Section A6.7 **Annex Point 6.7**	**Carcinogenicity study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	OIT is a chemically reactive substance. Because of the irritating/corrosive and sensitising capabilities of OIT a chronic exposure to humans can be ruled out. Given the lack of systemic toxicity, genotoxic potential and endocrine activity, it may be concluded that 2-n-octyl-4-isothiazolin-3-one is unlikely to demonstrate a carcinogenic potential.	
References	[redacted] 2007, 2-n-Octyl-4-isothiazolin-3-one - Justification for the non-submission of data: Chronic toxicity/Oncogenicity [redacted]	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*17/09/2009*	
Evaluation of applicant's justification		
Conclusion	*Acceptable*	
Remarks	*Further discussed in Doc IIA*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.7 Carcinogenicity

Annex Point IIA6.7

24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats

NOTE: This robust summary supports our justification for non-submission of data on carcinogenicity testing with OIT respective our waiving document referenced.

[redacted] 2007, 2-n-Octyl-4-isothiazolin-3-one - Justification for the non-submission of data: Chronic toxicity/Oncogenicity [redacted]

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 24-Month Drinking Water - Chronic/Oncogenic Study in Rats [redacted] unpublished.
1.2	**Data protection**	Yes.
1.2.1	Data owner	THOR GmbH Rohm and Haas
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. OECD 453, EPA 83-5.
2.2	**GLP**	*Yes*
2.3	**Deviations**	*No*

3 MATERIALS AND METHODS

3.1	**Test material**	Other substance comparable to test material as given in section 2
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Technical grade. Deviating from specification given in section 2 as stated under Purity.
3.1.2.1	Description	light brown liquide
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	test material was stable during the study
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 3-6 weeks

Section A6.7 Carcinogenicity

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3.2.6	Number of animals per group	90 males and 80 females per group; 40 per sex (sentinel animals to monitor intercurrent disease)
3.2.6.1	at interim sacrifice	10 per sex /group 12, 18 months
3.2.6.2	at terminal sacrifice	70 males/60 females /group (24 month survivors + nonsurvivors)
3.2.7	Control animals	Yes. Tap water- as well as salt control (groups 1 and 2).
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	rats 104 weeks
3.3.2	Interim sacrifice(s)	Following 12 and 18 months of treatment, 10 rats per sex/group for gross pathology recording.
3.3.3	Final sacrifice	after 104 weeks
3.3.4	Freqency of exposure	daily
3.3.5	Postexposure period	Other: no.
		Oral
3.3.6	Type	in drinking water
3.3.7	Concentration	0 (tap water), 0 (Salt control), 30, 100, 300 ppm (ad libitum) Decreased water consumption relative to control: 0-22% in 30 ppm group, 3-30% in 100 ppm group, 15-40% in 300 ppm group. Water consumption in tap water control was comparable to salt control throughout the study. Mean compound intake of test item (14,2%) over 24 month period:

		24 month mean intake mg/kg bw	
Group	ppm test item	Males	Females
3	30	2.0 +/- 0.6	3.1 +/- 0.5
4	100	6.6 +/- 1.9	9.8 +/- 1.3
5	300	17.2 +/- 4.7	25.7 +/- 3.3

3.3.8	Vehicle	aqueous solution
3.3.9	Concentration in vehicle	
3.3.10	Total volume applied	ad libitum
3.3.11	Controls	Other: vehicle (water) as well as salt control. Two samples of aqueous MgCl2/Mg(NO3)2 inorganic salt solutions (Group 2) were used in this study. [redacted]

Section A6.7 **Carcinogenicity**

Annex Point IIA6.7 *24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats*

3.4	**Examinations**	
3.4.1	Body weight	Yes. Weekly.
3.4.2	Food consumption	Yes. Individually, weekly.
3.4.3	Water consumption	Yes. Individually, weekly.
3.4.4	Clinical signs	Yes
3.4.5	Makroskopic investigations	Palpable masses, skin tumours
3.4.6	Ophthalmoscopic examination	Yes. Prior initiation and during treatment.
3.4.7	Haematology	Yes
3.4.7.1. Number of animals:		10 animals/sex/group
3.4.7.2. Time points:		After 3, 6, 12, 18, 24 months of treatment
3.4.7.3. Parameters:		Haematocrit, haemoglobin concentration, erythrocyte count and morphology, total and differential leukocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration.
3.4.7.4. Other		
3.4.8	Clinical Chemistry	Yes
3.4.8.1. Number of animals:		All animals, 10 animals/sex/group or other
3.4.8.2. Time points:		After 3, 6, 12, 18, 24 months of treatment
3.4.8.3. Parameters:		Sodium, potassium, calcium, chloride, glucose, total cholesterol, urea nitrogen, total bilirubin, total protein and albumin, glutamic pyruvic aminotransferase, glutamic oxaloacetic aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, triglycerides, inorganic phosphorus, A/G ratio, creatine phosphokinase.
3.4.8.4. Other		
3.4.9	Urinalysis	Yes
3.4.9.1. Number of animals		All animals during pretest period; at end of 3rd and 6th month urine was collected from all males and from 10 females per group; after 12th, 18th and 24th months urine was collected from 10 animals/sex/group.
3.4.9.2. Time points		After 3, 6, 12, 18, 24 months of treatment
3.4.9.3. Parameter		Color, clarity, microscopy of sediment, specific gravity, pH, protein, glucose, blood, ketones, bilirubin.
3.4.9.4. Other		
3.4.10	Pathology	Yes
Organ Weights		Yes
3.4.10.1.1. from:		all animals killed during scheduled necropsy (at interim sacrifice, at terminal sacrifice)
3.4.10.1.2 Organs:		adrenals, brain, kidneys, Liver, spleen, testes.
3.4.10.1.3 Other		
Histopathology		Yes
3.4.11.1 from:		all dose groups

Section A6.7 Carcinogenicity

Annex Point IIA6.7 *24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats*

3.4.11.2	from:	all surviving animals at interim sacrifice at terminal sacrifice
3.4.11.1	Tissues:	Adrenals, Aorta, bone marrow (sternum and femur), bone marrow smear, Brain, epididymides, esophagus, eye and harderian gland, gross lesions, heart, small and large intestines, kidneys, liver, lung, lymph node, female mammary gland, masses,sceletal muscle, peripheral nerve, ovaries, pancreas, pituitary, prostate, salivary glands, seminal vesicles skin, spinal cord, spleen, stomach, testes, tongue, trachea, thymus, thyroid/parathyroid, uterus and cervix, vagina, zymbal's gland.
3.4.11.1	Other	
3.4.11	Other examinations	Kidney ultrasonographyat 6th and 12th month for dilated renal pelves incidence (possible bias from rats of this strain)

3.5 Statistics

Prior to the evaluation of treatment-related effects, the "salt" control (Group 2) and tap water control (Group 1) groups were compared using two-sampie Student t-tests to determine the effect, if any, of the salt constituents (MgCl2 and Mg(NO3)2) of Kathon biocide an the various response parameters. Data were pooled across control groups for body weight, cumulative body weight change, water consumption arid feed consumption if the test for "salt" effect was not statistically significant (p e 0.05) for most time points. Ail subsequent tests for treatment effects in these parameters utilized pooled data from both control groups. Data were also pooled across contra! groups for hematology, clinical chemistry, urinalysis and Organ weight parameters if the test for "salt" effect was not statistically significant ($p<0.05$). All subsequent tests for treatment effects in this situation utilized pooled data from both control groups. Data was not pooled across control groups for hematology, clinical chemistry, urinalysis or Organ weight parameters if the test for "salt" effect was statistically significant (i.e., Group 2 was statistically different from Group 1, $p<0.05$). In this case, the Kathon biocioe-treated groups (Groups 3, 4 and 5) were compared to the "salt" control (Group 2) in all subsequent tests for treatment effect.

Distributions of the following parameters were inspected for normality and homogeneity of variance across treatment groups and sampling times: body weight, cumulative body weight change, water consumption, feed consumption, hematology, clinical chemistry, urinalysis arid Organ weights. Square root transformations were used for parameters of the White blood cell differential prior to further analysis. Analysis of variance (or covariance if pre-fest scores were available) was used to determine whether or not statistically significant riifferences existed among the various treatment group means. When significant differences were found (i.e., when the p-value for group effect was lese than 0.05 or the product of the F-value for group effect and the degrees of freedom for group effect axceeded 4.0), pairwise comparisons of least square means were made between Bach Kathon biocide-treated group and control using Dunnett's t-test. Statistical significance was indicated when a p-value of 0.05 or less (Dunnett's criterion) was obtained.

3.6 Further remarks

4 RESULTS AND DISCUSSION

Section A6.7 Carcinogenicity

Annex Point IIA6.7 *24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats*

4.1 Body weight

There were no treatment-related effects an body weight or cumulative body weight change in males or females at doses up to and including 100 ppm Kathon biocide (Group 4).

A treatment-related decrease in body weight and cumulative body weight change was seen in Group 5 males (300 ppm Kathon biocide) throughout the study. The change was considered treatment related, although the magnitude of the of the effect was not dramatic (body weight decrease generally in the 2-4% range; cumulative body weight gain decrease generally in the 6-12% range during the first 4 weeks of treatment and the 3-7% range for the remainder of the study). The body weight effects in were consistent and statistically significant at the majority of time points.

An equivocal decrease in body weights and body weight gain was noted in Group 5 females (300 ppm Kathon biocide). This effect was judged equivocal for a number of reasons. Throughout most of the study, female. body weight and cumulative body weight gain appear to be slightly decreased at both 100 (Group 4) and 300 (Group 5) ppm compared to the pooled control groups. For the first year of the study the effect was generally slight and more pronounced in the mid-dose group (100 ppm) than in the high dose group (300 ppm). The lack of a dose response is interpreted as normal variability and not indicative of a treatment-related effect. During the second year of treatment the effect was more pronounced in the high dose group (300 ppm) and does appear to be a treatment-related effect at that dose.

Body weight and cumulative body weight change in the salt control group (Group 2) was consistently higher than that of the tap water control group (Group 1). Since pooled control values were used for comparison to the Kathon biocide treated groups, the higher salt control values may have contributed to the large number of statistically significant differences that were seen. In light of the conflicting data, a decrease in body weight and cumulative body weight change in females at 300 ppm was considered equivocal. There were no treatment-related effects at lower doses.

4.2 Food consumption

There were no treatment-related effects on feed consumption In males at doses up to and including 100 ppm Kathon biocide (Group 4).

In the high dose (300 ppm) male group there was a slight (statistically significant at a number of time points) decrease in feed consumption throughout the study. The decrease was considered treatment related.

In females, there were a number of statistically significant differences at all dose levels throughout the study, however in the majority of cases no dose response was evident and the magnitude of the response among the Kathon biocide treated groups varied only slightly. These changes were judged normal biologic variation and no treatment-related effects on feed consumption were seen in females.

Section A6.7 Carcinogenicity

Annex Point IIA6.7

24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats

4.3 Water consumption

A treatment-related and concentration-dependent decrease in water consumption was seen in both sexes in all Kathon biocide-treated groups (Groups 3, 4 and 5; 30, 100 and 300 ppm, respectively) throughout the study. These decreases ranged from 0-22% at 30 ppm, 3-30% at 100 ppm and 15-40% at 300 ppm a.i.. These decreases appear to be attributable to the unpalatability of the active ingredient in Kathon biocide and not its inorganic stabiliser salts (MgCl2/Mg(NO3)2), since the water consumption in Group 2 (MgCl2/Mg(NO3)2 salt control) was comparable to the tap water control throughout the study. Based an the average daily water consumption, the 300 ppm dose was judged to be a maximum tolerated dose.

Mean compound intake over 24 months

Group	Dose (test item)	Mean Intake (mg a.i./kg/day)	
		Males	Females
1	tap water	-/-	-/-
2	salt contr	-/-	-/-
3	30 ppm	2.0+/-0.6	3.1+/-0.5
4	100 ppm	6.6+/-1.9	9.8+/-1.3
5	300 ppm	17.2+/-4.7	25.7+/-3.3

4.4 Clinical signs

There were no effects on the survival of males or females in any dose group following chronic administration of Kathon biocide or MgCl2/Mg(NO3)2 stabiliser salts in the drinking water for 24 months. The 24-month survival (adjusted for scheduled interim necropsies) for the controls and Kathon biocide-treated groups was as follows:

Adjusted Survival

Group	Males	Females	Dose (test item)
1	36%	35%	water
2	41%	35%	salt
3	30%	33%	30 ppm
4	37%	38%	100 ppm
5	46%	32%	300 ppm

No treatment-related clinical signs of toxicity were evident in treated animals. Clinical signs were noted throughout the study in both the controls and Kathon biocide treated animals, with no apparent difference between the controls and treated animals.

4.5 Macroscopic investigations

No effects / describe significant effects referring to data in results table

4.6 Ophthalmoscopic examination

No effects . The abnormalities observed occur commonly in laboratory rats of this strain and age and were judged to be unrelated to treatment.

4.7 Haematology

No treatment-related effects on haematology parameters were seen at any dose in either sex throughout the 24 months of treatment.

Statistically significant differences were occasionally noted in some parameters throughout the 24 month study. These differences were considered random events and not related to treatment since the changes lacked a dose response relationship and/or were seen only sporadically over the time intervals when haematology parameters were evaluated.

Section A6.7 Carcinogenicity

Annex Point IIA6.7 *24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats*

4.8	**Clinical Chemistry**	No treatment-related effects an clinical chemistry parameters were seen in males or females at any dose throughout the 24-month treatment period. Statistically significant differences were occasionally noted in various parameters throughout the 24 month study. These differences were considered random events and not related to treatment since the changes lacked a dose response relationship and/or were seen only sporadically over the time intervals when clinical chemistry parameters were evaluated.
4.9	**Urinalysis**	No changes in urinary parameters were seen which were considered indicative of treatment-related systemic toxicity. Statistical significant increases in urinary specific gravity were seen in males and females at 100 and 300 ppm after 3 and 6 months of treatment and in males at 30 ppm after 6 months of treatment. Other than a random occurrence of statistical significance similar effects were not evident at any dose, in either sex, after 12, 18 or 24 months of treatment. Increases in urinary specific gravity are not unexpected in light of the decreased water consumption seen in this study and are most likely secondary to that effect and not considered indicative of systemic toxicity. The single occurrence in the 30 ppm (Group 3) males was considered incidental.
4.10	**Pathology**	The only treatment-related effects observed were indicative of gastric irritation.
4.11	**Organ Weights**	There were no treatment related effects an organ weights, in males or females, at any dose up to and including 300 ppm (Group 5) following 24 months of treatment with Kathon biocide. Statistically significant differences were occasionally noted in some absolute or relative organ weights throughout the 24 month study however none were considered treatment related. A statistically significant increase in relative kidney weights was seen in both sexes at 300 ppm after 12 months of treatment. This change was judged to be related to a decrease in terminal body weight seen in both Sexes at this point. No changes in absolute kidney weights were seen at 12 months and no changes in absolute or relative kidney weights were seen at 18 or 24 months. No histopathologic changes were seen in the kidneys at any time point. Other statistically significant differences occurred sporadically and were considered spurious and not related to treatment since the changes lacked a dose response relationship and were not observed consistently over several time intervals.

Section A6.7 Carcinogenicity

Annex Point IIA6.7

24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats

4.12	**Histopathology**	The following comments were abstracted from the gross and microscopic pathology report. The administration of Kathon biocide in the drinking water for up to 24 months had no effect on the type or incidence of any neoplasms that were observed in this study. There were no treatment-related gross or microscopic changes in any of the male or female rats given 30 ppm of Kathon biocide during the study. Treatment-related gross and microscopic changes were limited to the stomach and were seen in male and female rats of Group 4 (100 ppm) and 5 (300 ppm). The predominant change was an increased thickness or prominence of the mucosa, frequently at the limiting ridge of the forestomach which was due to hyperplasia and hyperkeratosis of the squamous mucosa. There were a few other changes which occurred sporadically in the stomach of these groups of rats but only focal necrosis of the superficial glandular mucosa and edema and inflammatory cell infiltration of the submucosa of the forestomach in the 300 ppm dosage group male rats were significantly increased when compared to both of the control groups. There were no significant differences in the incidence of these changes among the tap water control, "salt" control and 30 ppm dosage groups. The chronic administration of Kathon biocide in the drinking water to rats at or above 100 ppm caused gastric irritation and the no-observed effect level in male and female rats was 30 ppm.
4.13	**Other examinations**	Kidney Ultrasonography No treatment-related effects were seen via ultrasound in the renal pelves of any male rats at any dose up to and including 300 ppm Kathon biocide (Group 5) at 6 or 12 months of the study.
4.14	**Time to tumours**	Not applicable.
4.15	**Other**	

Section A6.7 Carcinogenicity

Annex Point IIA6.7

24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was done according to OECD 452. The test item used was a technical grade mixture of CIT/MIT [redacted], which is comparable to the test material given in section 2. The NOAEL was established irrespective to sex on local effects.

5.2 Results and discussion

Administration of 30, 100 or 300 ppm a.i. Kathon biocide in the drinking water of male and female rats for 24 months had the following effects:

Group 3 (30 ppm)
Water Consumption: Decreased in males and females

Grouo 4 (100 ppm)
Water Consumption- : Decreased in males and females
Urinalysis: Sporadic increases in specific gravity in males and females, secondary to decreased water consumption.
Gross and Histopathology: Gross and microscopic changes were limited to the stomach and included:

1. Increased thickness of the forestomach mucosa due to hyperplasia and hyperkeratosis of the squamous mucosa (both sexes).

Group 5 (300 ppm)
Body Weight: Decreased in males; equivocal decrease in females
Water Consumption: Decreased in males and females
Feed Consumption: Decreased in males
Urinalysis: Sporadic increases in specific gravity in males and females,. secondary to decreased water consumption.
Gross and histopathology: Gross and microscopic change were limited to the stomach and included:

1. Increased thickness of the forestomach mucosa due to hyperplasia and hyperkeratosis of the squamous mucosa (both sexes).

2. Focal necrosis of the superficial glandular mucosa (males only).

3. Edema and inflammatory cell infiltration of the forestomach submucosa (males only).

5.3 Conclusion

Administration of Kathon® biocide to male and female rats in the drinking water for 24 months at concentrations up to and including 300 ppm active ingredient (a.i.)(17.2 mg a.i./kg of body weight/day in males and 25.7 mg a.i./kg of body weight/day in females) showed no effects on the type or incidence of neoplasm's in any group.

No treatment-related signs of toxicity were seen at 30 ppm active ingredient (a.i.)(2.0 mg a.i./kg of body weight/day in males and 3.1 mg a.i./kg of body weight/day in females), the No-Observed Effect Level (NOEL) in this study.

5.3.1 Reliability: 1

5.3.2 Deficiencies: Yes. 24-months survival in each dose group is below 50% - but no treatment related differences.

Section A6.7	**Carcinogenicity**
Annex Point IIA6.7	*24-Month Drinking Water - Chronic/Oncogenic Study with CIT/MIT (3:1) , CAS 55965-84-9 in Rats*
Comment on relevance	The data of the chronic exposure test with CIT/MIT are of relevance to OIT as both A.S. share corporate toxophoric structure elements, the mechanism of action/deactivation, and an overall lack of systemic toxicity/bioavailability of parent compound. However, because of the chlorine in the 5 position of the ring CIT in CIT/MIT (3:1) is of higher reactivity towards tissues of first contact. Therefore, an potential epigenetic mechanism of carcinogenicity in tissues of first contact would be more prominent for CIT than for OIT. The OIT is more lipophilic due to its Octyl side chain in comparison to the Methyl side chain of CIT or MIT, respectively. This difference is deemed not relevant with regard to toxicokinetics as it is ruled out by the reactivity/stability of this class of substances.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*17/04/2009*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	*This study has not been evaluated by the UK CA, although the results are considered to support the justification for the non-submission of carcinogenicity data.*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_7-1. Table for Clinical Chemistry, Haematology and Urinalysis (modify if necessary)

Use this table, if relevant effects occur and if time sequence is important. Symbols for increases or decreases (↑ / ↓) will be sufficient. Give figures or percentages if in doubt.

Not needed due to results of the study.

Table A6_7-2. Results of Carcinogenicity study

Parameter	control groups				low dose		medium dose		high dose		dose-response + / -	
	tap water -		salt control									
	m	f	m	f	m	f	m	f	m	f	m	f
	If differing numbers of animals are examined, give number affected/number of animals examined for each individual finding.											
Number of animals examined	90	80	90	80	90	80	90	80	90	80		
Mortality	46	40	43	40	51	40	45	39	40	41	-	-
body weight gain									↓	↓	+	+?
food consumption									↓	↓	+	-?
water consumption					↓	↓	↓	↓	↓	↓	+	+
Urinalysis - specific gravity							↑	↑	↑	↑	+	+
Overall tumour incidence:	See: Appendix 14, Table 12. Incidence of Primary Neoplasms and Histomorphologic Observations (below)										-	-
Stomach												
non-neoplastic changes								↑	↑	↑	+	+