot/Batch number	21,274A	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99%	
3.1.4	Description	White crystalline powder	
3.1.5	Stability	Not required	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	[redacted]	
3.2.4	Sex	male, female	
3.2.5	Age/weight at study initiation	Animals were about 7 weeks of age. Mean males pre-exposure weight was 261 g, females 165 – 178 g.	*
3.2.6	Number of animals per group	5 animals/sex/group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Post-exposure period	14 days	
3.3.2	Concentrations	Nominal target concentrations: 0 and 5 mg/L	

Section A6.1.3/01	**Acute Toxicity**	
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, Limit test**	
	Nominal concentrations tested: 0 and 6.89 mg/L	
3.3.3 Particle size	The particle size distribution of Technical IPBC is given in [redacted], 2001, Doc. No. 111-001, Document IIIA, Section A3.3.	*
3.3.4 Type or preparation of particles	Dust was generated by use of a Wright Dust Feeder.	
3.3.5 Type of exposure	not indicated	
3.3.6 Vehicle	not applicable	
3.3.7 Concentration in vehicle	not applicable	
3.3.8 Duration of exposure	4 h	
3.3.9 Controls	Control animals were exposed to 0 mg/L test substance.	
3.4 Examinations	Clinical observations, body weights, gross necropsy with special emphasis on lungs and respiratory tracts.	
3.5 Method of determination of LC_{50}	Limit test	
3.6 Further remarks	None	
	2 RESULTS AND DISCUSSION	
4.1 Clinical signs	At one hour of exposure, all animals exhibited dyspnoea, salivation and rhinorrhoea; these persisted throughout the exposure period of 4 hours. All animals showed lacrimation as well during the last one and one-and-a-half hours of exposure. During the 14-day post-exposure period, some of the predominant clinical signs were bloody crusts on nose, eyes, mouth, chest, and ears as well as rough hair coat, languid, wheezing, and urine stains. The majority of clinical signs had disappeared by day 4 post-exposure except for bloody crust around the nose. Four of the five males were normal in appearance by day 13 post-exposure; all surviving females were normal in appearance by day 11 post-exposure. One female of the treated group was found dead on day 5 post-exposure.	
4.2 Pathology	Findings at gross pathology were observed in control and treated animals in comparable incidences. The premature decedent had several findings at gross pathology in spleen, intestines, stomach, lung, and nares.	
4.3 Other	The mean body weight of all groups had increased by day 14 post-exposure when compared to pre-treatment values. However, the mean body weights of both sexes in the treated groups declined from day 2 post-exposure through day 4 post-exposure. Afterwards, animals gained successfully weight.	
4.4 LC_{50}	LC_{50} > 6.89 mg/L for males and females. One treated female was found dead on day 5.	

Section A6.1.3/01	**Acute Toxicity**
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, Limit test**

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	This study was performed to assess the acute toxicity of IPBC via the inhalation route (OECD guidelines 403). Groups of 5 animals per sex were treated with 0 or 6.89 mg/L IPBC over a period of 4 hours and were observed over a period of 14 days post-exposure.
5.2	**Results and discussion**	The LC_{50} was greater than 6.89 mg/L. One treated female was found dead on day 5 post-exposure. Clinical signs during exposure were dyspnoea, salivation, lacrimation, and rhinorrhoea. During the 14-day post-exposure period, the predominant clinical signs were bloody crusts, rough hair coat, thin, languid, wheezing and urine stains which had disappeared by day 4 post-exposure except for bloody crusts. These had disappeared by day 13. The mean body weight of the treated animals declined from day 2 to day 4 post-exposure. After 14 days, animals had gained weight. At gross pathology there were no differences between control and treated animals noted.
5.3	**Conclusion**	LC_{50} > 6.89 mg/L
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	23 November 2004. 08 November 2022.
Materials and Methods	Applicant's version is acceptable except for point 3.3.3. No data were available for the CA regarding the particle size distribution in the reference stated. Answer from the Notifier: The particle size was not measured at the time the study was conducted. However, the particle size of the representative products and products on the market are as given in the reference in 3.3.3. The proportion of particle with a size of ≤ 10 µm is ≤ [redacted]%. Therefore, the products are not considered to be respirable and consequently not toxic. Minor remark at renewal: Ad 3.2.5: This age range is below the recommendation of 8 to 12 weeks in the current OECD TG 403.
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is acceptable.
Reliability	2
Acceptability	Acceptable for non-respirable technical IPBC.
Remarks	The particle size of the test substance used in this study is stated to be not respirable. No data were available for the CA regarding the particle size distribution for the tested IPBC in this study. Therefore, strictly speaking, this study is not an OECD TG 403 study. Minor remark at renewal:

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, Limit test**

	The study was performed in 1985 according to OECD TG 403 as adopted in May 1981. The version (September 2009) of the guideline applicable at the time of renewal of IPBC is more flexible, reduce animal usage, and is designed to (better) fulfil regulatory needs. However, the changes to the guideline have no implication for the reliability of the study.

Table A6.1.3/01-1: Summary of Acute Inhalation Toxicity – Limit Test

Dose [mg/L]	Sex	Number of dead / number of investigated	Time of death	Clinical observations (incidence/number of animals per group)
0	male	0/5	-	none
0	female	0/5	-	none
6.89	male	0/5	-	during exposure: dyspnoea (5/5), salivation (5/5), rhinorrhoea (5/5), lacrimation (5/5) post-exposure: bloody crusts on nose (5/5), eyes (4/5), mouth (3/5), chest (1/5), and forepaws (1/5), languid (4/5), wheezing(4/5), urine stains (3/5), rough hair coat (4/5), soft faeces (1/5), thin (4/5), swollen penis (1/5) and face (1/5), squinted eyes (1/5)
6.89	female	1/5	day 5 post-exposure	during exposure: dyspnoea (5/5), salivation (5/5), rhinorrhoea (5/5), lacrimation (5/5) post-exposure: bloody crusts on nose (5/5), eyes (4/5), ears (2/5), mouth (1/5), and forepaws (1/5), languid (4/5), wheezing(4/5), urine stains (4/5), rough hair coat (5/5), low body temperature (1/5), soft faeces (1/5), thin (1/5), swollen abdomen (1/5)
LC_{50} value	**> 6.89 mg/L**			

3.3.1.2 Acute inhalation toxicity, rat, LC50

Section A6.1.3/02 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] (1990): (Troysan Polyphase P-100) – Acute Inhalation Toxicity Study in the Rat; [redacted]; Doc. No. 523-002; (unpublished)	TROY
1.2	**Data protection**	Yes	

Section A6.1.3/02		**Acute Toxicity**	
Annex Point IIA, VI.6.1.3		**Inhalation, Rat, LC_{50}**	
1.2.1	Data owner	Troy Corporation	
1.2.2	Companies with letter of access	Arch Chemicals, Bayer Chemicals, Sostram Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US EPA guidelines, Subdivision F: Hazard Evaluation: Human and Domestic Animals; Section 81-3 “Acute Inhalation Toxicity Study”, November 1984 which is comparable to OECD 403.	Current applic-able OECD guide-line (TG 403) adopted SEptem ber 2009 *
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the chamber temperatures were occasionally below the required range of 20 to 24°C. On occasion, the relative humidity exceeded the desired range of 40 to 60%. However, this was not considered to have affected the outcome of the study.	*
		3 MATERIALS AND METHODS	
3.1	**Test material A**	Technical active substance IPBC (Troysan Polyphase P-100)	
3.1.1	Lot/Batch number	90045420	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Light yellow powder	
3.1.2.2	Purity	98.2%	
3.1.2.3	Stability	Not required, single dosing only	
3.2	**Test material B**	Technical active substance IPBC (Troysan Polyphase AF1)	
3.2.1	Lot/Batch number	90045420	*
3.2.2	Specification	Not applicable	
3.2.2.1	Description	Yellow liquid	
3.2.2.2	Purity	40.1% active ingredient	
3.2.2.3	Stability	Not required, single dosing only	

Section A6.1.3/02	**Acute Toxicity**	
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, LC_{50}**	
3.3	**Test Animals**	
3.3.1	Species	Rat
3.3.2	Strain	Sprague-Dawley CD®
3.3.3	Source	[redacted]
3.3.4	Sex	male, female
3.3.5	Age/weight at study initiation	Animals were about 7 to 10 weeks of age. Males body weight on the day of exposure ranged from 225 to 331 g, females from 193 to 246 g. *
3.3.6	Number of animals per group	5 animals/sex/group; 7 groups
3.3.7	Control animals	Not required
3.4	**Administration/ Exposure**	Inhalation
3.4.1	Post-exposure period	14 days
3.4.2	Concentrations	Test material A: Dust exposure, Groups I, II, and VI Nominal concentrations: 13, 5.2, and 11 mg/L Gravimetric concentrations: 1.7, 0.38, and 0.72 mg/L Test material B: Liquid Aerosol, Groups III, IV, V, and VII Nominal concentrations: 24, 7.0, 1.6, and 4.6 mg formulation/L * Gravimetric concentrations: 3.4, 1.8, 0.45, and 0.75 mg formulation/L The differences in nominal and measured exposure concentrations are attributed to impaction or sedimentation of the dust or liquid aerosol on the surfaces in the exposure chamber and are considered typical for these types of exposure.

Section A6.1.3/02		**Acute Toxicity**	
Annex Point IIA, VI.6.1.3		**Inhalation, Rat, LC_{50}**	
3.4.3	Particle size	The particle size distribution is displayed in Table 6.3.1/02-1. Particle size distribution analysis of the dust showed a group average MMAD of 4.3 µm and a group average GSD of 2.9. An average of 8.1% of the particles were less than or equal to 1 µm. An average of 82% of the particles were 10 µm or less in size. Particle size determination of the liquid aerosol showed a group average MMAD of 2.4 µm and a group average GSD of 2.7. An average of 19% of the particles were less than or equal to 1 µm. An average of 94% of the particles were 10 µm or less in size.	
3.4.4	Type or preparation of particles	The dust derived from test material A was generated by use of a Wright Dust Feeder. The liquid aerosol derived from test material B was generated by a Spraying Systems air atomising nozzle.	
3.4.5	Type of exposure	whole body	
3.4.6	Vehicle	Not applicable	*
3.4.7	Concentration in vehicle	Not applicable	*
3.4.8	Duration of exposure	4 h	
3.4.9	Controls	Not required for this type of study.	
3.5	**Examinations**	Clinical observations, body weights, gross necropsy on all animals including the nasal passages, and trachea.	
3.6	**Method of determination of LC_{50}**	The method of Litchfield and Wilcoxon was used for LC_{50} determination.	
3.7	**Further remarks**	The dust was used as received, thus the particle size was unadulterated.	

Section A6.1.3/02	**Acute Toxicity**
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, LC_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality rates are displayed in Table 6.1.3/02-2. Deaths occurred between test days 1 to 8.

During the exposure period, the most commonly noted signs of toxicity were decreased activity, eye closure and excessive lacrimation. During the high levels exposures (Group I) gasping was noted.

After removal from the exposure chamber up to and including the first two hours after exposure, responses noted included laboured breathing, gasping, and rales as well as lacrimation, nasal discharge, and salivation. Signs similar to those seen following exposure continued throughout the first week of the recovery period, after which they decreased in incidence and/or became sporadic.

4.2 Pathology

Common findings in animals which die for naturally reasons without exsanguination are oedema, emphysema, and reddened lungs as well as red facial staining from Harderian gland and yellow/red ano-genital staining. These pathological conditions were also observed in the premature decedents and are, thus, of unclear relevance.

Pale lungs and black/red/tan/brown foci in lungs were seen in some of the animals killed at the terminal sacrifice after dust application. However, the toxicological significance of such findings is unclear on the basis of a post-mortem examination.

Other post-mortem findings observed grossly occurred sporadically and were not considered to be related to treatment.

4.3 Other

Substantial body weight losses were observed during the first week following the dust and liquid aerosol exposures. Recovery of weight occurred over time and most surviving animals had gained weight by termination of the study.

4.4 LC_{50}

dust:
0.68 mg/L for combined sexes
0.67 mg/L for males
0.67 mg/L for females

liquid aerosol:
0.78 mg formulation/L for combined sexes
0.63 mg formulation/L for males
0.99 mg formulation/L for females

Section A6.1.3/02	**Acute Toxicity**
Annex Point IIA, VI.6.1.3	**Inhalation, Rat, LC_{50}**

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	This study was performed to assess the acute toxicity of IPBC via the inhalation route (US-EPA guideline 81-3). Three groups of 5 animals per sex were treated once with 0.38, 0.72, and 1.7 mg/L IPBC dust. Other 4 groups of 5 animals per sex were treated once with 0.45, 0.75, 1.8, and 3.4 mg formulation/L as a liquid aerosol. Animals were exposed for 4 hours. After exposure animals were observed for 14 days.	
5.2	**Results and discussion**	The LC_{50} after dust application for males and females was 0.67 mg/L. A single treatment with the liquid aerosol resulted in an LC_{50} of 0.63 mg formulation/L in males and of 0.99 mg formulation/L in females. Signs of irritation such as excessive lacrimation or eye closure or gasping were commonly noted during exposure. Similar signs of toxicity persisted during the first week after exposure and declined thereafter. Substantial decreases in body weight were observed during the first week following exposure, however, recovery occurred over time. Most surviving animals had gained weight at terminal sacrifice. Post-mortem findings included discoloured respiratory tissues.	*
5.3	**Conclusion**	Comb. Sexes: LC_{50}: 0.68 mg/L dust LC_{50}: 0.78 mg formulation/L liquid aerosol	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.1.3/02 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24 November 2004. 08 November 2022.
Materials and Methods	Applicant's version is acceptable. One minor remark: Ad 3.2.1: the batch number is 90045448. Minor remarks at renewal: Ad 3.3.5: This age range is slightly below the recommendation of 8 to 12 weeks in the current OECD TG 403. Ad 3.4.6: The composition of the 'vehicle' used to generate *Test material B: Liquid Aerosol*, was not stated) Ad 3.4.7: The concentration of the active ingredient in *Test material B: Liquid Aerosol* was 40.1%. Minor remark at renewal: Ad. 3.4.2: The unit for the Nominal concentrations of *Test material B: Liquid Aerosol* has been revised from mg/L to mg formulation/L.
Results and discussion	Applicant's version is adopted. Minor remarks at renewal: Ad 5.2 and Table A61.3/02-2: The unit for the LC_{50} values for *Test material B: Liquid Aerosol* has been revised from mg/L to mg formulation/L.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable

Section A6.1.3/02 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

Remarks	Remark at renewal: The Abstract of the study states that it was "… designed to assess the toxic effects and determine the median lethal concentration of Troysan Polyphase P-100, when administered by inhalation using a powder and a liquid formulation to Sprague-Dawley CD® rats …" (this objective is reiterated at the beginning of the Conclusions section). The Conclusion of the study report states: "Although the powder (98.2% Active ingredient) had over twice the active ingredient content of the liquid formulation (40.1% Active ingredient), the powders' LC_{50} was only slightly lower than [that of] the liquid formulation. The reason for this difference was unclear but may have been attributable to particle size/lung deposition differences, the liquid formulation may be more easily absorbed than the dust or toxicity of other ingredients in the liquid formulation." As the composition of the vehicle in *Test material B: Liquid Aerosol* was not reported, potential inhalation toxicity of the vehicle cannot be discounted, though use of an inhalation toxic vehicle would be inconsistent with the objectives of the study. Pro-rata correction of the LC_{50} values obtained for *Test material B: Liquid Aerosol* was not performed in the study report. Minor remark at renewal: The study was performed in 1990 according to US-EPA TG 81-3, comparable to OECD TG 403 as adopted in May 1981. The version Seprember 2009) of the guideline applicable at the time of renewal of IPBC was more flexible, reduce animal usage, and was designed to (better) fulfil regulatory needs. However, the changes to the guideline have no implication for the reliability of the study.

Table A6.1.3/02-1: Particle Size Distribution

Group number	Dose [mg/L]	Type of exposure	MMAD	GSD	≤ 1.0 µm	≤ 10 µm
					[% particles in diameter]	
I	1.7	Dust	4.4	2.5	4.9	84
II	0.38	Dust	4.5	2.4	4.3	83
VI	0.72	Dust	3.9	3.8	15	78
III	3.4	Liquid Aerosol	2.9	2.4	12	92
IV	1.8	Liquid Aerosol	2.5	2.3	14	96
V	0.45	Liquid Aerosol	1.9	3.7	31	90
VII	0.75	Liquid Aerosol	2.2	2.4	18	96

3.3.1.3 Acute inhalation toxicity, rat, LC50

Section A6.1.3/03 **Acute Toxicity**

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994): Acute inhalation toxicity in rats 4-hour exposure to Omacide IPBC; [redacted]; Doc. No. 523-003 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	ARXADA (former Lonza Ltd, former ARCH Chemicals)	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH, Microbial Control (former Specialty Electronic Materials Switzerland GmbH), Troy Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA FIFRA 81-3	Current OECD TG 403 version adopted Septem-ber 2009 *
2.2	**GLP**	Yes	
2.3	**Deviations**	Particle size for the non-micronised material is above the respirable fraction for the rat. Only between 19.2 to 26.7 % was respirable.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Omacide IPBC (3-iodo-2-propynyl butylcarbamate)	
3.1.1	Lot/Batch number	2DR-293-TS1	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	97%	
3.1.4	Description	White powder	
3.1.5	Stability	At least 1 year from September 1993	

3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	[redacted]	
3.2.4	Sex	male, female	
3.2.5	Age/weight at study initiation	6 to 8 weeks, mean body weight was 200 g	*
3.2.6	Number of animals per group	5 animals/sex/group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Post-exposure period	14 days	
3.3.2	Concentrations	Nominal target concentrations: 0 and 17.4 mg/L Concentrations measured: 0 and 2.44 mg/L (micronised and non-micronised)	*
3.3.3	Particle size	Micronised material: MMAD 3.5 µm ± 1.9 GSD Non-micronised material: MMAD between 9.6 and 14.2 µm ± 2.8 and 3.6 GSD	*
3.3.4	Type or preparation of particles	Dust was generated by use of a Wright Dust Feeder.	
3.3.5	Type of exposure	not indicated	*
3.3.6	Vehicle	not applicable	
3.3.7	Concentration in vehicle	not applicable	
3.3.8	Duration of exposure	4 h	
3.3.9	Controls	Control animals were exposed to 0 mg/l test substance.	
3.4	**Examinations**	Clinical observations, body weights, food and water consumption, lung weight, macroscopic pathology	
3.5	**Method of determination of LC_{50}**	Log probit method of Miller and Tainter, 1944	
3.6	**Further remarks**	none	*

4 RESULTS AND DISCUSSION

4.1 Clinical signs

During exposure: signs consistent with exposure to an irritant aerosol, including exaggerated respiratory movements, partial closing of the eyes, wetness around the snout and mouth and salivation were seen in all rats exposed to Omacide IPBC. Signs seen only in rats exposed to micronized Omacide IPBC were erythema of the ears and restless behaviour.

4.2 Pathology

During observation period: signs seen included abnormalities to respiration, brown staining, gasping, partial closing of the eyes, peripheral vasodilation, and death. There were also numerous signs associated with loss of condition or irritant effects, including dry or loose flaky skin, swollen stomachs, swollen limps, secretion from the eyes, and yellow staining in the urogenital region.

The persistence of clinical signs and the rate of recovery from the effects of exposure were variable and there was no obvious correlation with the exposure level.

4.3 Other

Reduced bodyweight or rate of bodyweight gain was observed in rats exposed to Omacide IPBC. The effects were not clearly dose-related. Resumption of a normal rate of weight gain was observed in most groups but 1 male rat exposed to Omacide IPBC at 0.49 mg/L and male rats exposed to 1.19 mg/L failed to gain weight normally during the observation period. A continual reduction in bodyweight to death was observed in rats exposed at 2.44 mg/L.

Food consumption was reduced for up to 7 days in exposed rats and then variable in male rats exposed at 1.19 mg/L. Water consumption was variable and the effects were not proportional to exposure level.

The lung weight to body weight ratios for rats that died as a result of exposure to Omacide IPBC were higher than those of the controls rats. Ratios for rats that survived exposure to Omacide IPBC were generally within normal limits.

4.4 LC_{50}

Calculated from all mortality data: 0.67 mg/L. (LC_{50}, 4-hour)

Calculated from mortality data for groups exposed to non-micronised material: 0.88 mg/L.

The LC_{50} for the micronized material could not be calculated from the available mortality data due to the lack of a dose-response relationship. All 3 groups showed the same mortality (3/10 animals).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was performed to assess the acute toxicity of IPBC via the inhalation route. The study design was in compliance with EPA FIFRA 81-3 guideline for acute inhalation studies. Two control groups and 6 test groups of 5 animals per sex were once treated with 0, 0.16, 0.29, 0.49, 0.58, 1.19, and 2.44 mg/L IPBC over a period of 4 hours and were observed over a period of 14 days post-exposure. The concentrations for the micronised material were 0.16, 0.29, and 0.58 mg/L IPBC and 0.49, 1.19, and 2.44 mg/L IPBC for the non-micronised material.	
5.2	**Results and discussion**	The LC_{50} (4-hour) for Omacide IPBC, using all the mortality data, was calculated as 0.67 mg/L and the standard error was 0.23 mg/L. The LC_{50} for the non-micronised material alone was calculated as 0.88 mg/L with a standard error of 0.13 mg/L. The LC_{50} for the micronised material could not be calculated from the available mortality data due to the lack of a dose-response relationship, i.e. same mortality (3/10 animals) died in each of the concentrations. The particle size and the calculated respirable fraction had no impact on the mortality under the experimental conditions of this study.	*
5.3	**Conclusion**	LC_{50} = 0.67 mg/L, SE 0.23 mg/L (Calculates from all mortality data) LC_{50} = 0.88 mg/L, SE = 0.13 mg/L (combined sexes, non-micronised material) LC_{50} = 0.79 mg/L, SE = 0.24 mg/L (male rats, non-micronised material) LC_{50} = 1.04 mg/L, SE = 0.42 mg/L (female rats, non-micronised material)	*
5.3.1	**Reliability**	2	
5.3.2	**Deficiencies**	< 30% respirable fraction for non-micronised material; no dose-respond relationship for mortality seen for the 3 doses of micronised material.	*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	08 November 2022.

Materials and Methods	Applicant's version is adopted. Minor remark at renewal: Ad 3.3.2: *Micronised material*: Nominal IPBC concentrations: 2.4, 4.2 and 7.9 mg/L Actual IPBC concentrations: 0.16, 0.29 and 0.58 mg/L *Non-micronised material*: Nominal IPBC concentrations: 4.5, 10.7 and 17.4 mg/L Actual IPBC concentrations: 0.49, 1.19 and 2.44 mg/L Ad 3.2.5: Approximately 7 to 9 weeks on exposure, as the animals were acclimitised to laboratory conditions for at least 5 days before the day of exposure. 7 to 9 weeks is slightly below the recommendation of 8 to 12 weeks in the current OECD TG 403. Ad 3.3.3: The MMAD (3.5 µm) and associated GSD (± 1.9 or 2.0) was the same for all 3 groups exposed to micronized test material. MMAD was between 9.6 and 14.2 µm (and the associated GSD between ± 2.8 and 3.6) for the 3 groups exposure to non-micronised test material. A large proportion the non-micronised material collected in the the particle size sample was collected at a sieve size with a cut-off size of 9.8 µm. Consequently, the MMAD values calculated calculated for the non-micronised material can only be considered approximate, with implications for the accuracy of the 4h LC_{50} values. Ad 3.6: The micronized material contained a proportion of large aggregates and was difficult to handle in the Wright dust generator. Technical problems encountered were clogging of the dust generator scraper blade, jet and nozzle, and atendency for the test substance to form a solid mass under pressure or at speed controller settings in excess of 40% of the maximum speed. Ad 3.3.5: The exposure was 'whole body' (stated under 'Duration of exposure' in the SUMMARY section on p. 8 of the study report).
Results and discussion	Applicant's version is adopted. Major remark at renewal: Ad 5.2: The eCA does not agree with this statement; it considers the data to indicate that micronised IPBC was more acutely toxic on inhalation than equivalent or higher levels of non-micronised IPBC. Exposure to 0.29 or 0.58 mg/L micronised IPBC resulted in the death of 3 of 5 females (1 on each of days 1, 2 and 3), and 3 of 5 females (all on day 1); no males died at these exposure levels. In contrast, exposure to 0.49 mg/L non-micronized IPBC resulted in the death of 1 female on day 4 and 1 male on day 5. The 4h LC_{50} of 0.88 mg/L for the non-micronised IPBC was derived from a dust with respirable fraction in the range 19.2 – 26.7%. The mortality data for micronised IBPC with a respirable fraction in the range 74.4 – 80.5% suggest that a 4h LC_{50} significantly below 0.88 mg/L could be predicted (especially for female rats) had an adequate upper dose been tested. In this regard, the 4h LC_{50} value based on all mortality data (non-micronised and micronised exposure) was 0.67 mg/L. Minor remark at renewal: Ad 5.2 and 5.3.2: The eCA agrees that not dose-respone is evident, though to properly explain the finding it should be noted that the dose range for the micronized IPBC test material was narrow (a factor of 3.6), such that the highest dose tested was too low to result in a mortality rate adequate to permit calculation of a LC_{50}.

Conclusion	Applicant's version is adopted. Minor remark at renewal: Ad 5.3: The approximate nature of the MMAD values calculated calculated for the non-micronised material has implications for the accuracy of the 4h LC_{50} values.
Reliability	2
Acceptability	Acceptable
Remarks	Minor remark at renewal: The study was performed in 1993 according to US-EPA TG 81-3, comparable to OECD TG 403 as adopted in May 1981. The version (September 2009) of the guideline applicable at the time of renewal of IPBC is more flexible, reduce animal usage, and is designed to (better) fulfil regulatory needs. However, the changes to the guideline have no implication for the reliability of the study.

3.3.2 Human data

3.3.2.1 No new relevant data

3.3.3 Other data

3.3.3.1 No new relevant data

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 No new relevant data

3.4.2 Human data

3.4.2.1 No new relevant data

3.4.3 Other data

3.4.3.1 No new relevant data

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 No new relevant data

3.5.2 Human data

3.5.2.1 No new relevant data

3.5.3 Other data

3.5.3.1 No new relevant data

3.6 Respiratory sensitisation

3.6.1 Animal data

3.6.1.1 No new relevant data

3.6.2 Human data

3.6.2.1 No new relevant data

3.6.3 Other data

3.6.3.1 No new relevant data

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 No new relevant data

(A brief report identified in the open literature of a local lymph node assay (LLNA) is mentioned in Section 3.5 Skin sensitisation of the CLH report, though it is not considered sufficiently reliable for use in the classification of IPBC, and thus is not relevant new data. A Study Summary and a full copy of the study are provided below.)

Section A6.1.5/04	**Skin sensitisation**	
Annex Point IIA, VI.6.1.5	Local Lymph Node Assay	
	1 REFERENCE	**Official use only**
1.1 Reference	Siebert, J. (2004): The sensitizing potential of iodopropynyl butylcarbamate in the local lymph node assay; Schülke & Mayr GmbH, Research & Development, Norderstedt, Germany; Contact Points - Contact Dermatitis 2004: Volume 51 page 318-319, © Blackwell Munksgaard 2004; Doc. No. 592-016; (published)	
1.2 Data protection	No	

Section A6.1.5/04		**Skin sensitisation**	
Annex Point IIA, VI.6.1.5		Local Lymph Node Assay	
1.2.1	Data owner	N.R.	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Not applicable, peer reviewed publication	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline for Testing of Chemicals; No. 429: "Skin Sensitisation: Local Lymph Node Assay"	Current OECD TG 429 adopted July 2010 *
2.2	**GLP**	Yes	*
2.3	**Deviations**	No details	
		3 MATERIALS AND METHODS	
3.1	**Test material**	IPBC from Skyanide Chem., Prien, Germany	
3.1.1	Lot/Batch number	301506	
3.1.2	Specification	No information available	
3.1.3	Purity	No information available	
3.1.4	Description	No information available	
3.1.5	Stability	No information available	
3.1.5.1	Preparation of test substance and controls for application	a) Test substance concentrations: 10, 5, 1, and 0.1 % (w/v) IPBC in acetone/olive oil (AOO) [3 + 1 (v/v)] b) Positive controls: Methyldibromo glutaronitrile (MDBGN) 10% in AOO; 5-chloro-2-methy-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CMI/MI) 0.1% in AOO c) Negative control: AOO	*
3.1.5.2	Pretest performed on irritant effects	No information available	

Section A6.1.5/04	**Skin sensitisation**	
Annex Point IIA, VI.6.1.5	Local Lymph Node Assay	
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	No information available
3.2.3	Source	No information available
3.2.4	Sex	No information available
3.2.5	Age/weight at study initiation	No information available
3.2.6	Number of animals per group	No information available
3.2.7	Control animals	No information available
3.3	**Administration/ Exposure**	
3.3.1	Treatment preparation and Administration	Mice were dosed topically on the dorsum of both ears with 4 test item concentrations. Each group received one concentration. A negative control group was similarly treated with AOO and two positive control groups with 10% MDBGB and 0.1% CMI/MI in AOO, respectively. Dosing occurred daily for three consecutive days. Five days after the first topical application, all mice were injected intravenously by the tail vein with ^{3}H-methyl thymidine. Five hours later, the mice were euthanized and the draining auricular lymph nodes were excised and weighted individually for each animal. After the weighting, a single-cell suspension of the lymph node cells for each animal was prepared for counting radioactivity. The proliferative response of lymph node cells was calculated as the ratio of ^{3}H-methyl thymidine incorporation into lymph node cells of test group and positive control animals, relative to that of negative control group animals. A stimulation index (SI) (ratio of test item/negative control) was calculated for each concentration and for the positive control groups.
3.3.2	Exposure	Topical
3.3.3	Concentrations	a) Test substance concentrations: 10, 5, 1, and 0.1 % (w/v) IPBC in acetone/ olive oil (AOO) [3 + 1 (v/v)]; b) Positive controls: Methyldibromo glutaronitrile (MDBGN) 10% in AOO; 5-chloro-2-methy-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CMI/MI) 0.1% in AOO; c) Negative control: AOO

Section A6.1.5/04	**Skin sensitisation**	
Annex Point IIA, VI.6.1.5	Local Lymph Node Assay	
1.1.1 Statistics	For each concentration of test substance and the positive controls, a stimulation index (SI) relative to the concurrent AOO-treated control was calculated. The EC3 value, or the estimated concentration of test substance required to elicit an SI of 3 or more, was derived from the dose-response data by linear interpolation.	
	4 RESULTS AND DISCUSSION	
4.1 Results	The stimulation index (SI) for 0.1% IPBC was 0.7 ± 0,3, for 1% IPBC 3.4 ± 1.4, for 5% IPBC 4.2 ± 1.8, and for 10% IPBC 12.0 ± 3.4. The EC3 value was at a concentration of 0.87% IPBC. On daily clinical observation the animals did not show any visible clinical symptoms.	
4.2 Discussion	The SI for the positive controls (MDBGN, CMI/MI) were in the range of the known data. The SIs were confirmed by the second endpoint, the weight of the single lymph nodes. There was an increase in weights for all doses which showed sensitising properties at the determination of radioactivity. However, no dose response was observed. The weight of the lymph nodes for 0.1% IPBC was 2.8 ± 0.4 mg, which was similar to the weight of the lymph nodes for the negative control 2.6 ± 0.3 mg. The weight of the lymph nodes for 1% IPBC, 5% IPBC, and 10% IPBC were equivalent and ranged between 5.3 ± 0.3 and 5.8 ± 0.8 mg. Major study details were not included in the brief publication. There is no characterisation of the test substance and its impurities. Furthermore, the test animals are not well described, e.g., information on strain, gender, age, number of animals per group is missing.	*
4.3 Overall results	IPBC has to be classified as a skin sensitiser with an EC3 value of 0.87%.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The study was performed according to OECD Guideline for Testing of Chemicals; No. 429: “Skin Sensitisation: Local Lymph Node Assay”.	
5.3 Results and discussion	The EC3 value was at a concentration of 0.87% IPBC. On daily clinical observation the animals did not show any visible clinical symptoms.	
5.4 Conclusion	IPBC is a skin sensitizer.	
5.4.2 Reliability	3	
5.4.3 Deficiencies	Brief publication: no details on test item specification, impurities, animal strain, number of animals per group, gender etc. Furthermore, individual animal data are not reported. Results of the pre-screen were not reported if it was performed.	

Evaluation by Competent Authorities

Section A6.1.5/04	**Skin sensitisation**
Annex Point IIA, VI.6.1.5	Local Lymph Node Assay
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09 November 2022
Materials and Methods	Applicant's version is acceptable. Minor remark at renewal: Ad 3.1.5.1: The two positive control substances are not among the 'Preferred PC test substances' identified in Point 9 of the version of OECD TG 429 applicable at the time, or in Point 11 of the current OECD TG 429. 5-chloro-2-methy-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CMI/MI) is listed in Table 1 `Recommended reference substances for the LLNA´, though the vehicle used was 0.1% AOO as opposed to the recommended vehicle DMF.
Results and discussion	Applicant's version is adopted. Minor remark at renewal: Ad 4.2: The SI for the positive controls (MDBGN, CMI/MI) were stated to be in the range of known data, though no such values (or historical data from the test laboratory) were provided.
Conclusion	Applicant's version is adopted.
Reliability	3
Acceptability	Unacceptable
Remarks	Major remark at renewal: The brief publication provides insufficient information regarding performance of the study. Minor remark at renewal: Ad 2.2: No information was presented to support the GLP status of the study. The study is assumed to have been performed in 2004 (the year of publication of the study report) according to OECD TG 429 version of April 2002. The version (July 2010) of the guideline applicable at the time of renewal of IPBC has a number of revisions to the test procedure (including the requirement to perform a pre-screen test in the absence of information to determine the highest dose to be tested), observations made, calculation of results (including statistical analysis for presence and degree of dose-response relationship in the data), and to data and reporting. The changes to the guideline have no implication for the (un)reliability of the study as reported.

Section A6.1.5/04 **Skin sensitisation**

Annex Point IIA, VI.6.1.5 Local Lymph Node Assay

Table A6.1.5/04-1: Stimulation index (SI) and weight of lymph nodes (mean ± SD) of different concentrations of IPBC and MDBGN and CMI/MI as positive controls in the local lymph node assay (LLNA)

Test item	Concentration (%)	SI	Weight of lymph nodes (mg)
IPBC	0.1	0.7 ± 0,3	2.8 ± 0.4
IPBC	1	3.4 ± 1.4	5.5 ± 0.6
IPBC	5	4.2 ± 1.8	5.3 ± 0.3
IPBC	10	12.0 ± 3.4	5.8 ± 0.8
MDBGN (positive control)	10	6.1 ±1.3	4.5 ± 0.5
CMI/MI (positive control)	0.1	8.7 ±1.3	4.3 ± 0.5
Negative control		1.0	2.6 ± 0.3

Siebert (2004)_Sensitizing p

3.7.2 Human data

3.7.2.1 No new relevant data

(Two articles identified in the open literature reporting the results longitudinal studies of the prevalence of allergy to IPBC (as determined by skin patch testing) are mentioned in Section 3.5 Skin sensitisation of the CLH report. Their findings are considered consistent with the information for skin sensitisation in the original CLH report, thus they are not considered relevant new data. A copy of the studies is provided below.)

Martin-Gorgojo & Johansen (2013)_IPB

Gimenez-Arnau et al. (2017)_Contact al

3.7.3 Other data

3.7.3.1 No new relevant data

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Gene mutation in bacteria – No new relevant data

3.8.1.2 Cytogenicity in Mammalian Cells (Chromosome Aberration in V79 cells) – No new relevant data

3.8.1.3 Gene Mutation in Mammalian Cells (HPRT, V79) – No new relevant data

3.8.2 Animal data

3.8.2.1 Mammalian Erythrocyte Micronucleus Test, Mice – No new relevant data

3.8.2.2 Genotoxicity *in vivo* (e.g. DNA damage) – No new relevant data

3.8.3 Human data

3.8.3.1 No new relevant data

3.8.4 Other data

3.8.4.1 No new relevant data

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 No new relevant data

3.9.2 Human data

3.9.2.1 No new relevant data

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

3.9.3.1 No new relevant data

3.9.4 Other data (e.g. studies on mechanism of action)

3.9.4.1 No new relevant data

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Sexual function and fertility: Two-generation reproduction toxicity study – No new relevant data

3.10.1.2 Sexual function and fertility: Two-generation reproduction toxicity study – No new relevant data

3.10.1.3 Developmental toxicity: Teratogenicity study (Pre-natal developmental toxicity study) – No new relevant data

3.10.1.4 Developmental toxicity: Teratogenicity study (Pre-natal developmental toxicity study) – No new relevant data

3.10.2 Human data

3.10.2.1 No new relevant data

3.10.3 Other data (e.g. studies on mechanism of action)

3.10.3.1 No new relevant data

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

3.11.1.1 No new relevant data

3.11.2 Human data

3.11.2.1 No new relevant data

3.11.3 Other data

3.11.3.1 No new relevant data

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 No new relevant data

3.12.2 Human data

3.12.2.1 No new relevant data

3.12.3 Other data

3.12.3.1 No new relevant data

3.13 Aspiration hazard

3.13.1 Animal data

3.13.1.1 No new relevant data

3.13.2 Human data

3.13.2.1 No new relevant data

3.13.3 Other data

3.13.3.1 No new relevant data

3.14 Neurotoxicity

3.14.1 Animal data

3.14.1.1 No new relevant data

3.14.2 Human data

3.14.2.1 No new relevant data

3.14.3 Other data

3.14.3.1 QSAR analysis of the neurotoxicity of IPBC

Expert rule-based (Quantitative) Structure Activity Relationship ((Q)SAR) analysis using Derek Nexus 6.1.0 (Derek KB 2020 1.0) software of the neurotoxicity of IPBC ([redacted], 2021; Doc. No. 581-013).

Methods

Derek Nexus

Knowledge base version:	Derek KB 2020 1.0
Species:	*Escherichia coli* and *Salmonella typhimurium* (bacteria); dog, guinea pig, hamster and human (mammal); monkey (primate) and mouse and rat (rodent) and rabbit
Endpoints searched:	Carcinogenicity, Genotoxicity (including mutagenicity and chromosome damage), Irritation, Miscellaneous Endpoints, Neurotoxicity, Organ Toxicity, Reproductive Toxicity, Respiratory Sensitisation, Skin Sensitisation
Processing constraints:	The option to perceive tautomers was selected.

Derek Nexus is a rule based expert system, which assigns various levels of non-numeric likelihood to its predictions ranging from CERTAIN to CONTRADICTED, via PROBABLE, PLAUSIBLE, EQUIVOCAL, DOUBTED, IMPROBABLE, IMPOSSIBLE and OPEN.
In the Derek lexicon, PLAUSIBLE indicates that the structure has activated an alert for the endpoint in question. There is sufficient information in the database to support the prediction. EQUIVOCAL – indicates that there is an equal weight of evidence for and against the proposition. OPEN – means that there is no evidence that supports or opposes the proposition. In addition to the above, Derek Nexus contains expert-derived functionality to provide negative predictions for bacterial *in vitro* mutagenicity. The query compound is compared to a Lhasa reference set of Ames test data and the software determine whether there are structures that have been misclassified by Derek Nexus or is unknown. In addition, Derek Nexus contains expert-derived functionality to provide negative predictions for Skin Sensitisation. A negative prediction model is stored within the Lhasa Certified Knowledge Base and supplied with Derek Nexus. The model contains the Lhasa skin sensitisation negative prediction dataset which is used to generate a fragment library.

Summary of results

A summary of the results from the *in silico* analysis is given below. Where applicable the details of these results can be found in appendix 1.

Structure	Derek Analysis
Analysed 11 May 2021; Derek Nexus 6.1.0 (Derek KB 2020 1.0)	
3-iodo-2-propynyl butylcarbamate (IPBC, CAS no. 55406-53-6)	• No alerts triggered for carcinogenicity, chromosome damage, genotoxicity, mutagenicity, neurotoxicity or developmental neurotoxicity. • Contains unclassified features, however deemed INACTIVE for bacterial mutagenicity. • No alerts triggered for skin sensitisation however contains misclassified skin sensitisation feature. Positive comparator structure is exact match for test structure – the negative result is considered incorrect. • 692 Carbamate. Hepatotoxicity. • No further human-relevant alerts triggered.

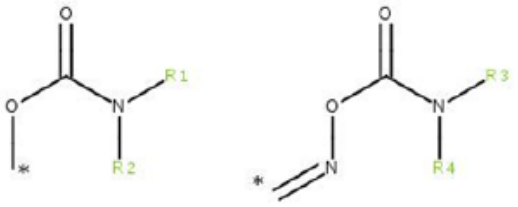
Comment and discussion

3-iodo-2-propynyl butylcarbamate (IPBC, CAS no. 55406-53-6) was analysed over a range of endpoints using Derek Nexus in a number of bacterial and mammalian species.

Derek Nexus analysis

IPBC triggered no alerts for carcinogenicity, chromosome damage, genotoxicity, mutagenicity, neurotoxicity or developmental neurotoxicity (a list of alerts related to neurotoxicity and developmental neurotoxicity may be found in appendix 2).

Alert 009 (*N*-methyl or *N,N*-dimethyl carbamate) was not triggered as the structure did not match the alert patternation (as shown below) examples include propoxur, carbofuran, aldicarb and oxamyl.



R1, R3 = CH3
R2, R4 = H, CH3

The combined number of atoms in all substituents attached to C* (excluding H) must be greater than 2

The lack of alert triggering is in line with that observed experimentally in a single dose study at up to 16 mg/kg-bw dosed via the lateral tail vein to rats where no change in erythrocyte cholinesterase was observed. Decreased plasma cholinesterase activity was observed in female rats fed 250 mg/kg-bw/day IPBC over a 4-week period but not at lower dose levels (Lanigan, 1998).

IPBC was found to contain an unclassified feature in Derek Nexus but was deemed INACTIVE for bacterial mutagenicity. In Derek Nexus, an unclassified feature is one that has not been found to occur in the Lhasa Ames database. Williams *et al*., examined the relevance of such features and found that substances predicted to be "inactive with unclassified" features were correctly predicted to be non-mutagenic 90.3% of cases. This conclusion is in line with the known negative mutagenicity and genotoxicity results for IPBC (Lanigan, 1998).

IPBC triggered no alerts for skin sensitisation however contains a misclassified skin sensitisation feature. Positive comparator structure is exact match for test structure – the negative result is considered incorrect.

Due to a carbamate moiety an alert for hepatoxicity was triggered by IPBC, this alert is in line with the expected toxicological profile of IPBC (Lanigan, 1998).

No further human-relevant alerts were triggered (further details may be found in appendix 1).

Conclusion

3-iodo-2-propynyl butylcarbamate (IPBC, CAS no. 55406-53-6) was analysed over a range of endpoints using Derek Nexus in a number of bacterial and mammalian species.

IPBC triggered no alerts for carcinogenicity, chromosome damage, genotoxicity, mutagenicity, neurotoxicity or developmental neurotoxicity (a list of alerts related to neurotoxicity and developmental neurotoxicity may be found in appendix 2).

IPBC was found to contain an unclassified feature in Derek Nexus but was deemed INACTIVE for bacterial mutagenicity. In Derek Nexus, an unclassified feature is one that has not been found to occur in the Lhasa Ames database. Williams *et al*., examined the relevance of such features and found that substances predicted to be "inactive with unclassified" features were correctly predicted to be non-mutagenic 90.3% of cases. This conclusion is in line with the known negative mutagenicity and genotoxicity results for IPBC (Lanigan, 1998).

IPBC triggered no alerts for skin sensitisation however contains a misclassified skin sensitisation feature. Positive comparator structure is exact match for test structure – the negative result is considered incorrect.

Due to a carbamate moiety an alert for hepatoxicity was triggered by IPBC, this alert is in line with the expected toxicological profile of IPBC (Lanigan, 1998).

No further human-relevant alerts were triggered (further details may be found in appendix 1).

3.14.3.2 Expert Evaluation of the neurotoxicity of IPBC

Expert Evaluation of the neurotoxicity of IPBC ([redacted], 2021; Doc. No. 581-012).

The studies identified in section 2.1 'Summary of repeated dose toxicity studies' and section 2.2 'Mechanistic investigations on acetyl cholinesterase inhibition' were included in the initial approval of IPBC in PT8. The QSAR analysis identified in section 2.3. 'QSAR results' is that presented in Section 3.14.3.1 above.

1) Introduction

The neurotoxicity potential of IPBC – a carbamate was investigated in details by means of results from repeated dose toxicity studies. In the studies, clinical signs as well as the acetyl cholinesterase activity in plamsa, RBC, and brain were investigated as parameters for neurotoxicity. In particular dedicated acute and subchronic neurotoxicity studies were taken into account. In addition, mechanistic *in vivo* und *in vitro* studies were considered which were conducted to investigate the ability of IPBC to inhibit the acetyl cholinesterase enzyme. Furthermore, QSAR considerations are presented to support the absence of relevant acetyl cholinesterase inhibition.

2) Results

2.1) Summary of repeated dose toxicity studies

In the key acute neurotoxicity study, a range-finding study in which Sprague-Dawley rats were gavaged with a single dose of IPBC of up to 1000 mg/kg bw and followed for 14 days post-dosing ([redacted] 2001a). Clinical signs (hunched posture, hypoactivity, laboured respiration) were only noted at 1000 mg/kg bw though had ceased by day 4. Low locomotor activity was observed at 1000 mg/kg bw on day 1 only. No changes to brain or RBC cholinesterase activity were observed. Plasma cholinesterase activity was decreased in females at 300 and 1000 mg/kg bw at day 3 and 15. Based on these findings, the LOAEL for acute systemic toxicity is considered to be 300 mg/kg bw yielding a NOAEL (Systemic toxicity) of 100 mg/kg bw, and the NOAEL for acute neurotoxicity to be 1000 mg/kg bw (the highest dose tested).

In the key sub-chronic neurotoxicity study in which Sprague-Dawley rats were fed IPBC at doses up to 120 mg/kg bw/day for 13 weeks, with satellite groups followed for 28 days post-dosing ([redacted] 2001b), no clinical signs (other than relating to body weight) were observed. No treatment-related effects on FOB and MA assessments, or on brain or RBC cholinesterase activity, were observed. Plasma cholinesterase activity was reduced (reversibly) in females at 50 and 120 mg/kg bw/day. No treatment-related histopathological findings for nervous system tissues were observed. Based on these findings, the LOAEL for sub-chronic systemic toxicity is considered to be 50 mg/kg bw/day, yielding a NOAEL (Systemic toxicity) of 10 mg/kg bw/day, and the NOAEL for sub-chronic neurotoxicity to be 120 mg/kg bw/day (the highest dose tested).

In the 104-week chronic toxicity/carcinogenicity rat study ([redacted] 1989a) and the 78-week mouse carcinogenicity study ([redacted] 1989b) Long-term oral toxicity, brain cholinesterase was unaffected by chronic oral IPBC exposure at doses up to 80 or 150 mg/kg bw/day in the rat and mouse respectively. No clinical signs suggestive of neurotoxicity were reported.

In the rat and rabbit studies on Sexual function and fertility ([redacted] 1996, [redacted] 1987) and on Developmental toxicity ([redacted] 1994a and 1994b), clinical signs suggestive of neurotoxicity were not reported, with the exception of post-dose salivation, aggressive behaviour (1 study), and occasional hunched posture or forepaw padding (1 study) in adult rats administered IPBC by gavage. Short-term oral toxicity, post-dosing salivation following gavage is likely to be a result of the irritating properties of IPBC, and potentially also the gavage process itself, rather than increased cholinergic activity; a position supported by the lack of reports of salivation in rats, mice and rabbits fed comparable or higher doses of IPBC. No signs of developmental neurotoxicity were reported in the key two-generation reproduction toxicity study (in which rats were administered oral doses of up to 30 mg/kg bw/day; up to 100 mg/kg bw/day to F0 parents)([redacted] 1996) or in the pre-natal developmental toxicity studies (in which rabbit and rats were administered oral doses of up to 40 or 250 mg/kg bw/day, respectively)([redacted] 1987).

Overall, the neurotoxicity studies in rodents are considered to provide sufficient data to evaluate the potential acute and delayed neurotoxicity (neurophysiological, neurobehavioral and neuropathological effects) and developmental neuropathy of IPBC. No clear signs of neurotoxicity or neuropathology are considered evident. The animal data are supported by the limited human data.

2.2) Mechanistic investigations on acetyl cholinesterase inhibition

Absence of relevant acetyl cholinesterase inhibition is supported by two studies performed with IPBC in addition to the information given above:
IPBC was tested *in vitro* for acetylcholinesterase inhibition in rat and human blood samples ([redacted] 1988). Briefly, plasma and RBC cholinesterase activity after addition of IPBC (called Polyphase at 50 µL of a 1 mg/mL Polyphase or 2 mg/mL solution added to 5 mL), the vehicle (polyethylene glycol, 50 µL/5 mL), or the positive control physostigmine salicylate (5×10^{-7} M) were measured *in vitro* using rat and human plasma and RBC. After 0, 15, 30, and 60 minutes, samples were removed and the subsamples assayed for acetyl cholinesterase activity. In none of the IPBC incubations, a reduction in both plasma and RBC acetyl cholinesterase activity were observed. The positive control produced the expected marked reduction in term of cholinesterase activity. Thus, IPBC was not able to inhibit acetyl cholinesterase *in vitro*.
In a key acute *i.v.* toxicity study ([redacted] 1988), SD rats (10animals/sex/dose) were treated with 0, 2, 4, 10 or 16 mg IPBC/kg bw using PEG 400/water; 70/30, v/v) as vehicle intravenously via the lateral tail vein. Blood samples were taken 15, 30, 60, 120 minutes and 5 hours after dosing for measurement of RBC acetyl cholinesterase activity. All animals dosed at 16 mg/kg bw showed convulsions and/or marked hyperkinesia. All the females and occasionally males dosed at 10 or 16 mg/kg bw showed red stained urine approximately 15 minutes after dosing. No clinical signs were noted for the other dose groups. One male died at 10 mg/kg bw; 3 males and 2 females at 16 mg/kg bw. The LD_{50} (*i.v.*) was > 16 mg/kg bw. Even when dosing up to lethal doses, RBC acetyl cholinesterase activity was normal and not different from the control values. Thus, IPBC did not inhibit RBC acetyl cholinesterase activity *in vivo*.

2.3) QSAR results

In a ((Quantitative) Structure Activity Relationship) (Q)SAR analysis using Derek Nexus 6.1.0 (Derek KB 2020 1.0) no alerts were triggered for the endpoints related to neurotoxicity. Within the software tool 8 alerts for neurotoxicity are incorporated (gamma-diketone or precursor; acrylamide or glycidamide; nitroimidazole; carbon disulphide or precursor; pyrethroid; 1-methyl-1,2,3,6-tetrahydropyridine; lead or lead compound; organophosphorus ester). The software further includes two alerts for cholinesterase inhibition (organophosphorus ester and *N*-methyl or *N,N*-dimethyl carbamate). Neither of them fired. Overall, this alter description indicates that *N*-methyl or *N,N*-dimethyl carbamates would be considered cholinesterase inhibitors but not a butyl-carbamate like IPBC.

The review from Fukuto (1990) is very useful in understanding the effects caused by acetyl cholinesterase inhibitors being carbamates. Methylcarbamates, *i.e.* carbamates carrying an H-atom and a methyl-group are usually very potent inhibitors of acetylcholinesterase. Substitution of the H atom with a methyl-group results in a reduced anticholinesterase activity along with a substantial improvement in acute mammalian toxicity.
Early work of Metcalf (1971) investigated different carbamate structures with regard to their anti-cholinesterase activity. Larger N-substituents such as *N*-propyl-, *N*-butyl- and *N*-hexyl reduce the acetyl cholinesterase inhibition to non-appreciable activity. Annex Table 12 of the paper generally confirms that the dimethyl-carbamates have lower anti-cholinesterase activity and lower toxicity as compared to their monomethyl-carbamates analogues. Likewise, Annex Table 13 shows that longer chain *N*-substituents generally reduce toxicity as compared to the non-*N*-substituted carbamate. Dimethylcarbamates act differently compared to the methylcarbamate at the enzyme. While the dimethylcarbamates are suggested to react via a nucleophilic attack on the C=O carbon with direct displacement of the other substituent, methylcarbamates react through a nucleophilic attack on the *N*-proton.
Jiang (2011) also reviewed structure–activity relationships of carbamate insecticides on acetylcholinesterase. Steric property is very important for carbamate compounds to access the catalytic site of acetyl cholinesterase. *N*-alkyl substituents are responsible for the reaction rate of carbamoylation. *N,N*-dimethyl carbamate is less reactive than *N*-monomethyl carbamate because the bulky substituent of the alkyl group on nitrogen blocks the access to catalytic site in the acetyl cholinesterases pocket (Thompson and Richardon 2004 cited in Jiang, 2011). This will cause a decreased rate of binding and reaction.
Sultatos (2006) discussed the role of organophosphate (OP) and carbamate structure in the inhibition of acetyl cholinesterase. Extensive structure–activity relationships with carbamate insecticides have revealed general similarities with those of the organophosphates. Specifically, the anticholinesterase activity of a carbamate is primarily a function of the reactivity of the carbonyl carbon (rather than the phosphorus of OPs) and the ability of the carbamate to diffuse into the active site gorge and form Michaelis complexes (steric properties). The steric properties of a carbamate modulate the access of the carbamate to the catalytic serine. Bulky substituents reduce access. Additions of larger, bulkier substituents reduced anticholinesterase activity.

3) Conclusion

To conclude, IPBC showed no indications for neurotoxicity potential and no signs of neurotoxicity in the reprotoxicity studies. Thus is also unlikely to cause developmental neurotoxicity. In particular, IPBC does neither inhibit acetyl cholinesterase *in vitro* nor *in vivo* using *i.v.* application. IPBC is a butyl-carbamate and already early research showed that large *N*-substituents do not inhibit the acetyl cholinesterase enzyme mainly because of steric hindrance at the catalytic site. Thus, further investigations are scientifically not justified.

3.15 Immunotoxicity

3.15.1 Animal data

3.15.1.1 Assessment of animal data

The assessment evaluated information related to immune parameters in studies peer-reviewed during the initial approval of IPBC in PT8. Summaries of the individual studies have not been provided here.

1) Introduction

To assess the endpoint 'Immunotoxicity', the Guidance on BPR: Volume III Parts B+C Version 4.0 December 2017 (Guidance on BPR) was consulted and used as the actual guidance document.

As recommended in the Guidance on BPR the available IPBC data from repeated dose, carcinogenicity and reproductive toxicity key-studies were evaluated with the aim to assess the immunotoxicity potential of the active substance. The studies which were taken in this assessment are listed in the Table 1.

According to the Guidance on BPR additional investigations (e.g. T-cell function test, host resistant models) are considered as Tier 2 assessment and should be conducted in case the screening (morphological changes or changes in haematology and of clinical chemistry parameters of Tier 1) shows concern.

Study reports were screened in in detail for the immunotoxicity relevant parameters which are listed in the Guidance on the BPR: Volume III Parts B+C Version 4.0 December 2017, p113/427 and repeated in the following:

- morphological changes of lymphoid organs and tissues including bone marrow (*e.g.* changes in thymus, spleen, lymph nodes, and/or bone marrow);
- weight changes of lymphoid organs;
- changes in haematology parameters (e.g. white blood cell number, differential cell counts of lymphocytic, monocytic and granulocytic cells);
- changes in clinical chemistry parameters (*e.g.* serum protein levels, immunoglobulin concentrations if determined).

Results

No alteration in the immune system organs (thymus, spleen, lymph nodes and bone marrow) in terms of gross and histopathology were observed (in rat, mouse and rabbit) under the treatment with IPBC. Furthermore, the treatment with IPBC had no effects on the weights of the lymphoid organs.

Additionally, the total and differential white blood cell counts (lymphocytic, monocytic and granulocytic cells) were not affected by the treatment in 9 to 11 studies. The parameter was not investigated in the remaining 2 studies.

The clinical chemistry parameters were reported in 9 of 11 studies. No toxicological relevant changes in the serum proteins (globulins) levels were observed in the studies. Again, the parameter was not investigated in the remaining 2 studies.

2) Conclusion

In conclusion, no indications for disturbance of proper functioning of the immune system through administration of IPBC were found in the available repeated-dose tests. Therefore, it can be reasonably concluded that IPBC does not raise concern for immunotoxicity. Therefore, in line with the guidance on BPR, further testing is not required.

3.15.2 Human data

3.15.2.1 No new relevant data

3.15.3 Other data

3.15.3.1 No new relevant data

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Section A7.1.1.2.1/01 Biodegradability (ready)

Annex Point IIA, VII.7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002): Ready biodegradability of IPBC in a Manometric Respirometry Test; [redacted]; Doc. No. 713-002; unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Corporation	
1.2.2	Companies with letter of access	Arch Chemicals, Bayer Chemicals and Sostram Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I for all references listed above.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 301 F	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	
3.1	**Test material**	Technical active substance IPBC	
3.1.1	Lot/Batch number	0004-1545	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99 %	
3.1.4	Description of test substance	solid	
3.1.5	Further relevant properties	Solubility in water: 156 mg/L, Vapour Pressure: < 1.8 x 10^{-6} Torr at 20°C	
3.1.6	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product	
3.1.7	TS inhibitory to microorganisms	Yes, the EC_{20} in a respiration inhibition test was determined to be 57 mg/L.	
3.1.8	Specific chemical analysis	Test principle: The biodegradation process consumed the dissolved oxygen in the liquid and generated CO_2. The CO_2 was adsorbed by soda lime and the total pressure decrease in the airtight test flasks was recorded.	
3.2 Reference substance		Yes Sodium benzoate	
3.2.1 Initial concentration of reference substance		50 mg/L, due to the inhibitory effect of IPBC on the activity of activated sludge microorganisms.	

Section A7.1.1.2.1/01 Biodegradability (ready)

Annex Point IIA, VII.7.6.1.1

3.3	**Testing procedure**	
3.3.1	Inoculum / test species	Details on inoculum are summarised in table A7.1.1.2/01-2.
3.3.2	Test system	Details on test system, laboratory equipment etc. are given in table A7.1.1.2/01-3.
3.3.3	Test conditions	Details on the relevant test conditions are given in table A7.1.1.2/01-4.
3.3.4	Method of preparation of test solution	The amounts of test item were directly weighed into the test flasks.
3.3.5	Initial TS concentration	50 mg/L or 52 / 63 mg O_2/L based on ThOD without / with nitrification.
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	CO_2 evolution
3.3.8	Sampling	Continuous detection of the pressure decrease in the test flasks
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	Yes
3.3.11	Controls	Inoculum Control: inoculum, but no test substance Abiotic control: test substance, but no inoculum Procedure control: inoculum with reference substance Toxicity control: inoculum with test substance and with reference substance
3.3.12	Statistics	According to the relevant guideline
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Details not provided
4.1.2	Degradation	-24 to –26 % after 28 days based on $ThOD_{NH4}$ -20 to –22 % after 28 days based on $ThOD_{NO3}$ The biochemical oxygen demand (BOD) of the test item IPBC in the test media was lower than in the inoculum controls. Consequently, IPBC was found to be not biodegradable under the test conditions within 28 days.
4.1.3	Other observations	No
4.1.4	Degradation of TS in abiotic control	No abiotic degradation of the test item occurred under the test conditions
4.1.5	Degradation of reference substance	The reference item was degraded by an average of 97 % at day 14 confirming the suitability of the activated sludge.

Section A7.1.1.2.1/01 Biodegradability (ready)

Annex Point IIA, VII.7.6.1.1

4.1.6	Intermediates/ degradation products	Not investigated.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	A manometric respirometry test was conducted according to OECD Guideline 301 F over a period of 28 days.
5.2	**Results and discussion**	Due to the inhibitory effect of IPBC on the activity of the activated sludge (EC_{20} = 57 mg/L), a test concentration of 50 mg/L was used. The biochemical oxygen demand (BOD) of the test item IPBC in the test media was lower than in the inoculum controls. Consequently, IPBC was found to be not biodegradable under the test conditions within 28 days.
5.3	**Conclusion**	According to the guideline all validity criteria were fulfilled. IPBC was not ready biodegradable under the test conditions within 28 days.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004, May 2022
Materials and Methods	The applicant's version is adopted
Results and discussion	The applicant's version is adopted
Conclusion	IPBC was not ready biodegradable under the test conditions within 28 days.
Reliability	Reliability indicator 1
Acceptability	Acceptable
Remarks	The study is conducted in accordance to OECD 301F. This guideline is from 1992, hence the study and its conclusion is still considered acceptable.

Table A7.1.1.2/01-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready

MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7.1.1.2/01-2: Inoculum / Test organism

Criteria	**Details**
Nature	Aerobic activated sludge
Species	Not specified
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	[redacted], Switzerland
Laboratory culture	Not applicable
Method of cultivation	Not applicable
Preparation of inoculum for exposure	After sampling the sludge was washed once with tap water.
Pre-treatment	The sludge was aerated at room temperature until use. Dilution with test water
Initial cell concentration	30 mg dry material per litre

Table A7.1.1.2/01-3: Test system

Criteria	**Details**
Culturing apparatus	500 mL Erlenmayer flasks.
Number of culture flasks/concentration	2 with inoculum and test item at 50 mg/L 2 with inoculum only (inoculum control) 2 with inoculum and reference item at 100 mg/L (procedure control) 1 with test item at 51 mg/L, without inoculum (abiotic control) 1 with inoculum, with test item at 51 mg/L and with reference item at 100 mg/L (toxicity control)
Aeration device	Consumed O_2 was replaced

Measuring equipment	The formed CO_2 was adsorbed by soda lime. The pressure decrease was detected by an electrode type manometer
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2/01-4: Test conditions

Criteria	**Details**
Composition of medium [g/L]	KH_2PO_4 8.50 K_2HPO_4 21.75 $NaHPO_4.2H_20$ 33.40 NH_4Cl 0.50 $CaCl_2.2H_2O$ 36.40 $MgSO_4.7H_20$ 22.50 K_2HPO_4 0.25
Additional substrate	Yes, drop of concentrated HCl
Test temperature	22°C
PH	Start of the test: 7.4 End of the test: 7.4 – 7.8
Aeration of dilution water	Not indicated.
Suspended solids concentration	Not indicated.
Other relevant criteria	Not indicated.

Table A7.1.1.2/01-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	**not fulfilled**
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	-	X
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	X	-
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	X	-
Percentage of removal of reference substance reaches pass level by day 14	-	97 %

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2004): Inherent biodegradability of IPBC in a modified "Zahn-Wellens / EMPA Test"; [redacted]; Doc. No. 713-007; unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	European Union IPBC Task Force (Arch Chemicals, Bayer Chemicals. Sostram Corporation and Troy Corporation).	
1.2.2	Companies with letter of access	No companies with letter of access	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 302 B	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes: instead of DOC (COD) measurement, biodegradation was monitored with specific analysis of IPBC and of its degradation product PBC in the aqueous phase and in the activated sludge	
		3 MATERIAL AND METHODS	
3.1.1	**Test material**	Technical active substance IPBC	
3.1.1.1	Lot/Batch number	0306-8715A	
3.1.1.2	Specification	As given in section 2	
3.1.1.3	Purity	99.2 %	
3.1.1.4	Description of test substance	White powder	
3.1.1.5	Further relevant properties	Solubility in water: very slightly soluble in cold and hot water	
3.1.1.6	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product	
3.1.1.7	TS inhibitory to micro-organisms	No, confirmed by DOC analysis of the toxicity control	
3.1.1.8	Specific chemical analysis	The biodegradation process was monitored by specific analysis (HPLC-MS/MS) of the test item IPBC in the aqueous phase and in the activated sludge.	
3.1.2	**Test material used as analytical reference item**	PBC (2-propynyl-butylcarbamate)	

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

3.1.2.1	Lot/Batch number	20030728002
3.1.2.2	Specification	Not relevant
3.1.2.3	Purity	99.3 %
3.1.2.4	Description of test substance	Amber liquid
3.1.2.5	Further relevant properties	Solubility in water: 4.5 g/L at 20°C, Density: 1.004 g/mL at 20°C
3.1.2.6	Composition of Product	Not relevant because a metabolite of the active substance was tested, not, however, a formulated product
3.1.2.7	TS inhibitory to micro-organisms	No, confirmed by DOC analysis of toxicity control
3.1.2.8	Specific chemical analysis	The biodegradation process was monitored by specific analysis (HPLC-MS/MS) of the test item PBC in the aqueous phase and in the activated sludge.
3.2	**Reference substance**	Yes, Sodium benzoate
3.2.1	Initial concentration of reference substance	170 mg/L
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	Details on inoculum are summarised in table A7.1.1.2/01-1.
3.3.2	Test system	Details on test system, laboratory equipment etc. are given in table A7.1.1.2/01-2.
3.3.3	Test conditions	Details on the relevant test conditions are given in table A7.1.1.2/01-3.
3.3.4	Method of preparation of test solution	0.5 mL (low dose), and 25 mL (high dose) of a 0.1 mg/mL (50.2 mg/500mL) solution of IPBC in test water were added to the corresponding test flasks (total volume 2500 mL).
3.3.5	Initial TS concentration	High dose: 1.0 mg/L Low dose: 20µg/L (0.02 mg/L)
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	Specific analysis of IPBC and PBC in the aqueous phase and the activated sludge , with HPLC-MS/MS

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

3.3.8	Sampling	Low dose: at 0, 2, 4, 8, 24, 48 hours and on day 3, only the aqueous phase was analysed for IPBC and PBC. High dose: sampling at 0, 2, 4, 8, 24, 48 hours and on day 3, 7, 14, 21, 28. Samples taken at 0, 2, 4,and 8: the aqueous phase and the sludge was analysed. Samples taken at 24 hours and later: only the aqueous phase was analysed.
3.3.9	Intermediates/ degradation products	The degradation product PBC was monitored by specific analysed.
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Inoculum Control: inoculum, but no test substance Procedure control: inoculum with reference substance Toxicity control: inoculum with test substance and with reference substance
3.3.12	Statistics	Not relevant, because the results allowed for a conclusive interpretation.
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Not relevant, because degradation was extremely fast (see below)

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

4.1.2	Degradation	High dose: (1mg/L) aqueous phase: IPBC: After 2 hours, IPBC concentrations of 0.01 (sample 1) and 0.02 mg/L (sample 2) were determined, corresponding to a degradation of 99%. After 4 hours: no IPBC was detected (LOQ 0.01 mg/L). **Conclusion:** IPBC was almost completely degraded to PBC within 2 hours PBC: After 2 and 4 hours, a maximum PBC concentration of ca. 0.5 mg/L was determined. This corresponds to 87 % of the theoretically expected amount, indicating that IPBC is completely transformed into PBC. At later time points, a continuous degradation of PBC was observed. On day 21, the PBC concentration was below the LOQ (0.01 mg/L). Sludge: IPBC: No IPBC was detected in the sludge (LOQ 0.01 mg/kg). PBC: Sludge samples were analysed after 2, 4 and 8 hours. The maximum PBC concentration was 1 mg/kg dry weight, corresponding to max 0.5 % of the theoretical expected PBC amount. **Conclusion:** PBC which is formed from the IPBC is not absorbed to the sludge, it is almost completely dissolved in the aqueous phase. Low dose: IPBC: 80% degradation of IPBC to PBC was observed already in the 0-hour aqueous phase samples. In the 2-hours samples no IPBC was detected (LOQ 0.01mg/L) No IPBC was detected in the sludge (LOQ 0.01 mg/kg). PBC PBC could not be determined because of interferences at this low concentration level.
4.1.3	Other observations	No
4.1.4	Degradation of procedure control	DOC analyses: the reference item sodium benzoate in the procedure control was completely degraded within the first three days of exposure, thus confirming the suitability of the activated sludge.
4.1.5	Degradation of toxicity control	DOC analyses: The DOC content rapidly decreased by 100% within the first three days of exposure. Degradation was clearly above the trigger value of 35% within 14 days of incubation. Thus, it can be assumed that the test item IPBC is not inhibitory to activated sludge.
4.1.6	Intermediates/ degradation products	Intermediates were not investigated. The degradation product PBC was monitored by specific analysis.

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	A modified Zahn Wellens / EMPS test was conducted according to OECD Guideline 302 B. The biodegradation process was monitored by specific analysis of the test item IPBC and the degradation product PBC in the aqueous phase and in the activated sludge
5.2	**Results and discussion**	Low dose (0.02 mg/L IPBC): IPBC was rapidly degraded in the aqueous phase to PBC. Concerning the sludge no statement could be made to the fate of PBC at the low IPBC concentrations because of analytical interference due to contaminants in the sludge. High dose (1.0 mg/L IPBC): In the aqueous phase, the IPBC concentrations decreased to 1.5% and 0.9% of the nominal values within two hours of incubation. In these samples, 85% and 89% of the maximum theoretical amount of the degradation product PBC were detected. The concentration of PBC in the water phase continuously decreased during the test period and was below 0.01 mg/L (LOQ) after 21 days of incubation. No significant amounts of IPBC and IPBC were found in the activated sludge samples.
5.3	**Conclusion**	Based on the measured concentrations of IPBC and PBC, rapid biodegradation of IPBC to PBC and further biodegradation of PBC was shown.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22. June 05, May 2022
Materials and Methods	The applicant's version is adopted
Results and discussion	The applicant's version is adopted
Conclusion	It is not possible to conclude on IPBCs inherent biodegradability from the study. Primary degradation of IPBC is shown.
Reliability	2
Acceptability	Acceptable; However, the ultimate biodegradation is not covered due to the lack of DOC or COD data. Therefore this test only shows that IPBC undergoes primary biodegradation.

Section A7.1.1.2.2/01 Biodegradability (inherent)

Annex Point IIA, VII.7.6.1.2

Remarks	The nominal test concentrations of IPBC were 0.02 mg/L (low dose) and 1.0 mg/L (high dose). The limit of quantification was 0.01 mg/L. An unknown substance, which had a similar retention time and mass as PBC was found in the aqueous phase resulting in high PBC-signals. A control sample of the activated sludge showed, that this substances was a contaminant of the sludge. Due to interference, the degradation of PBC could not be assessed in the low dose test It should be mentioned that 2-Propynyl and propargyl are synonyms. The study is conducted in accordance to OECD 302 B. This guideline is from 1992, hence the study and its conclusion is still considered acceptable.

Table A7.1.1.2/01-1: Inoculum / Test organism

Criteria	Details
Nature	Aerobic activated sludge
Species	Not specified
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	[redacted], Switzerland
Laboratory culture	Not applicable
Method of cultivation	Not applicable
Preparation of inoculum for exposure	After sampling the sludge was washed twice with tap water.
Pre-treatment	The sludge was aerated at room temperature until use, and filled up to 2500 mL with the test medium (see table A7.1.1.2/01-4).
Initial cell concentration	0.9 g dry material per litre

Table A7.1.1.2/01-2: Test system

Criteria	Details
Culturing apparatus	5000 mL Erlenmeyer flasks filled with 2500 mL test medium.
Number of culture flasks/concentration	14 with inoculum and test item at 1.0 mg/L 3 with inoculum and test item at 0.02 mg/L 2 with inoculum only (inoculum control) 2 with inoculum and reference item at 170 mg/L (procedure control) 1 with inoculum, with test item at 1.0 mg/L and with reference item at 170 mg/L (toxicity control)
Measuring equipment	Specific analysis of IPBC and PBC in the aqueous phase and in the activated sludge. IPBC and PBC were measured by HPLC-MS/MS.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2/01-3: Test conditions

Criteria	Details
Composition of medium [g/L]	KH_2PO_4 8.50 K_2HPO_4 21.75 $NaHPO_4.2H_20$ 33.40 NH_4Cl 0.50 $CaCl_2.2H_2O$ 36.40 $MgSO_4.7H_20$ 22.50 $FeCl_3$ $6H_2O$ 0.25
Additional substrate	Yes, drop of concentrated HCl
Test temperature	22-25°C
pH	6.6-7.5 (before adjustment) If necessary, before each sampling the pH was adjusted to 7.5+/- with a diluted sodium hydroxide solution
Aeration of dilution water	Not indicated.
Suspended solids concentration	Not indicated.
Other relevant criteria	Not indicated.

Table A7.1.1.2/01-4: Pass levels and validity criteria for tests on inherent biodegradability

	fulfilled	not fulfilled
Pass levels		

70% removal of DOC within 14 days		
Criteria for validity		
Percentage of removal of reference substance reaches pass level by day 14		

Not applicable for the modified Zahn Wellens test because of the specific analysis of the test items IPBC and the degradation product PBC.

4.1.2 BOD_5/COD

4.1.2.1 No new relevant data

4.1.3 Aquatic simulation tests

A10.1.3.2.b_Aero

A10.1.3.2.b_Degra

Section 7.1.2.2.2/01 Water/sediment study under anaerobic conditions

Annex Point IIIA, XII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1992): Anaerobic aquatic metabolism study of P-100; EPL [redacted]; Doc. No. 715-001; 24.08.1992; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Corporation	
1.2.2	Companies with letter of access	Arch Chemicals, Bayer Chemicals and Sostram Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I for all references listed above	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, U.S. EPA Pesticide Assessment Guidelines, Subdivision N, § 162-3, Chemistry: Environmental Fate	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	

Section 7.1.2.2.2/01 Water/sediment study under anaerobic conditions

Annex Point IIIA, XII.2.1

3.1	**Test material**	1) IPBC (technical grade; Troysan Polyphase P-100) 2) ^{14}C-IPBC (3-iodo-2propynyl-N-[1-^{14}C]- butyl[^{14}C]carbamate; Troysan Polyphase ^{14}C-P100)
3.1.1	Lot/Batch number	1) N.J. AS-2; 3D-95-C; 30-142-A; 9010-5951; 910261.11 2) NSN/1/77/1
3.1.2	Specification	As given in section 2
3.1.3	Purity	1) 97 % (minimum) 2) 99.4 % (radiochemical purity)
3.1.4	Description of test substance	Not indicated.
3.1.5	Further relevant properties	2) specific activity: 108 µCi/mg 1) and 2) water solubility: 156 ppm at 20°C
3.1.6	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product
3.1.7	TS inhibitory to microorganisms	Yes The EC_{20} in a respiration inhibition test was determined to be 57 mg/L.
3.1.8	Specific chemical analysis	Analytical methods used in the anaerobic aquatic metabolism study are described in detail in the study report (Appendix 5).
3.2	**Reference substance**	No
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Test solution**	A test solution was prepared by mixing technical grade and radiolabelled IPBC in degassed acetone.
3.3	**Testing procedure**	
3.3.1	Water/sediment systems	See table A7.1.2.1.2/01-1.

Section 7.1.2.2.2/01 **Water/sediment study under anaerobic conditions**
Annex Point IIIA, XII.2.1

3.3.2	Test system	Three different incubation conditions were included. Test A (Nonsterile and sterile static samples) Test B (Nonsterile enclosed static samples) Test C (Nonsterile continuous N_2 flow samples) For Nonsterile and Sterile Static Samples 20 g sediment were added to 250 mL polycarbonate centrifuge bottles and flooded with 100 mL N_2-purged pond water. The bottles were sealed with a Teflon gasket. Sample headspace was purged with N_2 at regular intervals to remove and trap volatile ^{14}C components. Set-up for the Nonsterile Enclosed Static Samples was the same as for the previous samples except that the 250 mL Polycabonate bottles were placed in individual chambers constructed of polyvinyl chloride (PVC). An air flow stream passed through the PVC chambers and into a 1 M NaOH solution to trap any CO_2 which escaped from the sealed bottles. For the Continuous N_2 Flow Samples, 50 g of sediment were added to 500 mL sidearm filter flasks and flooded with 250 mL of N_2-purged pond water. The filter flask sidearms were connected to a N_2 inlet with Tygon tubing. The N_2 outlet from each sample passed through a silica solid phase extraction column and a polyurethane foam plug and a gas washing bottle containing 1 M NaOH.
3.3.3	Temperature	22 ± 2°C
3.3.4	Initial TS concentration	Test A (Nonsterile and sterile static samples): 1.04 ppm Test B (Nonsterile enclosed static samples): 1.02 ppm Test C (Nonsterile continuous N_2 flow samples): 0.94 ppm
3.3.5	Duration of test	Test A: 244 days Test B: 180 days Test C: 118 days
3.3.6	Analytical parameter	Monitoring of - IPBC dissipation in aqueous phase and sediment; - metabolite formation; - CO_2 evolution; - bound residues accumulation
3.3.7	Sampling	Duplicate samples were taken at each specified interval: Test A: 0, 2, 4, and 8 hours, 1, 7, 14, 28, 59, 93, 162 and 244 days Test B: 28, 45, 90, 119 and 180 days Test C: 104 and 118 days

Section 7.1.2.2.2/01 **Water/sediment study under anaerobic conditions**
Annex Point IIIA, XII.2.1

3.3.8	Sampling and Analytical methods	Headspace of samples was purged with nitrogen passing through a polyurethane foam plug and a 1 M NaOH solution to trap organic volatiles and CO_2 NaOH solutions of the PVC chambers were radioassayed by LSC. Selected NaOH samples were precipitated with $BaCl_2$ to confirm the presence of CO_2. Polyurethane foam plugs were extracted with methanol and radioassayed. The silica solid phase extraction columns were eluted with methanol/1 N HCl (9/1 v/v). Aliquots were radioassayed and extracts partitioned with methylene chloride. Organic extracts were concentrated. Water layers were separated from the sediment by filtration, and extracted twice with methylene chloride after acidification with 1N HCl. Aliquots of the aqueous and organic fractions were radioassayed by LSC. Sediment samples were extracted with methanol for 18 hours in Soxhlet tubes. Aliquots of the extracts were radioassayed by LSC. All sample extracts containing > 1 % of applied radioactivity were analysed by HPLC with UV and ^{14}C detector. Confirmatory analyses were performed on isolated metabolites using GC/MS. All extracted sediment samples were combusted and analysed by LSC to determine the amount of non-extractable residues.
3.3.9	Statistics	The half-life of IPBC and its degradation product propargyl butyl carbamate (PBC) was calculated assuming pseudo first-order kinetics.

4 RESULTS

4.1	**Material balance**	In Nonsterile Static Samples the material balance values averaged 99.2 % through the day 14 sampling interval but had decreased to 62.1 % by the day 59 and 46.7 % by the day 244 sampling interval. The low material balance values from the later samplings was most probably related to CO_2 evolution (see table A7.1.2.1.2/01-2). To accomplish this, Nonsterile Enclosed Static Samples were placed in individual PVC chambers which were connected to an air flow passing through 1 M NaOH solution to trap any CO_2 that had escaped the sealed incubation flasks. However, despite these efforts, the material balance declined rapidly between the 28 and 90 days sampling intervals (see table A7_1_2_1_2-3). Based on this data it was assumed that the unaccounted ^{14}C appears to be a volatile, low molecular weight molecule (e.g. CH_4) formed by bacteria which are able to convert CO_2 to methane. Additional samples were incubated under a continuous N_2 flow to limit CO_2 conversion to CH_4 by removal of the CO_2 as it was formed and/or by inhibition of the methanogenic bacterial population. The material balance from the continuous flow samples were significantly greater than from static samples ranging from 75.4 to 81.7 % of applied radioactivity (see table A7.1.2.1.2/01-4). Again, it is likely that the unaccounted ^{14}C was in the form of $^{14}CH_4$. Although the continuous N_2 flow appeared to inhibit CH_4 formation, it did not stop it completely. The material balance in Sterile Static Samples averaged 95.3 % of applied radioactivity (see table A7.1.2.1.2/01-5).

Section 7.1.2.2.2/01 **Water/sediment study under anaerobic conditions**
Annex Point IIIA, XII.2.1

4.2	**Distribution of radioactivity between water and sediment**	Residues resulting from ^{14}C-IPBC application to anaerobic aquatic systems remained predominantly in the water layer. Less than 21 % of the applied radioactivity was associated with the sediment (extractable and bound residues) at any sampling interval (see tables A7.1.2.1.2/01-6 to A7.1.2.1.2/01-8).
4.3	**Concentration – time data**	Please refer to tables A7.1.2.1.2/01-6 to A7.1.2.1.2/01-8.
4.4	**Dissipation time**	Due to the rapid degradation of IPBC under nonsterile anaerobic aquatic conditions, dissipation rates were calculated for the total system rather than separating the data between its sediment and water components. The DT_{50} and the corresponding DT_{90} values were calculated to be 1.5 and 5.0 hours, respectively (table A7.1.2.1.2/01-9). DT_{50} and DT_{90} values for propargyl butyl carbamate (PBC) were calculated using the day 1 sampling interval as the initiation point for its dissipation. A DT_{50} of 11.5 days and a DT_{90} of 34.8 days calculated based on data collected up to 59 days after treatment. Under sterile conditions the degradation of IPBC was significantly slower with DT_{50} and DT_{90} values of 13.3 and 44.3 hours, respectively. However, it is apparent that nonbiological processes also contribute to the anaerobic aquatic degradation of IPBC.
4.5	**Specification of the transformation products**	The initial degradation product of IPBC was propargyl butyl carbamate (PBC) accounting for > 97 % of the applied radioactivity one day after treatment (see tables A7.1.2.1.2/01-6 and A7.1.2.1.2/01-8). PBC was further degraded to 2-propenyl-butyl carbamate and several non-identified compounds prior to complete mineralisation. Residue levels of 2-popenyl-butyl carbamate in sediment and water of nonsterile static systems peaked at 8.0 and 34.7 % of the applied radioactivity, respectively, at day 59. Total residue levels of either of the non-identified metabolites accounted for less than 3 % at any sampling interval. Under sterile conditions PBC was again the main degradation product accounting for maximum values of >80 % of applied radioactivity in the total system 29 days after treatment (table A7.1.2.1.2/01-8).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The anaerobic biodegradation test was conducted according to U.S. EPA Pesticide Assessment Guidelines, Subdivision N, § 162-3, Chemistry: Environmental Fate. ^{14}C-IPBC was applied to a natural water/sediment system and incubated under anaerobic conditions at 22 ± 2°C in the dark. Water and sediment samples were taken up to 244 days after treatment.

Section 7.1.2.2.2/01 Water/sediment study under anaerobic conditions

Annex Point IIIA, XII.2.1

5.2	**Results and discussion**	IPBC was rapidly degraded in anaerobic aquatic systems with a half-life of 1.5 hours. IPBC remained predominantly in the water layer but degradation products were similar between water and sediment. The initial degradation product of IPBC was propargyl butyl carbamate accounting (PBC) for > 97 % one day after treatment. It was further degraded to 2-propenyl butyl carbamate and two nonidentified compounds prior to complete mineralisation. The calculated half-life for propargyl butyl carbamate was 11.5 days. Material balance values declined with time probably due to the formation of $^{14}CH_4$. The terminal degradation products of IPBC in anaerobic aquatic systems appear to be CO_2 and CH_4.
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. IPBC was rapidly degraded in anaerobic aquatic systems with a half-life of 1.5 hours. The major degradation product was propargyl butyl carbamate (PBC) which degraded itself with a half-life of 11.5 days. The terminal degradation products of IPBC in anaerobic aquatic systems appear to be CO_2 and CH_4. IPBC degradation in anaerobic aquatic systems was primarily microbially mediated but nonbiological mechanisms also contribute to the degradative process.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004, May 2022
Materials and Methods	The applicant's version is acceptable
Results and discussion	In the study on IPBC degradation in anaerobic aquatic systems (water/sediment system) IPBC remained predominantly in the water layer with less than 20 recovered from the sediment layer. Degradation products were similar between water and sediment. The initial degradation product of IPBC was propargyl butyl carbamate accounting (PBC) for > 97 % one day after treatment. It was further degraded to 2-propenyl butyl carbamate and two non-identified compounds prior to complete mineralisation. Bound residues remained below 10%. Material balance values declined with time probably due to the formation of $^{14}CH_4$. Thus, the terminal degradation products of IPBC in anaerobic aquatic systems appear to be CO_2 and CH_4.

Section 7.1.2.2.2/01 Water/sediment study under anaerobic conditions

Annex Point IIIA, XII.2.1

Conclusion	The dissipation of IPBC in an anaerobic aquatic system (water/sediment system) was relatively fast with a DT50 value for the total system of 1.5 hours and a DT90 of 5 hours at 22ºC. This corresponds to with a DT50 value for the total system of 3.3 hours and a DT90 of 11 hours at 12ºC using Arrhenius equation. The major degradation product was propargyl butyl carbamate (PBC) which had a dissipation half-life of 11.5 days and a DT90 of 38.4 days at 22ºC. This corresponds to with a DT50 value for the total system of 26 days and a DT90 of 86 days at 12ºC using Arrhenius equation. The terminal degradation products of IPBC in anaerobic aquatic systems appear to be CO_2 and CH_4. In a sterile water/sediment system the DT50 was 13.3 hours and DT90 44.3 hours at 22ºC. Therefore it was concluded that IPBC degradation in anaerobic aquatic systems was primarily microbially mediated but non-biological mechanisms also contribute to the degradative process. Considering the low recovery and the fact that the conversion of CO_2 into CH_4 (although probable) has not be formally proven, a reliability of 2 should apply. The metabolite 2-propenyl-butyl carbamate is not transient and is found at percentage >10% in water. It should therefore normally been considered as major and its ecotoxicological relevance assessed. However, this metabolite is only found in significant amount in the water under anaerobic conditions and a QSAR estimate using ECOSAR indicates the toxicity to be at the level of IPBC. Based on this we will not ask for experimental data for this metabolite.
Reliability	Reliability indicator 2
Acceptability	Acceptable
Remarks	Dissipation rate for IPBC was presented for the total water/sediment system. Based on the data A7.1.2.2.2/01-6 it appears that 78% remained in the water phase and less than 10% was recovered from the sediments. These data indicated that the DT50 in the water phase was 1.4 hours and DT90 4.7 hours, and the corresponding values for the sediment were 2.2 hour and 7.5 hours assuming pseudo first order degradation kinetics. The dissipation rates on IPBC was calculated from data from samples 1-8 hours and the dissipation rates on PBC from data covering day 0 to 59. This study has been performed in accordance with U.S. EPA Pesticide Assessment Guidelines, Subdivision N, § 162-3. This guideline is from 1982, hence the study and its conclusions are still considered valid.

Table A7.1.2.2.2/01-1: Water/sediment systems used

	System I[1)]	System II[2)]
Origin	Pond, Macon County, IL, U.S.A.	Pond, Macon County, IL, U.S.A.
	Sediment properties	
Sediment texture	Sandy loam	Sand
% sand (50 µm-2 mm)	80	92
% silt (2 –50 µm)	8	2.5
% clay (< 2 µm)	12	5.5
Organic matter (%)	1.2	0.74
Organic carbon (%)	0.70	0.43
pH value	7.5	7.8
Cation exchange capacity [meq/100 g]	7.9	12.1
	Water properties	
Hardness [mg equivalent $CaCO_3$/L]	346.0	138.0
pH value [a)]	7.85	8.5

1) Systems I was used for Test A and C
2) Systems II was used for Test B

Table A7.1.2.2.2/01-2: Material balance for Nonsterile Static Samples

Sampling times (days)	Sediment & Water extracts	Foam plug extract	NaOH	Bound residues	Material balance
0	105.0	na	na	0.1	105.1
2 h	98.3	0.0	0.0	0.0	98.3
4 h	104.9	0.0	0.0	0.0	104.9
8 h	100.6	0.0	0.0	0.0	100.6
1	99.8	0.0	0.1	0.1	100.0
7	94.8	0.0	0.8	0.3	95.9
14	88.3	0.0	1.1	0.5	89.9
28	72.7	0.0	3.0	1.0	76.6
59	52.1	0.0	8.3	1.7	62.1
93	10.1	0.2	41.6	2.8	54.7
162	4.5	0.1	46.4	3.9	54.8
244	2.1	0.0	43.3	1.4	46.7

na: not analysed

Table A7.1.2.2.2/01-3: Material balance for Nonsterile Enclosed Static Samples

Sampling times (days)	Sediment & Water extracts	Foam plug extract	NaOH	Bound residues	Material balance
28	87.4	0.0	2.0	2.0	91.3
45	83.9	0.0	3.8	1.0	88.7
90	23.8	0.0	20.3	3.2	47.2
119	10.4	0.0	20.5	6.3	37.1
180	9.2	0.0	20.9	3.3	33.4

Table A7.1.2.2.2/01-4: Material balance for Nonsterile Continuous N_2 Flow samples

Sampling times (days)	Sediment & Water extracts	Silica column extract	Foam plug extract	NaOH	Bound residues	Material balance
104	49.3	5.8	18.0	8.0	0.7	81.7
118	57.8	4.8	2.0	10.0	0.8	75.4

Table A7.1.2.2.2/01-5: Material balance for Sterile Static Samples

Sampling times (days)	Sediment & Water extracts	Foam plug extract	NaOH	Bound residues	Material balance
0	99.7	na	na	0.0	99.7
1	93.7	0.0	0.0	0.1	93.8
29	91.7	0.0	0.0	0.7	92.4

na: not analysed

Table A7.1.2.2.2/01-6: Degradation of IPBC and formation of metabolites in Nonsterile Static water/sediment systems

Sampling times (days)	IPBC	PBC	2-PBC	Compound #2	Compound #3
	Water				
0	78.24	11.6	nd	nd	nd
2 h	4.56	78.7	nd	nd	nd
4 h	5.21	84.9	nd	nd	nd
8 h	0.91	88.6	nd	0.6	nd
1	nd	85.7	nd	nd	nd
7	nd	70.6	7.9	0.7	0.6
14	nd	56.9	15.1	0.2	0.7
28	nd	29.1	32.2	0.4	2.1
59	nd	2.6	34.7	1.7	2.9
93	nd	nd	3.1	1.1	nd
162	nd	nd	nd	0.7	nd
244	nd	nd	nd	nd	nd
	Sediment				
0	6.78	6.8	nd	nd	nd
2 h	0.19	13.3	nd	nd	nd
4 h	0.07	13.3	0.2	nd	nd
8 h	0.31	9.2	0.2	nd	nd
1	nd	12.1	0.7	nd	nd
7	nd	10.5	2.2	nd	nd
14	nd	9.6	3.6	nd	0.5
28	nd	3.0	3.3	nd	0.3
59	nd	0.6	8.0	nd	nd
93	nd	0.2	1.7	nd	nd
162	nd	0.3	1.8	nd	nd
244	nd	0.2	1.2	nd	nd

PBC: Propargyl butyl carbamate
2-PBC: 2-propenyl butyl carbamate
na: not analysed
nd: not detected

Table A7.1.2.2.2/01-7: Degradation of IPBC and formation of metabolites in Nonsterile Enclosed Static water/sediment systems

Sampling times (days)	IPBC	PBC	2-PBC	Compound #2	Compound #3
	Water				
28	nd	41.1	25.6	0.7	0.4
59	nd	29.3	35.4	1.6	1.5
93	nd	nd	8.6	2.9	0.5
162	nd	nd	0.2	0.9	0.1
244	nd	nd	nd	nd	nd
	Sediment				
28	nd	12.0	6.8	nd	nd
59	nd	7.7	8.1	nd	nd
93	nd	2.3	8.8	0.1	nd
162	nd	2.9	4.6	nd	nd
244	nd	3.0	3.7	nd	1.7

PBC: Propargyl butyl carbamate
2-PBC: 2-propenyl butyl carbamate
na: not analysed
nd: not detected

Table A7.1.2.2.2/01-8: Degradation of IPBC and formation of metabolites in Sterile Static water/sediment systems

Sampling times (days)	IPBC	PBC	2-PBC	Compound #2	Compound #3
	Water				
0	87.2	1.5	nd	nd	nd
1	21.4	43.0	nd	nd	nd
29	nd	68.1	nd	0.6	4.9
	Sediment				
0	10.0	0.3	nd	nd	nd
1	6.6	20.9	nd	nd	nd
29	0.04	14.5	nd	nd	1.1

PBC: Propargyl butyl carbamate
2-PBC: 2-propenyl butyl carbamate
na: not analysed
nd: not detected

Table A7.1.2.2.2/01-9: DT_{50} and DT_{90} values of IPBC and Propargyl butyl carbamate in total water/sediment systems

Parameter	IPBC		Propargyl butyl carbamate
	Nonsterile static samples[1)]	Sterile static samples	Nonsterile static samples
DT_{50}	1.5 hours	13.3 hours	11.5 days
DT_{90}	5.0 hours	44.3 hours	38.4 days
k	0.464 h^{-1}	0.052 h^{-1}	0.060 d^{-1}

[1)] Data collected up to 8 hours after treatment in nonsterile static samples (Test A; sandy loam water/sediment systems) were used for half-life determination

4.1.4 Other degradability studies

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA, VII.7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2001): Preventol MP 100 – Abiotic degradation; [redacted]; Doc. No. 711-004; 29.06.2001; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer Chemicals	
1.2.2	Companies with letter of access	Arch Chemicals, Sostram Corporation and Troy Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I for all references listed above	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. EG guideline C7. 92/69	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	
3.1	**Test material**	Preventol MP 100 (technical IPBC)	
3.1.1	Lot/Batch number	147-270300-1	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	98.3 %	
3.1.4	Description of test substance	Not indicated.	
3.1.5	Further relevant properties	Not indicated.	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test solution**	The preparation of test solutions corresponded to test method C.7 for determination of abiotic degradation , EG guideline 92/69 (see Table A7.1.1.1.1/01-1).	
3.4	**Testing procedure**		

Section A7.1.1.1.1/01 **Annex Point IIA, VII.7.6.2.1**		**Hydrolysis as a function of pH and identification of breakdown products**
3.4.1	Test system	See tables A7.1.1.1.1/01-2 and A7.1.1.1.1/01-3
3.4.2	Temperature	50°C (pH 4, 7 and 9) = Pretest 65°C (pH 9) = Main test 80°C (pH 9) = Main test
3.4.3	pH	Only initial pH indicated: pH 4, 7 and 9
3.4.4	Duration of the test	See table A7.1.1.1.1/01-4
3.4.5	Number of replicates	One replicate
3.4.6	Sampling	See table A7.1.1.1.1/01-4
3.4.7	Analytical methods	The aqueous samples were analysed by HPLC / UV. Quantification was done by area normalisation.
3.5	**Preliminary test**	Yes. A preliminary test was conducted at pH 4, 7 and 9 at 50°C. Buffer systems: see Table A7.1.1.1.1/01-1
		4 RESULTS
4.1	**Concentration and hydrolysis values**	Results are presented in Table A7.1.1.1.1/01-4. The preliminary test indicated that less than 10 % has been hydrolyzed after 5 days at pH 4 and 7. Therefore, no further testing was required at these pH values. At pH 9 and 50°C the initial IPBC concentration decreased to ca. 77.7 % after 119 hours (= 5 days) and decreased further to about 50 % after 287 hours (= ca. 12 days). At higher incubation temperatures the IPBC concentration decreased to 49.7 % within 31 hours (at 65°C) and to 36.2 % (at 80°C), respectively.
4.2	**Hydrolysis rate constant (k_h)**	The hydrolysis rate constants at pH 9 (k_h) were determined to be 0.0025 h^{-1} at 50°C (r = 0.998), 0.0224 h^{-1} at 65°C (r = 0.993) and 0.125 h^{-1} at 80°C (r = 0.999) assuming pseudo-first order reaction. The hydrolysis rate constant at 25°C was extrapolated according to the Arrhenius equation and calculated to be 5.36 x 10^{-5} h^{-1}.
4.3	**Dissipation time**	Dissipation times of IPBC at pH 9 at different incubation temperatures are presented in Table A7.1.1.1.1/01-5. DT_{50} values ranged from 5.56 to 12,942 hours. DT_{90} values ranged from 18.5 to 42,993 hours.
4.4	**Concentration – time data**	Results are presented in Table A7.1.1.1.1/01-4.
4.5	**Specification of the transformation products**	Not indicated.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The aqueous hydrolysis test was conducted according to test method C.7 for determination of abiotic degradation, EG guideline 92/69.

Section A7.1.1.1.1/01 **Annex Point IIA, VII.7.6.2.1**	**Hydrolysis as a function of pH and identification of breakdown products**	
		IPBC was dissolved in buffer solutions of pH 4, 7 and 9 and incubated at 50°C. Test solutions buffered at pH 9 were additionally incubated at 65 and 80°C.
5.2	**Results and discussion**	See below.
5.2.1	k_H	The test substance IPBC is not degradable at pH 4 and pH 7. At pH 9 the hydrolysis rate constants k_H are 0.0025 h^{-1} at 50°C, 0.0224 h^{-1} at 65°C and 0.125 h^{-1} at 80°C. The hydrolysis rate constant at 25°C was extrapolated according to the Arrhenius equation and calculated to be 5.36 x 10^{-5} h^{-1}.
5.2.2	DT_{50}	The test substance IPBC is not degradable at pH 4 and pH 7. DT_{50} values at pH 9: 5.6 h = 0.2 days (80°C) 31 h = 1.3 days (65°C) 282 h = 11.8 days (50°C) 12942 h = 539 days (25°C)
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. IPBC was found to be stable under acidic and neutral conditions. Under alkaline conditions IPBC degraded slowly at ambient temperatures with a DT_{50} of 539 days.
5.3.1	Reliability	1
5.3.2	Deficiencies	Yes Formation of degradation products was not investigated. However, the study is acceptable to predict the hydrolysis rate constant and dissipation times of the parent substance IPBC.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	23. June 05, May 2022
Materials and Methods	The applicant's version is acceptable.
Results and discussion	Adopt applicant's version; however the DT50 of hydrolysis should be recalculated to 12°C pH 4 at 12°C = 755 d pH 7 at 12°C = 702 d pH 9 at 12°C = 648 d = 1525 d
Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	The study is still considered reliable

Table A7.1.1.1.1/01-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
4	citric acid/potassium hydroxide/sodium chloride	Exact composition not indicated; ready to use solution.
7	potassium dihydrogen phosphate/di-sodium hydrogen phosphate	Exact composition not indicated; ready to use solution
9	borax/hydrochloric acid	Exact composition not indicated; ready to use solution

Table A7.1.1.1.1/01-2: Description of test solution

Criteria	Details
Purity of water	Not indicated, however test method corresponded to EG guideline C.7 92/69.
Preparation of test medium	Not indicated
Test concentrations (mg a.i./L)	Not indicated
Temperature (□C)	50°C (pH 4, 7 and 9) 65°C (pH 9) 80°C (pH 9)
Controls	Not indicated
Identity and concentration of co-solvent	Not indicated
Replicates	One replicate.

Table A7.1.1.1.1/01-3: Description of test system

Glassware	Not indicated
Other equipment	Not indicated
Method of sterilization	Not indicated

Table A7.1.1.1.1/01-4: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9.

pH 4

Compound	Sampling times (*hours*)					
	0	**0.7**	**4.1**	**26.1**	**95.2**	**119**
Parent compound	100	98.5	97.4	97.3	97.9	94.2

pH 7

Compound	Sampling times (*hours*)					
	0	**3.7**	**4.8**	**26.1**	**95.2**	**119**
Parent compound	100	103.1	103.5	102.1	100.1	99.2

pH 9

Compound	Sampling times (*hours*)							
Parent compound	**50°C**							
	0	**119**	**126.7**	**142.5**	**166.1**	**190.5**	**266.9**	**286.8**
	100.0	77.7	75.9	73.2	67.3	63.9	52.4	50.2
	65°C							
	0	**22.6**	**27.3**	**29.3**	**30.6**	**-**	**-**	**-**
	100.0	64.9	54.9	51.8	49.7	-	-	-
	80°C							
	0	**2.9**	**4.1**	**5.1**	**6.1**	**6.9**	**8.3**	**-**
	100.0	70.3	60.0	52.5	47.3	41.5	36.2	-

Table A7.1.1.1.1/01-5: Dissipation times of IPBC at pH 9 (expressed in hours)

25°C*		50°C		65°C		80°C	
DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**
12942	42993	281.5	935.2	30.89	102.6	5.56	18.5

* DT_{50}/DT_{90} values at 25°C were extrapolated according to the Arrhenius equation

Section A7.1.1.1.1/02 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA, VII.7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994): Hydrolysis of ^{14}C-3-Iodo-2-propynyl-n-butylcarbamate (^{14}C-IPBC); [redacted]; Doc. No. 711-003; 13.12.1994; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Arch Chemicals	
1.2.2	Companies with letter of access	Bayer Chemicals, Sostram Corporation and Troy Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I for all references listed above	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, U.S. EPA Pesticide Assessment Guidelines, Subdivision N, § 161-1, Chemistry: Environmental Fate	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	
3.1	**Test material**	^{14}C-IPBC, labelled in the n-butyl-1-position of the molecule	
3.1.1	Lot/Batch number	3048-141	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	> 98 % radiochemical purity	
3.1.4	Description of test substance	Not indicated.	
3.1.5	Further relevant properties	Specific activity: 3.16 mCi/mmol / 24,965 dpm/µg	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable.	
3.3	**Test solution**	The preparation of test solutions is described in tables A7.1.1.1.1/02-1 and A7.1.1.1.1/02-2.	
3.4	**Testing procedure**		
3.4.1	Test system	The test system is described in detail in table A7.1.1.1.1/02-3.	

Section A7.1.1.1.1/02 Annex Point IIA, VII.7.6.2.1		Hydrolysis as a function of pH and identification of breakdown products
		Test systems were capped and incubated in a constant temperature incubator at 25 ± 1°C in the dark.
3.4.2	Temperature	25 ± 1°C
3.4.3	pH	Only the initial pH is indicated: pH 5, 7 and 9 However, the pH of all buffered test solutions was monitored upon harvesting.
3.4.4	Duration of the test	30 days
3.4.5	Number of replicates	Duplicate samples
3.4.6	Sampling	Samples were taken at 0, 3, 7, 14, 21, and 30 days post-treatment.
3.4.7	Analytical methods	Aliquots of the test solutions were assayed by LSC to determine the levels of ^{14}C. Subsequently, samples were analysed by solid-phase extraction using C_{18} Sep-Pak cartridges followed by reversed phase and normal phase HPLC with radiometric detection.
3.5	**Preliminary test**	No
		4 RESULTS
4.1	**Concentration and hydrolysis values**	The results are presented in detail in table A7.1.1.1.1/02-4. The average total ^{14}C recoveries were 96.1 %, 95.9 % and 95.1 % for pH 5, 7 and 9 buffered solutions, respectively. The only significant radioactive component throughout all sampling intervals was parent IPBC. Six other degradants were observed during the study, but none of these exceeded 1.48 % of the total recovered radioactivity.
4.2	**Hydrolysis rate constant (k_h)**	The hydrolysis rate constants (k_h) for IPBC were 0.0026 d^{-1} at pH 5, 0.00279 d^{-1} at pH 7 and 0.00302 d^{-1} at pH 9 (see table A7.1.1.1.1/02-5). Correlation coefficients ranged from 0.657 to 0.796.
4.3	**Dissipation time**	The dissipation time was determined by linear regression analysis assuming pseudo-first order kinetics. The calculated DT_{50} values of IPBC in pH 5, 7 and 9 buffered aqueous solutions were 267, 248 and 229 days, respectively (see table A7.1.1.1.1/02-5). The corresponding DT_{90} values were 886, 825 and 762 days, respectively.
4.4	**Concentration – time data**	Please refer to table A7.1.1.1.1/02-4.
4.5	**Specification of the transformation products**	Six transformation products were observed during the study, but none of these exceeded 1.48 % of the total recovered radioactivity (see table A7.1.1.1.1/02-4). Therefore, no further identification work was required.

Section A7.1.1.1.1/02 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA, VII.7.6.2.1

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The aqueous hydrolysis test was conducted according to U.S. EPA Pesticide Assessment Guidelines, Subdivision N, § 161-1, Chemistry: Environmental Fate. ^{14}C-IPBC was dissolved in buffer solutions of pH 5, 7 and 9 and incubated at 25°C for up to 30 days.
5.2	**Results and discussion**	Under sterile conditions at 25°C in the dark, IPBC is stable and does not undergo any appreciable hydrolytic degradation in buffered aqueous solutions at pH levels of 5, 7 or 9. In all samples analysed, the only significant radioactive component detected in the organic fractions throughout the sampling intervals was the parent compound IPBC. Six minor degradants not exceeding 1.48 % of applied radioactivity were also detected.
5.2.1	k_H	pH 5: 0.0026 d^{-1} pH 7: 0.00279 d^{-1} pH 9: 0.00302 d^{-1}
5.2.2	DT_{50}	pH 5: 267 days (25°C) pH 7: 248 days (25°C) pH 9: 229 days (25°C)
5.2.3	r^2	Correlation coefficients ranged from 0.657 to 0.796.
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. IPBC was found to be hydrolytically stable in sterile aqueous solutions at ambient temperatures.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2005, May 2022
Materials and Methods	The applicant's version is adopted
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	The study is still considered reliable

Table A7.1.1.1.1/02-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
5	0.01 M	0.01 M sodium acetate in water adjusted to pH 5 with acetic acid
7	0.01 M	0.01 M monobasic sodium phosphate in water adjusted to pH 7 with dibasic sodium phosphate in water
9	0.01 M	0.01 M boric acid in 0.01 M potassium chloride adjusted to pH 9 with sodium hydroxide

Table A7.1.1.1.1/02-2: Description of test solution

Criteria	Details
Purity of water	All water used throughout the experiment was processed through a NANOPure® II water purification system and sterilised in an autoclave at ca. 118°C for about 30 min.
Preparation of test medium	Approximately 1.6 mL of acetonitrile-diluted radiolabelled test material was applied individually to sterilised glass bottles containing 300 mL of the respective buffer solution.
Test concentrations (mg a.i./L)	5 mg a.i./L (= 5 ppm)
Temperature (□C)	25 ± 1°C
Controls	Not applicable
Identity and concentration of co-solvent	Acetonitrile (< 1 %); approximately 9.47 mg of the radiolabelled test substance was diluted in 10 mL of acetonitrile.
Replicates	Duplicate samples of each buffer type

Table A7.1.1.1.1/02-3: Description of test system

Glassware	Sterilised glass bottles
Other equipment	Constant temperature incubator (VWR Scientific, Model 2020); Autoclave, Market Forge Company; Corning® 340 pH meter;
Method of sterilisation	Autoclave

Table A7.1.1.1.1/02-4: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of total recovered radioactivity, at pH 5, pH 7 and pH 9.

pH 5

Compound	Sampling times (days)					
	0	**3**	**7**	**14**	**21**	**30**
Parent compound	102.68	93.90	94.74	90.30	91.95	92.28
D1 (Rt 9 min)	0.03	0.14	0.04	0.41	nd	nd
D2 (Rt 20 min)	nd	0.04	0.04	0.81	nd	1.40
D3 (Rt 28 min)	nd	0.06	0.13	0.63	0.08	0.22
D4 (Rt 32 min)	0.06	0.15	0.40	0.63	0.12	0.94
D5 (Rt 15 min)	nd	0.33	nd	0.72	0.16	nd
D6 (Rt 5 min)	nd	nd	nd	0.39	0.27	nd
Total % recovery	103.04	94.94	95.82	94.27	93.00	95.44

Rt: HPLC retention time

nd: not detected

pH 7

Compound	Sampling times (days)					
	0	**3**	**7**	**14**	**21**	**30**
Parent compound	102.88	92.85	95.30	91.84	93.61	90.43
D1 (Rt 9 min)	0.23	0.14	0.06	0.09	0.06	nd
D2 (Rt 20 min)	0.16	0.06	0.25	0.51	0.19	0.86
D3 (Rt 28 min)	nd	nd	0.11	0.33	0.04	0.50
D4 (Rt 32 min)	0.22	0.12	nd	0.22	0.08	1.33
D5 (Rt 15 min)	nd	nd	nd	0.18	0.08	nd
D6 (Rt 5 min)	nd	nd	nd	0.54	0.28	nd
Total % recovery	103.72	93.47	96.10	94.08	94.70	93.51

Rt: HPLC retention time

nd: not detected

pH 9

Compound	Sampling times (days)					
	0	**3**	**7**	**14**	**21**	**30**
^{14}C-IPBC	100.73	94.12	92.03	90.56	91.58	89.04
D1 (Rt 9 min)	0.14	nd	nd	0.48	0.09	nd
D2 (Rt 20 min)	0.13	0.20	0.40	1.09	0.57	0.64
D3 (Rt 28 min)	0.07	0.13	0.32	0.96	0.56	1.48
D4 (Rt 32 min)	nd	0.24	0.17	0.69	0.33	0.38
D5 (Rt 15 min)	nd	nd	0.19	0.40	nd	nd
D6 (Rt 5 min)	nd	nd	nd	0.52	nd	nd
Total % recovery	101.46	95.04	93.52	95.16	93.57	92.07

Rt: HPLC retention time

nd: not detected

Table A7.1.1.1.1/02-5: Dissipation times of ^{14}C-IPBC at pH 5, pH 7 and pH 9 (expressed in days

	pH 5		**pH 7**		**pH 9**	
	DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**
	266.5	885.6	248.4	825.3	229.5	762.4
Hydrolysis rate constant (k_h) [d^{-1}]	0.00260		0.00279		0.00302	
Correlation coefficient	0.65728		0.70041		0.79632	

Section A7.1.1.1.2/01/02	**Phototransformation in water including identity of transformation products**
Annex Point IIA, VII.7.6.2.1	

			Official use only
		1 REFERENCE	
1.1	**Reference**	A7.1.1.1.2/01: Lee, D. et al. (1991): Photostability of organoiodine wood preservatives I, Progressive degradation and loss in fungal inhibition rate through photoirradiation; Mokuzai Gakkaishi Vol. 37, No. 1, p. 76 – 81; Doc. No. 792-005, (published). A7.1.1.1.2/02: Lee, D. et al. (1991): Photostability of organoiodine wood preservatives II, The photolytic process of preservatives; Mokuzai Gakkaishi Vol. 37, No. 3, p. 261 – 265; Doc. No. 792-004, (published).	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	No data protection claimed.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, publication	
2.2	**GLP**	No, publication	
2.3	**Deviations**	Not applicable	
		3 MATERIAL AND METHODS	
3.1	**Test material**	IPBC	
3.1.1	Lot/Batch number	Not indicated.	
3.1.2	Specification	Not indicated.	
3.1.3	Purity	Not indicated.	
3.1.4	Description of test substance	Not indicated.	
3.1.5	Radiolabelling	Not indicated.	
3.1.6	UV/VIS absorption spectra and absorbance value	Not indicated.	
3.1.7	Further relevant properties	Not indicated.	
3.2	**Reference substance**	Not indicated.	

Section A7.1.1.1.2/01/02	**Phototransformation in water including identity of transformation products**
Annex Point IIA, VII.7.6.2.1	

3.3	**Test solution**	a) IPBC was dissolved in ethanol at a concentration of 400 ppm (w/w). b) IPBC was dissolved in ethanol at a concentration of 1.0 % (w/w) for dip treatment of wood slices.
3.4	**Testing procedure**	
3.4.1	Test system	Irradiation of test solutions: The test solution (400 mL) was filled in a 500 mL Pyrex Erlenmeyer flask. The flask was plugged to exclude light of a wavelength of less than 200 nm. The flasks were exposed to six sunlight (Toshiba DR 400/t) and to six ultraviolet lamps (Toshiba GL 15) with wave length of 290 – 400 nm at 25 ± 1°C and 65 % relative humidity. The test solutions were stirred magnetically to obtain even irradiation and to minimise the heat effects. Treatment of wood slices: Sapwood slices of *Cryptomeria japonica* measuring 0.4 x 20 x 120 mm were dipped in the dip-treatment solution for 30 seconds. After excess solution had been wiped off the treated sliced were air-dried for 3 weeks in the dark. Afterwards, the treated wood slices were set in a slide stand and irradiated for 0, 2, 5, 10, 15, 25 and 50 days.
3.4.2	Properties of light source	▪ sunlight lamp (Toshiba DR 400/t) ▪ ultraviolet lamp (Toshiba GL 15) Wavelength of both lamps: 290 – 400 nm
3.4.3	Determination of irradiance	Not indicated.
3.4.4	Temperature	25 ± 1°C
3.4.5	pH	Not indicated
3.4.6	Duration of the test	Up to 50 days.
3.4.7	Number of replicates	Three replicates.
3.4.8	Sampling	0, 2, 5, 10, 15, 25 and 50 days.
3.4.9	Analytical methods	Test solution: After irradiation, two mL of solution were collected from each flask. The ethanol was evaporated, and the residues were dissolved in 0.5 mL of chloroform or acetone. The acetone solutions were then subjected to GC-MS analysis using a non-polar column, chemically bonded with methyl silicone. Wood slices: Each irradiated wood slice was torn to shreds and extracted with chloroform for 24 hours. The chloroform was evaporated, and the residues were dissolved in 0.5 mL of acetone. The acetone solutions were then subjected to GC-MS analysis using a non-polar column, chemically bonded with methyl silicone.

Section A7.1.1.1.2/01/02	**Phototransformation in water including identity of transformation products**
Annex Point IIA, VII.7.6.2.1	

3.4.10	Calculations	The decomposition rate of IPBC was calculated on the basis of its initial amount before irradiation.
3.5	**Transformation products**	Transformation products identified: Yes
3.5.1	Method of analysis for transformation products	The photodegradation products were identified by MS analysis.
		4 RESULTS
4.1	**Screening test**	Not performed
4.2	**Actinometer data**	Not available.
4.3	**Controls**	No controls used.
4.4	**Photolysis data**	
4.4.1	Concentration values	Decomposition of IPBC in irradiated solutions: The decomposition rate of IPBC in irradiated ethanol solutions reached 25 % after 400 hours (= ca. 17 days). The peak of the main photodegradation product appeared after 108 hours(= ca. 4.5 days) of irradiation. After 270 hours (= ca. 11 days) of irradiation a second photodegradation product was detected. Decomposition of IPBC in wood IPBC impregnated into wood was also degraded by exposure to light. The recovery rates decreased progressively with the time of irradiation. After 25 to 50 days of irradiation the recovery rate of IPBC decreased to approximately 40 to 50 %.
4.4.2	Mass balance	Not indicated.
4.4.3	k^c_p	Not indicated.
4.4.4	Kinetic order	Not indicated.
4.4.5	k^c_p / k^a_p	Not indicated.
4.4.6	Reaction quantum yield (ϕ^c_E)	Not indicated.
4.4.7	k_{pE}	Not indicated.
4.4.8	Half-life ($t_{1/2E}$)	Not indicated.
4.5	**Specification of the transformation products**	IPBC was converted to propargyl butyl carbamate (PBC) by photolytic cleavage of the carbon-iodine bond and release of the iodine.

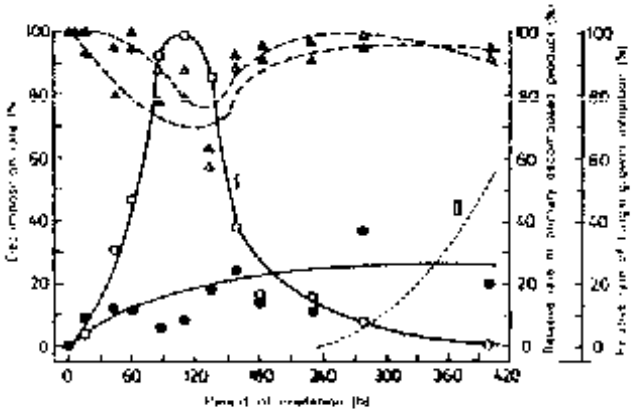
Section A7.1.1.1.2/01/02	**Phototransformation in water including identity of transformation products**
Annex Point IIA, VII.7.6.2.1	

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	IPBC was dissolved in ethanol and exposed to sunlight lamps and UV lamps for up to 50 days. In addition, wood slices were dipped in an IPBC solution and irradiated for up to 50 days. Extracts were analysed by GC-MS.
5.2	**Results and discussion**	In irradiated ethanol solutions approximately 25 % of the initial IPBC was degraded within 17 days of exposure. Results on the decomposition of IPBC in wood suggest that photodegradation will also occur in a thin layer of the wood surface. After 25 to 50 days of irradiation the recovery rate of IPBC decreased to approximately 40 to 50 %. IPBC was converted to propargyl butyl carbamate (PBC) by photolytic cleavage of the carbon-iodine bond and release of the iodine.
5.2.1	k^c_p	Not indicated
5.2.2	K_{pE}	Not indicated
5.2.3	ϕ^c_E	Not indicated
5.2.4	$t_{1/2E}$	Not indicated
5.3	**Conclusion**	The test results show that IPBC may be subject to photolytical degradation.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The publication shows methodological and reporting deficiencies. However, the test results show that IPBC may be subject to photolytical degradation.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2004, May 2022
Materials and Methods	The applicant's version is adopted.

Section A7.1.1.1.2/01/02 **Annex Point IIA, VII.7.6.2.1**	**Phototransformation in water including identity of transformation products**
Results and discussion	The degradation of IPBC increased to 20 to 25% during the first 200 hours. Based on figure 4 in reference A7.1.1.1.2/01 the decomposition levels off within the remainder of the 400 hours (approx. 17 days) light exposure period. The first decomposition product (PBC) reaches a peak after 108 hours and then disappears after 400 hours. A second decomposition product appears after 270 hours and still increases after 400 hours.  Fig. 4. Effects of light exposure on the decomposition rate (●—●), the relative rate of primary decomposed product (I) (○···○), the appearance of a secondary decomposed product (II) (·····), and the fungal-growth inhibitory rate of IPBC (△···△ : against *T. palustris*, ▲···▲ : against *C. versicolor*). The second reference finds that the photolytic process of IPBC involves cleavage of the C-I bond followed by the hydrogen substitution for iodine
Conclusion	IPBC may be subject to photolytical degradation but since only 25% was degraded after 400 hours (17 days) may still be considered stable to photolysis
Reliability	Reliability indicator 2 (lack of primary data, test solution in ethanol and not water)
Acceptability	Acceptable
Remarks	It should be noted that IPBC test solution was an ethanol solution and not a water solution at 400 ppm w/w and there was no dark control. The publication shows methodological and reporting deficiencies. However, the test results show that IPBC may be subject to photolytical degradation. The study is still considered reliable

Section A7.1.1.1.2/03

Annex Point IIA, VII.7.6.2.2

Addendum 2 to Dossier

Phototransformation in water including identity of transformation products

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2005): Aqueous photolysis of IPBC and determination of the quantum yield; [redacted]; Doc. No. 712-001 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	European Union IPBC (3-Iodo-2-propynyl-butylcarbamate) Task Force	
1.2.2	Companies with letter of access	No companies with Letter of Access	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I for all references listed above	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline for Testing of Chemicals, draft document, August 2000	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	
3.1	**Test material**	IPBC (3-Iodo-2-propynyl-butylcarbamate)	
3.1.1	Lot/Batch number	0111-4008	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.8% (wt/wt)	
3.1.4	Description of test substance	Crystalline solid	
3.1.5	Radiolabelling	Not indicated.	
3.1.6	UV/VIS absorption spectra and absorbance value	Absorption spectra were measured. The UV-absorption spectrum (200 to 400 nm) of IPBC in methanol/purified water 1:1 (v/v) at a concentration of 1 mg/ml is presented. Natural pond water was used as background. As a absorption maximum only a small shoulder at 230 nm could be detected.	
3.1.7	Further relevant properties	Not indicated.	
3.2	**Reference substance**	Propargyl-butyl-carbamate (PBC), batch: 20030728002, purity: 99.3%	
3.3	**Test solution**	1.977 mg IPBC/l solutions in sterilised aqueous pH 7 buffer and in natural water.	

Section A7.1.1.1.2/03 **Annex Point IIA, VII.7.6.2.2** **Addendum 2 to Dossier**	**Phototransformation in water including identity of transformation products**

3.4	**Testing procedure**	Simulated sunlight was used to irradiate solutions in sterilised aqueous pH 7 buffer and in natural water maintained at 25.3 ± 0.2 °C, respectively. Irradiation was for a continuous period of 3 days, with corresponding samples maintained under the same conditions but in the dark. At a range of time intervals (0, 1.65 and 2.92 days), samples were taken and subjected to chromatographic analysis and quantification by high performance liquid chromatography/mass spectrometry (LC/MS).
3.4.1	Test system	Irradiation of test solutions: The "Suntest" apparatus was equipped with a xenon arc lamp. Filters were used to cut off ultraviolet light with a wavelength below 290 nm. For a representative range (300 nm to 400 nm) of the whole visual light spectrum, the intensity of light was determined to be 39.7 W/m² at the surface of the photodegradation vessel. Duplicate 100 ml aliquots of treated buffer solution, previously sterilised by autoclaving (30 minutes at 120 °C) and duplicate 100 ml aliquots of treated natural pond water, sterilised by γ-irradiation, were continuously illuminated in the photolysis apparatus. The vessels were also sterilised by rinsing with an ethanol/water solution (70:30; v/v). All test solutions were cooled by means of a water jacket connected to a water bath at a temperature of 25.3 ± 0.2 °C and continuously stirred with magnetic stirrers. In addition to the irradiated samples, duplicate aliquots of 100 ml sterilised treated buffer solution and natural pond water were incubated under identical conditions in the dark using similar vessels as for the irradiated solutions
3.4.2	Properties of light source	"SUNTEST CPS, Original Hanau" equipped with a 1.8 kW xenon burner and an UV filter system (UV filter with a 290 nm cut-off to simulate natural sunlight.)
3.4.3	Determination of irradiance	The intensity of light was determined to be 39.7 W/m² at the surface of the photodegradation vessel.
3.4.4	Temperature	25.3 ± 0.2 °C
3.4.5	pH	Sterilised aqueous buffer solution: pH 7.0 to 7.1 Natural pond water: pH 8.2 to 8.5
3.4.6	Duration of the test	Up to 3 days.
3.4.7	Number of replicates	Duplicate aliquots of treated buffer solution and duplicate of treated natural pond water
3.4.8	Sampling	0, 1.65 and 2.92 days

Section A7.1.1.1.2/03
Annex Point IIA, VII.7.6.2.2
Addendum 2 to Dossier

Phototransformation in water including identity of transformation products

3.4.9	Analytical methods	Duplicate 4 ml aliquots were taken from the irradiated and dark control test solutions after 0, 39.5 and 70 hours of irradiation/incubation. The samples were directly analysed by LC/MS using a reversed phase column without clean-up of the sample. For elution a gradient is used consisting of solvent A: 0.1% formic acid in purified water/methanol (70/30 v/v) and solvent B: 0.1% formic acid in methanol. MS conditions were electron spray ionisation (ESI) as ionisation mode and the positive ion mode as the detection mode. A m/z 282 is used for the detection of IPBC and m/z 156 used for detection of PBC.
3.4.10	Calculations	A calibration curve for IPBC and its degradation product PBC was obtained by correlation of the peak areas of the calibration solutions with the corresponding concentration of the respective compound in the injected sample (mg/l). The concentration of IPBC in each sample was calculated by the calibration curve and the obtained peak area.
3.5	**Transformation products**	In both systems no degradation of IPBC was detected throughout the irradiation period. After 3 days the amounts quantified in the irradiated samples were virtually identical with those of the dark controls.
3.5.1	Method of analysis for transformation products	As described under 3.4.9
		4 RESULTS
4.1	**Screening test**	Not indicated
4.2	**Actinometer data**	Not indicated
4.3	**Controls**	Dark control samples were analysed in the same way as the irradiated solutions. Mean amounts of IPBC in the samples incubated for 70 hours represented 100.9% in the dark controls of the buffered system, while for the natural pond water amounts of 100.9% were detected in the in the dark control samples (all percentages given in % of the applied amount.)
4.4	**Photolysis data**	Mean amounts of IPBC in the samples incubated for 70 hours represented 100.7% in the irradiated samples of the buffered system, while for the natural pond water amounts of 100.6% were detected. (all percentages given in % of the applied amount)
4.4.1	Concentration values	In both systems (buffer solution and natural pond water) no degradation of IPBC was detected throughout the irradiation period.
4.4.2	Mass balance	The total amounts recovered during the 70 hours irradiation period ranged from 100.0% to 102.0% of the initially applied amount in sterilised buffer solutions. The recovered amounts from the dark control solutions represented between 99.0% and 105.0%. In the irradiated samples from the natural pond system recoveries ranged from 100.6% to 104.7%. Corresponding values for the dark controls were 99.5% and 102.9%.

Section A7.1.1.1.2/03 **Annex Point IIA, VII.7.6.2.2** **Addendum 2 to Dossier**	**Phototransformation in water including identity of transformation products**

4.4.3	k^c_p	Not indicated.
4.4.4	Kinetic order	Not indicated.
4.4.5	k^c_p / k^a_p	Not indicated.
4.4.6	Reaction quantum yield (ϕ^c_E)	Not indicated.
4.4.7	k_{pE}	Not indicated.
4.4.8	Half-life ($t_{1/2E}$)	Not indicated.
4.5	**Specification of the transformation products**	Not indicated.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The rate of photochemical degradation of IPBC was determined under simulated sunlight in sterilised aqueous buffer solution at pH 7 and natural pond water at a pH value of about 8.5. Irradiation was for a continuous period of 3 days, with corresponding samples maintained under the same conditions but in the dark. Samples of these solutions were analysed by LC-MS.
5.2	**Results and discussion**	The results show that IPBC was stable within 3 days of continuous irradiation (corresponding to 6.1 days natural summer sunlight at latitude 50° N). Since IPBC was stable during the incubation period no half-lives and no quantum yield could be calculated.
5.2.1	k^c_p	Not indicated
5.2.2	K_{pE}	Not indicated
5.2.3	ϕ^c_E	Not indicated
5.2.4	$t_{1/2E}$	Not indicated
5.3	**Conclusion**	The results of the study demonstrate that IPBC is stable to direct and indirect photolysis in the aquatic environment.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 06, May 2022
Materials and Methods	Applicant's version is acceptable.

Section A7.1.1.1.2/03 **Annex Point IIA, VII.7.6.2.2** **Addendum 2 to Dossier**	**Phototransformation in water including identity of transformation products**

Results and discussion	Adopt applicant's version
Conclusion	The applicant’s version is adopted
Reliability	Reliability indicator: 1
Acceptability	Acceptable
Remarks	The study is still considered reliable

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

4.2.1.1 No new relevant data

4.2.2 Bioaccumulation test with other organisms

4.2.2.1 No new relevant data

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

4.3.1.1 No new relevant data

(One article identified in the open literature search for the environment reported results on IPBC tested in the fish embryo toxicity test corresponding to OECD 236, and is mentioned in Section A.4.2 Effects on environmental organisms of the CLH report. The findings are considered consistent with the information on acute fish toxicity of the original CLH report, thus are not considered relevant new data. A copy of the study is provided below.)

Coors et al. (2012) - Mixture toxicity of wo

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 No new relevant data

4.3.3 Algal growth inhibition tests

Section A7.4.1.3/01	**Growth inhibition test on algae**	
Annex Point IIA, VII.7.3	***Scenedesmus subspicatus***	
	1 REFERENCE	**Official use only**
1.1 Reference	[redacted] (2001): Toxicity of Polyphase P-100 to *Scenedesmus subspicatus* in a 72-hour Algal Growth Inhibition Test; [redacted]; Doc. No. 823-003; (unpublished)	

Section A7.4.1.3/01 **Annex Point IIA, VII.7.3**		**Growth inhibition test on algae** ***Scenedesmus subspicatus***
1.2	**Data protection**	Yes
1.2.1	Data owner	Troy Corporation
1.2.2	Companies with letter of access	Arch Chemicals, Bayer Chemicals and Sostram Corporation
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD No. 201 (1984) and European Commission Directive 92/69/EEC, C.3 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIAL AND METHODS
3.1	**Test material**	Technical active substance IPBC (Polyphase P-100)
3.1.1	Lot/Batch number	0011-1954
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.1 %
3.1.4	Description of test substance	Off-white solid
3.1.5	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product
3.1.6	Further relevant properties	Detailed information is given in Section A3 (physical and chemical properties of active substance).
3.1.7	Method of analysis	Extraction with dichloromethane Elution with purified water and acetonitrile Analysing with HPLC with MS detection.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant. The stock solution was prepared with test water.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Details are given in table A7.4.1.3/01-2

Section A7.4.1.3/01 **Annex Point IIA, VII.7.3**		**Growth inhibition test on algae** ***Scenedesmus subspicatus***
3.4.2	Test organisms	*Scenedesmus subspicatus*, details are given in table A7.4.1.3/01-3
3.4.3	Test system	Details are given in table A7.4.1.3/01-4
3.4.4	Test conditions	Details are given in table A7.4.1.3/01-5
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	Inhibition of cell growth
3.4.7	Sampling	Daily
3.4.8	Monitoring of TS concentration	Yes, at the beginning and at the end of the test
3.4.9	Statistics	EC_{50} values were calculated by Probit Analysis For the determination of LOEC and NOEC, the mean values of the test concentration were compared to the control values by a Dunnett-test
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1.0, 2.2, 4.6, 10, 22, 46 µg/L (nominal)
4.2.2	Actual concentrations of test substance	Measured concentrations at test termination showed sufficient stability of the test substance during the test period. Therefore, values are given as nominal values. Details are given in table A7.4.1.3/01-6
4.2.3	Growth curves	The figure of the growth curves is provided in the report (see reference above)
4.2.4	Concentration / response curve	No slope is given
4.2.5	Cell concentration data	Effects on cell concentration (growth inhibition) are given in table A7.4.1.3/01-7
4.2.6	Effect data (cell multiplication inhibition)	EC_{50} values as well as NOEC values are given in table A7.4.1.3/01-8
4.2.7	Other observed effects	The test substance did not affect size or shape of the cells
4.3	**Results of controls**	

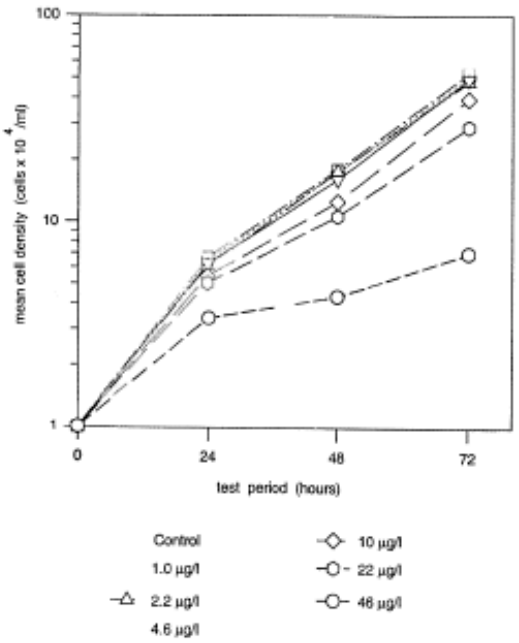
Section A7.4.1.3/01 **Annex Point IIA, VII.7.3**		**Growth inhibition test on algae** ***Scenedesmus subspicatus***	
4.3.1	Nature of adverse effects	No adverse effects were observed in the control	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was conducted according to OECD guideline 201 and EU Commission Directive 92/69/EEC, C.3. It was a static test-system and *Scenedesmus subspicatus* was used as test organism.	
5.2	**Results and discussion**	The properties of the test substance gave no indications to assume any relevant influences on the results. No effects on algae cells other than growth inhibition were noted. The analysis of the test media indicated a sufficient water solubility and stability of the test substance in the test medium within the course of the test (72 hours).	
5.2.1	NOEC (biomass)	4.6 µg/L	
5.2.2	NOEC (growth rate)	4.6 µg/L	
5.2.3	E_bC_{50}	22 µg/L	
5.2.4	E_rC_{50}	53 µg/L	
5.3	**Conclusion**	The mean increase in the number of cells in the control test vessel by a factor of about 50.5 during the course of the test (72 hours) was sufficient to fulfil the validity criteria (see table A7.4.1.3/01-9 Based on the results the EC_{50} (biomass) was calculated to be 22 µg/L, the EC_{50} (growth rate) was determined to be 53 µg/L. The NOEC for biomass as well as for growth rate was determined to be 4.6 µg/L.	
5.3.1	Other Conclusions	Not applicable	
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004, May 2022
Materials and Methods	The applicant's version is adopted
Results and discussion	The applicant's version is adopted

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA, VII.7.3 ***Scenedesmus subspicatus***

Conclusion	EC_{50} (biomass) was calculated to be 22 µg/L, EC_{50} (growth rate) was determined to be 53 µg/L. NOEC was determined to be 4.6 µg/L.
Reliability	Reliability indicator 1
Acceptability	Acceptable
Remarks	Include fig 1 growth rate from reference in section 4.2.3:



The effect data should include the EC10 values:

E_bC10 (72 h, biomass): 5.8 µg/l (95% confidence limits 3.1-8.4)

$E_\mu C10$ (72 h, growth rate): 13 µg/l (95% confidence limits 3.7-21)

The study is considered acceptable. The OECD guideline 201 was updated in 2011, however it is not considered to have an significant impact on the conclusion on toxicity towards algae.

Table A7.4.1.3/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	n.a.
Concentration of vehicle	n.a.
Vehicle control performed	n.a.
Other procedures	n.a.

Table A7.4.1.3/01-2: Culture medium (according to OECD 201)

Nutrient	Concentration
$NaHCO_3$	50.0 mg/L
$CaCl_2$ x 2 H_2O	18.0 mg/L
NH_4Cl	15.0 mg/L
$MgSO_4$ x 7 H_2O	15.0 mg/L
$MgCl_2$ x 6 H_2O	12.0 mg/L
KH_2PO_4	1.6 mg/L
Na_2EDTA x 2 H_2O	100 µg/L
$FeCl_3$ x 6 H_2O	80.0 µg/L
$MnCl_2$ x 4 H_2O	415.0 µg/L
H_3BO_3	185 µg/L
Na_2MoO_4 x 2 H_2O	7.0 µg/L
$ZnCl_2$	3.0 µg/L
$CoCl_2$ x 6 H_2O	1.5 µg/L
$CuCl_2$ x 2 H_2O	0.01 µg/L

Table A7.4.1.3/01-3: Test organism

Criteria	Details
Species	*Scenedesmus subspicatus* CHODAT
Strain	No. 86.81 SAG
Source	Sammlung von Algenkulturen, Göttingen, Germany
Laboratory culture	Yes
Method of cultivation	The algae had been grown in the laboratory under standardized conditions.
Pre-treatment	An exponentially grown pre-culture was set up three days prior to the test at the same conditions as in the test.
Initial cell concentration	10^4 cells/mL

Table A7.4.1.3/01-4: Test system

Criteria	Details
Volume of culture flasks	50 mL
Culturing apparatus	Electronic particle counter
Light quality	Fluorescent tubes
Procedure for suspending algae	Algae suspension was continuously stirred by magnetic stirrers
Number of vessels/ concentration	Three

Test performed in closed vessels due to significant volatility of TS	The flasks were covered with glass dishes to avoid contamination

Table A7.4.1.3/01-5: Test conditions

Criteria	Details
Test temperature	21 °C
pH	7.9 – 8.2
Aeration of dilution water	No
Light intensity	8500 to 9270 Lux
Photoperiod	Continuous illumination

Table A7.4.1.3/01-6: Concentrations of test substance in test medium

Nominal concentrations of test substance (µg/L)	Measured concentration (µg/L)					
	Day 0		Day 3		Mean	Percent of Nominal
	A	B	A	B		
Control	< 0.001*	ND	< 0.001*	ND	—	—
1.0	NA	NA	NA	NA	—	—
2.2	NA	NA	NA	NA	—	—
4.6	4.4	3.6	3.5	3.5	3.8	82
10	10.2	9.2	8.7	8.9	9.3	93
22	20.1	19.2	20.6	21.7	20.4	93
46	42.0	41.0	42.5	39.9	41.4	90

* = the obtained value was below the LOQ of 0.001 mg/L

ND = not determined

NA = not analysed, because values were below the NOEC

Table A7.4.1.3/01-7: Cell concentration data

Test-Substance Concentration (nominal) [µg/L]	Cell concentrations (mean values) [10^4 cells/mL]							
	Cell density				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
control	1	6.47	17.32	50.48	–	–	–	–
1.0	1	6.68	17.90	52.50	100	103	103	104
2.2	1	6.07	17.52	47.78	100	94	101	94
4.6	1	6.23	16.03	49.30	100	96	93	97

10	1	5.43	12.52	39.50	100	84	72	78
22	1	5.02	10.60	28.77	100	78	61	57
46	1	3.40	4.35	7.00	100	53	25	14
Temperature [°C]	21	21	21	21				
pH	7.9	ND	ND	8.2				

ND = not determined

Table A7.4.1.3/01-8: Effect data

	EC_{50}[1]	95 % C.L.	NOEC[1]
24 h [µg/L]	ND	ND	4.6
48 h [µg/L]	ND	ND	4.6
72 h [µg/L] (biomass)	22	17 – 33	4.6
72 h [µg/L] (growth rate)	53	32 – ND	4.6

[1] data are based on nominal concentrations

ND = not determined

Table A7.4.1.3/01-9: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test[1]		yes

Criteria for poorly soluble test substances	n.a.	n.a.

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA, VII.7.3 ***Selenastrum capricornutum***

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994): Growth and Reproduction Test with Omacide® IPBC and the Freshwater Alga, *Selenastrum capricornutum*; [redacted]; Doc. No. 823-001; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Arch Chemicals	
1.2.2	Companies with letter of access	Bayer Chemicals, Sostram Corporation and Troy Corporation	

Section A7.4.1.3/02		**Growth inhibition test on algae**
Annex Point IIA, VII.7.3		***Selenastrum capricornutum***
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, U.S. EPA-FIFRA 122-2, which is comparable to OECD 201
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIAL AND METHODS
3.1	**Test material**	Technical active substance IPBC (Omacide® IPBC)
3.1.1	Lot/Batch number	2DR-293-TSI
3.1.2	Specification	As given in Section A2. The purity of the test substance was slightly lower than the specification given in section 2. This does not influence the integrity of the study.
3.1.3	Purity	97.5 %
3.1.4	Description of test substance	White lumpy solid
3.1.5	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product
3.1.6	Further relevant properties	Detailed information is given in Section A3 (physical and chemical properties of active substance).
3.1.7	Method of analysis	Solid Phase Extraction with a pre-conditioned BOND-ELUT® C18 column Elution with 1 mL of acetonitrile followed by 1 mL HPLC water Analysing with HPLC with UV detection at a wavelength of 215 nm
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Methanol was used as a solvent, details are given in Table A7.4.1.3/02-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Sterile enriched media, adjusted to a pH of 7.5 was used. The composition of the media is not given.
3.4.2	Test organisms	Details are given in table A7.4.1.3/02-2
3.4.3	Test system	Details are given in table A7.4.1.3/02-3

Section A7.4.1.3/02	**Growth inhibition test on algae**	
Annex Point IIA, VII.7.3	***Selenastrum capricornutum***	
3.4.4	Test conditions	Details are given in table A7.4.1.3/02-4
3.4.5	Duration of the test	120 hours
3.4.6	Test parameter	Inhibition of cell growth
3.4.7	Sampling	Daily
3.4.8	Monitoring of TS concentration	Yes, at the beginning and at the end of the test
3.4.9	Statistics	EC_{50} values were calculated by non-linear interpolation. The 95 % confidence limits were calculated by the binomial method and the 120 hour NOEC was calculated by ANOVA, followed by Dunnett's test
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.060, 0.12, 0.24, 0.30, 0.60 mg/L (nominal, according to the biological part of the study) 0.090, 0.15, 0.24, 0.36, 0.60 mg/L (nominal, according to the analytical part of the study) 0.089, 0.16, 0.30, 0.36, 0.68 (initial measured)
4.2.2	Actual concentrations of test substance	Measured concentrations at test termination (120 hour) were all less than the analytical detection limit of 0.050 mg/L Details are given in table A7.4.1.3/02-5
4.2.3	Growth curves	Not provided.
4.2.4	Concentration / response curve	No slope is given
4.2.5	Cell concentration data	Details are given in table A7.4.1.3/02-6
4.2.6	Effect data (cell multiplication inhibition)	Details are given in table A7.4.1.3/02-7
4.2.7	Other observed effects	Incubation of test media containing 0.16 mg/L of test substance in the presence of algae for a period of 9 days indicated that the effect of the test substance on the cells was algistatic rather than algicidal.
4.3	**Results of controls**	
4.3.1	Nature of adverse effects	No adverse effects were observed in the control

Section A7.4.1.3/02	**Growth inhibition test on algae**
Annex Point IIA, VII.7.3	***Selenastrum capricornutum***

4.4	**Test with reference substance**	Not applicable
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was conducted according to EPA-FIFRA guideline 122-2. It was a static test-system and *Selenastrum capricornutum* was used as test organism.
5.2	**Results and discussion**	The properties of the test substance gave no indications to assume any relevant influences on the results. No effects on algae cells other than growth inhibition were noted. The analysis of the test media indicated a sufficient water solubility and degradation of the test substance in the test medium within the course of the test. At test termination, concentration of test substance was below the limit of detection.
5.2.1	NOEC	< 0.089 mg/L (given as 120 hour value, based on cell number)
5.2.2	EC_{50}	0.10 mg/L (given as 120 hour value, based on cell number)
5.3	**Conclusion**	The mean increase in the number of cells in the control test vessel by a factor of about 70 during the first 72 hours was sufficient to fulfil the validity criteria (see table A7.4.1.3/02-8) Based on the results, the EC_{50} was calculated to be 0.10 mg/L, based on cell number and initial measured values. The NOEC was determined to be < 0.089, the lowest concentration tested, also based on initial measured values.
5.3.1	Other Conclusions	Not applicable
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004, May 2022
Materials and Methods	The applicant's version is acceptable
Results and discussion	The results of the test on *Selenastrum capricornutum* EC50 (120 h) 0.10 mg/L (based on cell number) NOEC (120 h) < 0.089 mg/L (based on cell number) At test termination, concentration of test substance was below the limit of detection (0.050 mg/l).
Conclusion	EC50 (72 h) 0.10 mg/L (based on cell number)
Reliability	Reliability indicator 3

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA, VII.7.3 ***Selenastrum capricornutum***

Acceptability	Not acceptable Test period 120 hours too long, The cell density values indicate that the algae population no longer increases exponentially. Effect data rely on 2 to 3 values which is insufficient for an adequate curve fitting NOEC value unbound, i.e. significant effect (inhibition) at the lowest concentration used already after 48 hours. pH was measured to 10.2 in the 2 controls and the lowest test concentration and 7.8 to 8.0 in the remaining concentrations. The high pH value and its potential effect was not discussed. The light intensity was 390 foot-candles equivalent to 4200 Lux. In the OECD guidance is recommended approximately 8000 lux although the US guideline does accept 400 foot-candles. Despite the flaws of the study another acceptable study exist and thus a repeat is not necessary
Remarks	In the study there appears to be some confusion about the nominal concentrations used although the initial measured concentrations are used to calculate the results.

Table A7.4.1.3/02-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, Methanol was used as a solvent
Concentration of vehicle	The concentration of the solvent did not exceed 0.1 mL/L
Vehicle control performed	Yes, a vehicle control was performed with 0.1 mg Methanol/L
Other procedures	No

Table A7.4.1.3/02-2: Test organisms

Criteria	Details
Species	Selenastrum capricornutum
Strain	UTEX 1648
Source	Culture Collection of Algae at the University of Texas at Austin
Laboratory culture	Yes
Method of cultivation	The culture was maintained at test conditions for more than 14 days
Pre-treatment	Subculture used for the testing had been growing for 6 days prior to testing

Initial cell concentration	10^4 cells/mL

Table A7.4.1.3/02-3: Test system

Criteria	Details
Volume of culture flasks	250 mL
Culturing apparatus	Microscope, Hemacytometer
Light quality	Cool-white fluorescent lights
Procedure for suspending algae	Arranged on a rotary shaker adjusted to 100 rpm
Number of vessels/ concentration	Three
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3/02-4: Test conditions

Criteria	Details
Test temperature	23.9 – 24.9 °C
pH	7.5 – 10.3
Aeration of dilution water	No
Light intensity	390 foot-candles
Photoperiod	24 hour light, 0 hour dark

Table A7.4.1.3/02-5: Concentrations of test substance in test medium

Nominal concentrations of test substance (mg/L)	Measured concentration (mg/L)			
	Day 0	Day 5	Mean	Percent of Nominal
Control	ND	ND	ND	—
Solvent Control	ND	ND	ND	—
0.090	0.089	ND	—	—
0.15	0.16	ND	—	—
0.24	0.30	ND	—	—
0.36	0.36	ND	—	—
0.60	0.68	ND	—	—

ND = not detected (LOQ = 0.050 mg/L)

Table A7.4.1.3/02-6: Cell concentration data

Test-Substance Concentration (initial measured) [mg/L]	Cell concentrations (mean values) [10^3 cells/mL]											
	measured						Percent of control					
	0 h	24 h	48 h	72 h	96 h	120 h	0 h	24 h	48 h	72 h	96 h	120 h
Control	10	41	247	703	2466	2388	–	–	–	–	–	–
Solvent control	10	41	221	733	1806	2028	–	100	89	104	73	85
0.089	10	39	172	419	943	1635	100	95	70	60	38	68
0.16	10	19	13	< 11	< 10	< 10	100	46	5	<2	<1	<1
0.30	10	17	11	< 10	< 10	< 10	100	41	4	<1	<1	<1
0.36	10	12	< 10	< 10	< 10	< 10	100	29	<4	<1	<1	<1
0.68	10	< 11	< 10	< 10	< 10	< 10	100	<27	<4	<1	<1	<1
Temperature [°C]	24.8	24.9	24.9	24.7	23.9	24.5						
pH	7.5	ND	ND	ND	ND	10.2						

ND = not determined

Table A7.4.1.3/02-7: Effect data

	EC_{50}[1]	95 % C.L.	NOEC[1]
24 h [mg/L]	0.15	0.089 – 0.36	ND
48 h [mg/L]	0.10	0.089 – 0.16	ND
72 h [mg/L]	0.095	0.089 – 0.16	ND
96 h [mg/L]	> 0.089	—	ND
120 h [mg/L]	0.10	0.089 – 0.16	< 0.089

[1] data are based on initial measured concentrations

ND = not determined

— = not given

Table A7.4.1.3/02-8: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test[1]		yes

[1] This is not a validity criterion according to EPA-FIFRA. Due to adsorption, the concentration fell below the limit of the detection of the test substance at test termination. Therefore, the initial measured values were used for calculation of the EC_{50} instead of the nominal values.

Criteria for poorly soluble test substances	n.a.	n.a.

4.3.4 *Lemna* sp. growth inhibition test

4.3.4.1 No new relevant data

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

Section A7.4.3.2/01 Effects on reproduction and growth rate of fish

Annex Point IIIA, XIII.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1992): Troysan Polyphase P-100 – Toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae; [redacted]; Doc. No 826-001; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Corporation	
1.2.2	Companies with letter of access	Arch Chemicals, Bayer Chemicals and Sostram Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA Guideline 72-4, which is comparable to OECD 210	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIAL AND METHODS	
3.1	**Test material**	Technical active substance IPBC (Tryosan Polyphase P-100)	
3.1.1	Lot/Batch number	9102-6111	
3.1.2	Specification	As given in section A2. The purity of the test substance was slightly lower than the specification given in section A2. This does not influence the integrity of the study.	
3.1.3	Purity	97.25 %	
3.1.4	Description of test substance	Off-white crystalline solid	
3.1.5	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product	
3.1.6	Further relevant properties	Detailed information is given in Section A3 (physical and chemical properties of active substance).	

Section A7.4.3.2/01 **Annex Point IIIA, XIII.2.2**		**Effects on reproduction and growth rate of fish**
3.1.7	Method of analysis	Extraction with acetone and transfer into 40% acetonitrile and 60% water HPLC-UV column: Phenomex Ultramex C_{18} (5 µm), 250 mm x 4.6 mm mobile phase: 70 % Acetonitrile : 30 % water (nanopure) flow rate: 1.0 mL/min wavelength: 240 nm injection volume: 100 µL
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Acetone was used as solubilizing agent. Details are given in table A7.4.3.2/01-1.
3.3	**Reference substance**	Yes IPBC (Tryosan Polyphase P-100), purity: 99.6 %, batch: QC2-192
3.3.1	Method of analysis for reference substance	see 3.1.7
3.4	**Testing procedure**	
3.4.1	Dilution water	Details are given in table A7.4.3.2/01-2.
3.4.2	Test organisms	Fathead minnow (*Pimephales promelas*), details are given in table A7.4.3.2/01-3).
3.4.3	Handling of embryos and larvae (OECD 210/212)	Fourteen unlabelled, unassigned petri dishes were set in a water bath. A small amount of water from the control aquaria was placed in each dish. The collected eggs were then counted into each dish sequentially (60 eggs per dish). Fourteen incubation cups were placed in control water. Each group of 60 eggs in the petri dishes was impartially transferred to one of the fourteen incubation cups. At test initiation the incubation cups were placed into the test aquaria. The 30 day post-hatch larval exposure was initiated when 40 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 5 and placed into their respective exposure aquaria.
3.4.4	Test system	Details are given in table A7.4.3.2/01-4.
3.4.5	Test conditions	Details are given in table A7.4.3.2/01-5.
3.4.6	Duration of the test	35 days
3.4.7	Test parameter(s)	Embryo survival at hatch, larval survival, larval growth (total length and wet weight)
3.4.8	Examination / Sampling	*Examination:* Dead embryos were counted daily until hatching was complete. Post hatching dead larvae were removed when observed and behaviour and appearance of larvae were observed and recorded daily. Larval survival was estimated at last twice weekly. *Sampling:* Water samples were taken from both replicate test solutions of each treatment level and the controls on days 0, 5, 12, 19, 26, 29, 33 and 35.

Section A7.4.3.2/01 Effects on reproduction and growth rate of fish

Annex Point IIIA, XIII.2.2

3.4.9	Monitoring of TS concentration	Yes The samples mentioned in 3.4.7 were analysed for TS concentration.
3.4.10	Statistics	ANOVA was conducted for each endpoint to compare the performance of the control organisms with that of the solvent control organisms. Since these comparisons indicated that the presence of acetone in the exposure solutions did not affect hatchability, survival or growth of the test organisms, the data for the controls and the solvent controls were pooled for subsequent statistical comparisons. All statistical comparisons of treatment levels with the pooled control were made using Williams´ Test.
		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentrations	Test I: 6.2 – 100 µg/L Test II: 31, 62, 120, 250 and 500 µg/L
4.1.2	Number/ percentage of animals showing adverse effects	Test I: After 13 days exposure, ≤ 20% mortality was recorded throughout the treatment level range. Test II: After 96 h of exposure, mortality of 90% was observed in the 500 µg/L treatment level. No mortalities were observed in the remaining treatment levels and in the control solutions
4.1.3	Nature of adverse effects	Sublethal effects (i.e. complete loss of equilibrium, lethargy) were observed among surviving fish in the 250 and 120 µg/L treatment levels.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	6.2, 12, 25, 50 and 100 µg/L; based on the purity of the (97.25 %) TS
4.2.2	Actual concentrations of test substance	4.0, 8.4, 19, 27 and 57 µg/L (mean measured concentrations), see table A7.4.3.2/01-6

Section A7.4.3.2/01	**Effects on reproduction and growth rate of fish**	
Annex Point IIIA, XIII.2.2		
4.2.3	Effect data	Data on survival of organisms at hatch (test day 5) and survival, total length and wet weight of fish larvae determined at test end (30 days post-hatch) are given in table A7.4.3.2/01-7. Further effect data: - time to start of hatching and end of hatching: Hatching was complete on day 5 since no unhatched viable embryos remained in any control or treatment level egg incubation cup. The time to hatch (to the nearest day) for each treatment level and control was thus identical (i.e., 5 days) - numbers of larvae hatching each day: No data available. - numbers of deformed larvae: No abnormal larvae appearance is reported. - numbers of fish exhibiting abnormal behaviour: No abnormal fish behaviour is reported. NOEC and LOEC values are represented in table A7.4.3.2/01-8. Values are based on mean measured concentrations.
4.2.4	Concentration / response curve	No data available.
4.2.5	Other effects	No other effects were observed.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	The effect data is given in table A7.4.3.2/01-7. No sublethal effects were observed in the controls.
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was conducted according to FIFRA Guideline 72-4. The test system was flow-through and fathead minnow was used as test organism.
5.2	**Results and discussion**	Concentrations above the water solubility were tested by using Acetone as solubilizing agent
5.2.1	NOEC	8.4 µg/L (35 days, parameter: larval growth (length and weight)
5.2.2	LOEC	19 µg/L (35 days, parameter: larval growth (length and weight)

Section A7.4.3.2/01 Effects on reproduction and growth rate of fish

Annex Point IIIA, XIII.2.2

5.3	**Conclusion**	The validity criteria as given in table A7.4.1.1/01-8 can be considered as fulfilled. Though the mean measured test concentrations averaged 64 % of the nominal test concentrations, the results obtained are considered as valid, since they were based on mean measured test concentrations. The 35 d-NOEC was determined to be 8.4 µg/L based on larval growth (length and weight).
5.3.1	Other Conclusions	No other conclusions.
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2004, May 2022
Materials and Methods	The applicant's version is adopted.
Results and discussion	The applicant's version is adopted.
Conclusion	The 35 days ELS test on the fish *P. promelas* resulted in a NOEC of 8.4 µg/l based on larval growth.
Reliability	Reliability indicator 1
Acceptability	Acceptable
Remarks	The test guideline was not amended since the study was conducted, hence the study is still considered acceptable.

Table A7.4.3.2/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	max. 0.014 mL Acetone/L
Vehicle control performed	Yes 0.010 mL Acetone/L
Other procedures	n.a.

Table A7.4.3.2/01-2: Dilution water

Criteria	Details
Source	well water
Salinity	20 – 22 mg CaCO3/L
Hardness	25 – 27 mg CaCO3/L

pH	7.1 – 7.3
Oxygen content	No data available
Conductance	110 – 140 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.2/01-3: Test organisms

Criteria	**Details**
Species/strain	Fathead minnow (*Pimephales promelas*)
Source	brood stock at [redacted]
Wild caught	No
Age/size	age of embryos: < 24 h length: n.a. (embryos) weight: n.a. (embryos)
Kind of food	The fry were fed live brine shrimp (*Artemia salina*) three times on weekdays and two times daily on weekends.
Amount of food	See above.
Feeding frequency	see above.
Post-hatch transfer time	Eggs were introduced at test initiation. The hatch was completed on day 5.
Time to first feeding	On day 5 after completion of hatch.
Feeding of animals during test	Yes See above.
Treatment for disease within 2 weeks preceding test	No. Eggs were introduced at test initiation.

Table A7.4.3.2/01-4: Test system

Criteria	**Details**
Test type	Flow-through
Renewal of test solution	flow-rate: 6.5 volume replacement/24 h
Volume of test vessels	11 L
Volume/animal	1.2 L per egg and day
Number of animals/vessel	At test initiation: 60 eggs
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2/01-5: Test conditions

Criteria	Details
Test temperature	24 – 26 °C
Dissolved oxygen	4.0 – 8.1 mg/L
pH	6.7 – 7.2
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	20 – 55 foot-candles
Photoperiod	16 h photoperiod daily

Table A7.4.3.2/01-6: Actual concentrations of test substance in replicate (A, B) solutions

Nominal concentrations of test substance [µg/l]		Measured concentrations of test substance [µg/l]							
		0 d	5 d	12 d	19 d	26 d	29 d	33 d	35 d
Control	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Solv. Control	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6.2	A	5.0	4.8	4.2	<LOQ	4.3	2.4	4.4	0.89
	B	5.2	5.2	4.1	3.6	4.7	3.1	3.9	2.0
12	A	9.8	21	5.2	6.2	6.0	7.3	7.6	3.4
	B	9.1	16	7.2	5.2	10	8.8	8.8	3.4
25	A	26	18	16	18	13	18	29	11
	B	18	18	14	17	13	20	21	4.6
50	A	34	34	24	20	25	17	36	26
	B	37	35	16	19	16	16	36	22
100	A	68	67	55	52	57	42	68	47
	B	71	71	57	49	66	51	50	49

Table A7.4.3.2/01-7: Data on survival of organisms at hatch (test day 5) and survival, total length and wet weight of fish larvae determined at test end (30 days post-hatch)

Mean measured test substance concentration [µg/l]	Survival of organisms at hatch [%]	Larval survival [%]	Total length [mm]	Total weight [mg]
Control	88	84	32.6	334
Solv. Control	95	95	32.9	346
Pooled Control	91	89	32.7	340
4.0	88	89	33.1	343
8.4	85	90	32.7	329
19	89	84	30.8*	284*
27	92	86	30.1*	286*
57	83*	76	27.1*	189*

*** Statistically ($p \leq 0.05$) different as compared to the pooled control data.**

Table A7.4.3.2/01-8: NOEC and LOEC values

Endpoint	NOEC [µg/L]	LOEC [µg/L]
Survival of organisms at hatch	27	57
Larval survival	57	> 57
Total larval length	8.4	19
Total larval weight	8.4	19

Table A7.4.3.2/01-9: Validity criteria for fish tests according to OECD Guidelines 210/212

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	-	yes*
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	yes	-
Overall survival of fertilized eggs in controls (and solvent controls) ≥ 70% value, specified for the specific test species	yes	

* 60% saturation of dissolved oxygen for a temperature of 26°C corresponds to 4.8 mg/L. Mean concentrations of dissolved oxygen were determined to be 6.5 ± 0.99 to 7.0 ± 0.72. It can be concluded that this criteria was fulfilled sufficiently.

Test substance concentrations maintained within ± 20% of mean measured values		yes*
No effect on survival nor any other adverse effect found in solvent control	yes**	
Further criteria for poorly soluble test substances	yes	

*** However, results obtained are considered as valid, since they were based on mean measured test concentrations.**
** Survival was distinctly greater than the trigger given in OECD 210.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

4.4.2.1 No new relevant data

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

4.4.3.1 No new relevant data

4.4.4 Chronic toxicity to aquatic invertebrates

Section A7.4.3.4/01 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA, XIII.2.4 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991): Troysan Polyphase P100: Chronic Toxicity to the Water Flea *Daphnia magna* under Flow-Through Test Conditions, [redacted]; Doc. No. 827-001; (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Corporation	
1.2.2	Companies with letter of access	Arch Chemicals; Bayer Chemicals and Sostram Corporation	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, U.S. EPA-FIFRA 72-4 (1982) and OECD 202 (1984)	
2.2	**GLP**	Yes	

Section A7.4.3.4/01		**Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA, XIII.2.4		*Daphnia magna*
2.3	**Deviations**	No
		3 MATERIAL AND METHODS
3.1	**Test material**	Technical active substance IPBC (Troysan Polyphase P100)
3.1.1	Lot/Batch number	9007-5747
3.1.2	Specification	As given in section A2. The purity of the test substance was slightly lower than the specification given in section 2. This does not influence the integrity of the study.
3.1.3	Purity	97 %
3.1.4	Description of test substance	Off-white crystalline solid
3.1.5	Composition of Product	Not relevant because the active substance was tested, not, however, a formulated product
3.1.6	Further relevant properties	Detailed information is given in Section A3 (physical and chemical properties of active substance).
3.1.7	Method of analysis	Solid Phase Extraction with pre-conditioned BOND-ELUT® C18 columns Elution with HPLC water and acetonitrile Analysing with HPLC with UV detection at a wavelength of 205 nm
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Dimethylformamide (DMF) was used as a solvent, details are given in table A7.4.3.4/01-1.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	Details are given in table A7.4.3.4/01-2
3.4.2	Test organisms	*Daphnia magna*, details are given in table A7.4.3.4/01-3
3.4.3	Handling of offspring	Survival and reproduction of the daphnids were monitored daily
3.4.4	Test system	Details are given in table A7.4.3.4/01-4
3.4.5	Test conditions	Details are given in table A7.4.3.4/01-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Mortality, growth, reproduction

Section A7.4.3.4/01		**Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA, XIII.2.4		*Daphnia magna*
3.4.8	Examination / Sampling	Survival and reproduction were monitored daily, growth was determined at the end of the test
3.4.9	Monitoring of TS concentration	Yes, samples were taken on day 0, 7, 10, 14, 17 and 21
3.4.10	Statistics	EC_{50} values were determined by moving average angle, probit, logit and non-linear interpolation, Confidence limits for EC_{50} values determined by non-linear interpolation were calculated by binomial probability Mortality, number of offspring and growth of daphnids were evaluated by ANOVA, followed by a Dunnett's test
		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentrations	10, 50, 100, 500, 1000, 5000 µg/L under static conditions for a period of 4 days
4.1.2	Number/ percentage of animals showing adverse effects	At the end of the exposure period, mortality of daphnids was 0 to 10 percent in nominal concentrations ≤ 100 µg/L and 100 percent in concentrations ≥ 500 µg/L.
4.1.3	Nature of adverse effects	Mortality
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	12.5, 25, 50, 100, 200, 400 µg/L
4.2.2	Actual concentrations of test substance	Details are given in table A7.4.3.4/01-6
4.2.3	Effect data	The cumulative number of dead animals as well as the number of offspring are given in table A7.4.3.4/01-7 EC_{50} (including 95 % C.L.), NOEC, LOEC as well as MATC (maximum acceptable toxicant concentration) values are given in table A7.4.3.4/01-8
4.2.4	Concentration / response curve	No plot given
4.2.5	Other effects	Lengths were determined after 21 days of exposure. There was a statistical significant reduction of growth at 99.3 µg/L in comparison to the solvent control.
4.3	**Results of controls**	Mortality in the dilution control and the solvent control were 2 and 8 percent, respectively. These values are within the range of validity. Details are given in table A7.4.3.4/01-7

Section A7.4.3.4/01		**Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA, XIII.2.4		*Daphnia magna*
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was conducted according to EPA-FIFRA 72-4 (1982) and OECD 202 (1984). It was a flow-through test system and *Daphnia magna* was used as the test species.
5.2	**Results and discussion**	The properties of the test substance gave no indications to assume any relevant influences on the results. The analysis of the test media indicated a sufficient water solubility and a degradation of the test substance between 50 and 74 percent of nominal during the course of the test (21 days).
5.2.1	NOEC	49.9 µg/L, based upon the lack of significant mortality, reproductive or growth effects at this concentration at test termination (based on mean measured values)
5.2.2	LOEC	99.3 µg/L, based upon mortality and significant reductions in growth and reproductive success at this concentration at test termination (based on mean measured values)
5.2.3	EC_{50}	133 µg/L at test termination, based on mortality mean measured values
5.3	**Conclusion**	The mortality in the control was below 20 %. Also the number of offspring produced per parent animal surviving at the end of the test is ≥ 60 %. Therefore, the validity criteria can be considered as fulfilled (see table A7.4.3.4/01-9). Based on the results, NOEC and LOEC were determined to be 49.9 µg/L and 99.3 µg/L, respectively. The EC50 was calculated to be 133 µg/L. All values based on mean measured test concentration.
5.3.1	Other Conclusions	Not applicable
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2004, May 2022
Materials and Methods	The applicant's version is adopted
Results and discussion	The applicant's version is adopted
Conclusion	Based on the mean measured test concentrations, NOEC and LOEC were determined to be 49.9 µg/L and 99.3 µg/L, respectively.
Reliability	Reliability indicator 1
Acceptability	Acceptable

Section A7.4.3.4/01 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA, XIII.2.4 *Daphnia magna*

Remarks	The EC50 was calculated to be 133 µg/l based on mortality. However, including the results on reproduction (number of offspring total and number per reproduction day) the EC50 value would appear to be approximately 100 µg/l. The effect levels on number of offspring at 99.3 µg/l appears to be very close to half the number of offspring in total and per reproduction day (cf. table A7.4.3.4/01-7). According to guidance this test should have been conducted in accordance to OECD 211 however the test conducted in accordance to OECD 202 was accepted during the evaluation of IPBC in PT13 in 2014, therefore it is still considered acceptable to apply the OECD 202.

Table A7.4.3.4/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	DMF was used as a solvent
Concentration of vehicle	80 µL/L
Vehicle control performed	Yes, the solvent concentration in the vehicle control was 80 µL/L in solvent control and in all test substance concentrations
Other procedures	No

Table A7.4.3.4/01-2: Dilution water

Criteria	Details
Source	Carbon-treated Jupiter, Florida, town water
Salinity	Not given
Alkalinity	22 to 34 mg/L as $CaCO_3$
Hardness	56 to 70 mg/L as $CaCO_3$
pH	7.4 – 8.1
Ca / Mg ratio	Not given
Na / K ratio	Not given
Oxygen content	7.7 – 8.9
Conductance	356 – 380 µmhos/cm
TOC	6.0 mg/L
Holding water different from dilution water	No

Table A7.4.3.4/01-3: Test organism

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	Environmental Protection Agency, Duluth, Minnesota
Age	Less than 24 hours
Breeding method	Not mentioned
Kind of food	Algae (*Selenastrum capricornutum*) yeast/trout chow/cerophyll mixture
Amount of food	0.98 x 10^3 cells/mL 3.7 mg/L food mix
Feeding frequency	Algae were added continuously during the course of the test (21 days). Algae were metered into the dilution during each diluter cycle. Food mix was added once daily
Pre-treatment	None
Feeding of animals during test	Yes, Daphnids were fed continuously during the course of the test (feed was metered into the dilution water during each dilution cycle)

Table A7.4.3.4/01-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	17 volume additions / day
Volume of test vessels	300 mL
Volume/animal/day	510 mL/animal/day
Number of animals/vessel	10
Number of vessels/ concentration	40
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4/01-5: Test conditions

Criteria	Details
Test temperature	19.0 – 21.8 °C
Dissolved oxygen	1.2 – 8.8 mg/L
pH	7.3 – 8.1
Adjustment of pH	No
Aeration of dilution water	Yes, dilution water was aerated prior to use
Quality/Intensity of irradiation	Fluorescent lightning with a light intensity of approx. 333 to 408 lux
Photoperiod	16 hours light, 8 hours dark

Table A7.4.3.4/01-6: Actual concentrations of test substance

Nominal concentrations of test substance (µg/L)	Measured concentration (µg/L)							
	Day 0	Day 7	Day 10	Day 14	Day 17	Day 21	Mean	Percent of Nominal
Control	ND	ND	ND	ND	ND	ND	—	—
Solvent control	ND	ND	ND	ND	ND	ND	—	—
12.5	9.76	4.39	11.0	8.00	7.27	5.08	7.59	61
25	19.7	11.2	22.5	16.0	16.0	13.2	16.4	66
50	28.6	38.9	30.6	27.9	28.1	23.0	29.5	59
100	61.8	39.7	64.2	53.9	39.8	39.9	49.9	50
200	114	60.9	118	111	108	84.4	99.3	50
400	300	383	279	286	291	252	298	74

ND = Not detected (LOD = 10 µg/L)

Table A7.4.3.4/01-7: Effect data

Mean measured concentrations (µg/L)	Cumulative number of dead animals		Number of offspring	
	Per treatment	% Mortality	Total	Per reproductive day
Control	1	2	5118	9.4
Solvent Control	3	8	6955	13.3
7.59	1	2	5367	9.4
16.4	5	12	5890	11.2
29.5	1	2	5715	9.7
49.9	1	2	5703	9.6
99.3	10	25	2595	5.7
298	40	100	—	—

— = not determined because all daphnids were dead by day 10

Table A7.4.3.4/01-8: Toxicity values

Exposure period (Day)	EC_{50}[1] (µg/L)	95 % C.L. (µg/L)	NOEC[1] (µg/L)	LOEC (µg/L)	MATC[2] (µg/L)
7	142	99.3 – 298	ND	ND	ND
14	136	99.3 – 298	ND	ND	ND
21	133	99.3 – 298	49.9	99.3	> 49.9, < 99.3

[1] data are based on mean measured concentrations

[2] maximum acceptable toxicant concentration

ND = not determined

Table A7.4.3.4/01-9: Validity criteria for invertebrate reproduction test according to OECD Guideline 202

	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination	yes	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	yes	
Criteria for poorly soluble test substances	n.a.	n.a.

4.4.5 Chronic toxicity to algae or aquatic plants

See short-term toxicity

4.5 M-factor

See the section M-factor under A.4.5.3 in the RAR.