Section A6.3.1 J **Annex Point A6.3**	**Short-term repeated dose toxicity test** 28 days oral exposure study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure []**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A 90 day oral toxicity study in the rat (Section A6.4.1-02) and a 90 day oral toxicity study in the dog (Section A6.4.1-01) is available. Sufficient data on the oral exposure of OIT is available, further studies are not deemed to be necessary. The risk assessment does not indicate that a further study is necessary.	
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	*01/04/2009*	
Evaluation of applicant's justification		
Conclusion	*Acceptable*	
Remarks	*The UK CA considers this justification acceptable according to the data requirements of the BPD ('[short term repeated dose toxicity studies]* are not required where an *adequate* sub-chronic toxicity study is available in a rodent').	
	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 A6.3.2 J **Annex Point A6.3**	**Short-term repeated dose toxicity test** 28 days dermal exposure study

Section A6.3.2 J **Annex Point A6.3**	**Short-term repeated dose toxicity test** 28 days dermal exposure study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure []**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A 90 day dermal toxicity study in the rat is available (Section A6.4.2-01). Sufficient data on the dermal exposure of OIT is available, further studies are not deemed to be necessary. The risk assessment does not indicate that a further study is necessary.	
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	*01/04/2009*	
Evaluation of applicant's justification		
Conclusion	*Acceptable*	
Remarks	*The UK CA considers this justification acceptable according to the data requirements of the BPD ('[short term repeated dose toxicity studies]* are not required where an *adequate* sub-chronic toxicity study is available in a rodent').	
	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 A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 2007, 90-Day oral dietary toxicity study with ACTICIDE® OIT (2-n-Octyl-4-isothiazolin-3-one) in male and female [redacted] dogs, [redacted] unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	THOR GmbH, Germany
1.2.2		
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD Guideline no. 409, 1998 EC Directive 87/302/EEC, Part B: No. L 133, 1988 OPPTS 870.3050, EPA 712-C-98-200, 1998
2.2	**GLP**	Yes
2.3	**Deviations**	Yes 1. No clinical observations were entered in the computer on 27 August 2004. Evaluation: Sufficient data were available to evaluate the clinical signs properly. 2. The following tissues were not available for histopathology: Animal 1: thymus. Animal 18: one parathyroid. Evaluation: Sufficient tissues were available for evaluation. 3. With the exception of male nos. 41 and 42, all group 7 animals inadvertently received 6000 ppm diet on day 12 of the pretest period. Evaluation: Pretest blood was already collected at an earlier stage. Animals were returned to pretest diet immediately on day 13. This incidental occurrence was therefore considered to have no adverse effect on the study results obtained in the treatment phase.

3 MATERIALS AND METHODS

3.1	**Test material**	*As given in section 2*
3.1.1	Lot/Batch number	Acticide® OIT, [redacted]
3.1.2	Specification	Technical grade
3.1.2.1	Description	Amber solid to liquid depending on ambient temperature
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Stable
3.2	**Test Animals**	Non-entry field

Section A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

3.2.1	Species	dog
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	Groups 1-4: Approximately 8-9 months Groups 5-7: Approximately 7-8 months
3.2.6	Number of animals per group	4 animals/sex/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	At least 90 days
3.3.2	Frequency of exposure	daily
3.3.3	Postexposure period	none
3.3.4	**Oral**	

Section A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

3.3.4.1 Type: in food

3.3.4.2 Dose:

Group	Dose Level ppm
1 [2]	0
2	100
3	300
4	1000
5 [3]	0
6	3000
7	4500 [1]

1 Animals received 6000 ppm diet on days 1-8. From day 9-12, treatment of group 7 animals at 6000 ppm was discontinued for ethical reasons, based on a continuous and significantly reduced food intake and reduced body weights observed so far at 6000 ppm.

From day 9 to 12, group 7 animals received Standard dog maintenance pelleted food (Altromin diet 4119 extrudat) supplied by Altromin GmbH (Lage, Germany) mixed with ½ can of Hill's Prescription Diet (Hill's Pet Nutrition BV., the Netherlands)).

On day 13, group 7 animals were fasted for blood collection on day 14.

From day 14-21, group 7 animals received diet with a dose level of 4500 ppm.

From day 22-30, group 7 animals inadvertently received the 6000 ppm instead of 4500 ppm diets.

From day 31 onwards, these animals again received test diet with a dose level of 4500 ppm.

The nominal dose level of 4500 ppm is mentioned throughout the document.

2 Control group for groups 2-4.

3 Control group for groups 6-7.

food consumption per day: 250 grams per animal.

From day 64 onwards, group 1-4 animals were offered the test diet once daily at 0.275 kg/animal/day and group 5-7 animals received 0.300 kg/animal/day from day 79 onwards in the early morning since a higher food supply was considered more appropriate based on their age/body weight development.

3.3.4.3 Vehicle: Not applicable.

3.3.4.4 Concentration in vehicle: Not applicable.

3.3.4.5 Total volume applied: Not applicable.

3.3.4.6 Controls: plain diet

3.4 Examinations

Section A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

3.4.1	Observations	
3.4.1.1	Clinical signs	yes, at least once daily during pretest and treatment
3.4.1.2	Mortality	Yes, at least twice daily
3.4.2	Body weight	Yes, twice during pretest, weekly during treatment and on the day of necropsy
3.4.3	Food consumption	Yes, daily, except over days when urine was collected.
3.4.4	Water consumption	Not measured.
3.4.5	Ophthalmoscopic examination	Yes at Pre-test: All animals at week 13: All animals
3.4.6	Haematology	yes – During pretest – Week 4 – Week 13 – additionally on group 5 and 7 animals in week 2 for health status monitoring. White blood cells, Red blood cells, Haemoglobin, Haematocrit, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, Platelets, Red blood cell distribution width c.v., Differential leucocyte count (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils), Reticulocytes, Prothrombin time, Partial thromboplastin time.
3.4.7	Clinical Chemisty	yes – During pretest – Week 4 – Week 13 – additionally on group 5 and 7 animals in week 2 for health status monitoring. Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Gamma glutamyl transferase, Lactate dehydrogenase, Glutamate dehydrogenase, Bilirubin, total, Glucose, Creatinine, Urea, Protein, total, albumin, globulin, Albumin Globulin ratio, Cholesterol, total, Triglycerides, Phospholipids, Sodium, Potassium, Chloride, Calcium, Phosphorus.
3.4.8	Urinalysis	yes – During pretest – Week 4 – Week 13 – additionally on group 5 and 7 animals in week 2 for health status monitoring. Volume, Colour, Clarity, Specific gravity, pH, Protein, Glucose Ketone, Bilirubin, Blood, Leucocytes, Nitrite, Urobilinogen, Sediment (white blood cells, red blood cells, casts, epithelial cells, crystals, bacteria, other)

Section A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes Adrenals, Pituitary gland, Brain, Prostate, Epididymides, Spleen Heart, Testes, Kidneys, Thymus, Liver, Thyroid with parathyroids Ovaries, Uterus
3.5.2	Gross and histopathology	yes - all tissues collected at the scheduled sacrifice from all animals of the control groups, and group 4 and 7 - all tissues from all animals of all dose groups which died spontaneously or were sacrificed *in extremis* - all gross lesions On detection of possible treatment-related changes in the organs of any animal in groups 4 and 7 the histological examination was extended to that particular organ of all animals of groups 2, 3 and 6 (males and/or females).

Tattoo (not processed)	Pituitary gland
Adrenal glands	Prostate gland
Aorta	Rectum
Brain (medulla, pons,	Salivary gland (parotid, sublingual,
Caecum	Sciatic nerve
Cervix	Skeletal muscle
Colon	Skin +Mammary gland area, males and females (pelvic, left and right)
Duodenum	
Eyes, optic nerve and lacrimal	Spinal cord (cervical, thoracic, lumbar)
Gall bladder	Spleen
Heart	Sternum
Ileum	Stomach
Jejunum	Testes and Epididymides
Kidneys	Thymus
Liver	Thyroids
Lung	Tongue
Lymph node (mandibular,	Trachea
Oesophagus	Urinary Bladder
Ovaries	Ureter
Pancreas	Uterus
Parathyroid glands	Vagina
Peyer's patches (jejunum,	All gross lesions

3.5.3	Other examinations	None
3.5.4	Statistics	None
3.6	**Further remarks**	Dose levels for groups 1-4 (0, 100, 300 and 1000 ppm) were based on results of a 7-day dietary range finding study with ACTICIDE® OIT [redacted]

Since no clear effect level (i.e. a LOAEL) could be discerned based on the results of groups 1-4, additional dose levels (groups 5-7) were added in consultation with and at request of the sponsor. Dose levels for groups 5-7 (0, 3000 and 6000 ppm) were selected based on a 14-day range finding study conducted with 3000 and 9000 ppm [redacted]. The high dose of 6000 ppm was eventually lowered to 4500 ppm during treatment based on study results/ethical considerations.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

There were no clinical signs evident of toxicity.

4.1.2 Mortality

One female at 4500 ppm was sacrificed on day 30. Inanition was considered to be the cause of the animal's clinical condition.

4.2 Body weight gain

During the first week of treatment at 6000 ppm notable weight loss was recorded for males and females. Body weights remained at approximately the same lower level during the intermittent 4500 ppm treatment (days 14-21), followed by a further reduction at 6000 ppm (days 22-30). Upon commencing treatment at 4500 ppm from day 31 onwards, body weights increased to control levels for males, but body weights of females remained lower throughout treatment (achieving a level of statistical significance on several occasions).

At 3000 ppm, body weights of females were reduced when compared to control levels essentially from week 4/5 on treatment onwards (achieving statistical significance for lower weight gain on days 50 and 71-92). Body weights of males at 3000 ppm remained similar to control levels.

4.3 Food consumption and compound intake

During the first week of treatment at 6000 ppm food consumption was significantly reduced for both males and females. In the intermittent period (days 9-12) when group 7 animals received standard pelleted food, food intake was similar to control levels. Subsequent treatment at 4500 ppm (days 14-21) and 6000 ppm (days 22-30) resulted in a decrease with partial recovery. Upon commencing treatment at 4500 ppm from day 31 onwards, food intake levels recovered to control levels for both males and females.

The average intake of active ingredient (OIT) achieved during the 13-week study period was as follows:

Dietary inclusion level (ppm)	Average OIT intake (mg OIT/kg body weight/day) [redacted]	
	Males	females
100	[redacted] *1.6*	[redacted] *1.6*
300	[redacted] *5.5*	[redacted] *5.6*
1000	[redacted] *22.4*	[redacted] *24.7*
3000	[redacted] *70.8*	[redacted] *87.7*

4500	[redacted] *114.5*	[redacted] *135.2*

[redacted]

4.4	**Ophthalmoscopic examination**	No toxicologically relevant ophthalmoscopic findings were noted.
4.5	**Blood analysis**	
4.5.1	Haematology	No toxicologically relevant alterations were noted in haematological parameters.
4.5.2	Clinical chemistry	The following statistically significant changes in clinical biochemistry parameters were observed: - Reduced calcium levels and increased chloride levels for females at 3000 and 4500 ppm at the end of treatment; - Reduced total protein and albumin levels for females at 4500 ppm at the end of treatment; - Increased albumin levels for males at 3000 and 4500 ppm in week 4 and in males at 4500 ppm at the end of treatment; - Increased albumin/globulin ratio for males at 4500 ppm in week 4 and at the end of treatment.
4.5.3	Urinalysis	No toxicologically relevant alterations in urinary parameters were noted.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Thymus weight and thymus to body weight ratio was reduced in females at 3000 and 4500 ppm.
4.6.2	Gross and histopathology	Necropsy: One female at 4500 ppm that was sacrificed on day 30 had an emaciated appearance. Histopathology: No toxicologically significant findings.
4.7	**Other**	None.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Based on a 7-day range finding study [redacted] and in consultation with the sponsor, the dose levels for this 90-day dietary study were selected to be 0, 100, 300 and 1000 ppm. Based on the results obtained at these dose levels and in consultation with/at request of the sponsor one control group and two test substance groups were added at a later stage. Dose levels for these additional dose groups were based on a 14-day range finding study [redacted], and were set in consultation with the sponsor at 0, 3000 and 6000 ppm. In consultation with the sponsor, the high dose of 6000 ppm was lowered to 4500 ppm during treatment based on study results/ethical considerations. The study was based on the following guidelines: – OECD 409, "Repeated Dose 90-day Oral Toxicity Study in Non-Rodents", 1998. – EC Directive 87/302/EEC, B.27: "90-days repeated Oral Dose Study using Non-rodent species", 1988. – EPA 712-C-98-200, 90-Day Oral Toxicity in Nonrodents, 1998. Beagle dogs received the test substance by dietary intake for at least 90

days. One control group and three treated groups were tested, each consisting of 4 males and 4 females. Based on the results obtained at these dose levels, in consultation with the sponsor and based on a range finding study, additional groups at 0, 3000 and 6000 ppm were dosed.

Chemical analysis of prepared diets was conducted on a regular basis during the study to assess homogeneity, accuracy and/or stability of preparations.

The following parameters were evaluated: clinical signs (daily), body weight (weekly), food consumption (daily), ophthalmoscopic examination (at pretest and end of treatment), clinical pathology (pretest, weeks 4 and end of treatment, and for groups 5 and 7 also in week 2), macroscopy and organ weights at termination. Histopathology was performed on selected tissues from dogs of the control groups (i.e. groups 1 and 5) and the high dose groups (i.e. groups 4 and 7).

5.2 Results and discussion

Homogeneity and accuracy of diet preparations were considered to be acceptable. Stability of diets over 4 weeks at room temperature was confirmed.

The average intake of active ingredient (OIT) achieved during the 13-week study period was as follows-

Dietary inclusion level (ppm)	Mean analytical accuracy (% of target conc.)	Average OIT intake (mg OIT/kg body weight/day)	
		males	females
100	50%	[redacted] *1.6*	[redacted] *1.6*
300	57%	[redacted] *5.5*	[redacted] *5.6*
1000	69%	[redacted] *22.4*	[redacted] *24.7*
3000	78%	[redacted] *70.8*	[redacted] *87.7*
4500 [redacted][2]	86%	[redacted] *114.5*	[redacted] *135.2*

[redacted]

[2] Between days 1-8 and 22-30 animals received 6000 ppm diets. Between days 14-21 and from day 31 onwards, animals received 4500 ppm diets. From days 9-12 animals received standard dog maintenance pelleted food with canned food. Mean analytical accuracy was 84% for 6000 ppm diet preparations.

It was concluded that the lower recoveries were most likely due to reaction of OIT with sulfur containing compounds in the diet and/or effects of irreversible binding of OIT. Extraction was however complete with regard to the extractable OIT.

One female at 4500 ppm was sacrificed in week 5. Inanition (evidenced by an emaciated appearance at necropsy) was considered to be the cause of the animal's clinical condition.

No mortality occurred at dosages up to 3000 ppm.

Section A6.4.1-01 **Subchronic toxicity (oral)**

Annex Point IIA6.4.1 *90 days dietary toxicity study in dogs*

The lower body weight and food intake at 3000 and 4500 ppm frequently occurred with food scatter observed throughout treatment at these dose levels. There were no clinical signs evident of toxicity up to the highest dose level tested, and there was no morphological indication of organ toxicity. Also, no toxicologically significant haematological alterations or histopathological abnormalities were noted up to 4500 ppm. Therefore, the lower food intake/body weight was considered to be related to palatability of the test diet, rather than being indicative of primary systemic toxicity.

Changes in clinical biochemistry parameters noted during treatment in animals at 3000 and 4500 ppm had no morphological correlates and were of a slight nature. These changes consisted of a.o. reduced total protein and albumin levels which are in line with the expected biochemistry changes in case of reduced body weights/food intake. Reduced thymus weights of females at 3000 and 4500 ppm were not supported by any histopathological evidence of organ dysfunction. These changes were therefore considered to be related to the lower body weights and food intake. No toxicological relevance was ascribed to these alterations.

It is concluded that at 3000-4500 ppm the maximum tolerated dose has been approximated with regard to palatability of the test diet. It is considered that the level at which signs of primary toxicity would emerge occurs beyond the level of palatability of the test substance.

5.3 Conclusion

5.3.1	LO(A)EL	Not applicable.
5.3.2	NO(A)EL	4500 ppm (3870 ppm based on overall analytical accuracy of the 4500 ppm diet preparations), corresponding to an actual intake of 133 and 157 mg active ingredient (OIT)/kg body weight/day for males and females respectively (115 and 135 mg active ingredient (OIT)/kg body weight/day for males and females respectively, based on overall analytical accuracy of the 4500 ppm diet preparations).
5.3.3	Other	Since no evidence of target organ toxicity was obtained with any of the examined parameters in this study, the observed effects were considered to be related to palatability of the test diets.
5.3.4	Reliability	1
5.3.5	Deficiencies	There were no deviations from the test guidelines/protocol that were considered to have adversely affected the study integrity.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*01/04/2009*
Materials and Methods	

Section A6.4.1-01 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in dogs

Results and discussion	
Conclusion	
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*In agreement with the applicant's assessment.*
	COMMENTS FROM ... *(specify)*
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_4-1. Results of clinical chemistry, haematology and urinalysis

parameter changed	Unit	Control			100 ppm			300 ppm			1000 ppm		
weeks after start of treatment		4		13	4		13	4		13	4		13
males													
No effects													
females													
No effects													

parameter changed	Unit	Control			3000 ppm			4500 ppm		
weeks after start of treatment		2	4	13	4		13	2	4	13
males										
Albumin					↑* +12%				↑* +13%	↑* +9%
Albumin/Globulin ratio									↑ +20%	↑* +30%
females										
Calcium							↓* -4%			↓* -8%
Chloride							↑* +4%			↑* +3%
Total protein										↓* -12%
Albumin										↓* -9%

* $p < 0,05$

Table A6_4-2. Results of repeated dose toxicity study

Parameter	Control		low dose 100 ppm		medium dose 300 ppm		high dose 1500 ppm	
	m	f	m	f	m	f	m	f
number of animals examined	4	4	4	4	4	4	4	4
Mortality	0	0	0	0	0	0	0	0
clinical signs	=	=	=	=	=	=	=	=
body weight	=	=	=	=	=	=	=	=
food consumption	=	=	=	=	=	=	=	=
clinical chemistry	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]
haematology	=	=	=	=	=	=	=	=
urinalysis	=	=	=	=	=	=	=	=
gross pathology	=	=	=	=	=	=	=	=
microscopic pathology	=	=	=	=	=	=	=	=

[1] Based on all groups, i.e. groups 1-7.

[2] See Table A6_4-1. Results of clinical chemistry, haematology and urinalysis.

= No toxicologically significant change/incidence similar to control group.

Parameter	3000 ppm		4500 ppm		dose-response +/- [1]	
	m	f	m	f	m	f
number of animals examined	4	4	4	4	4	4
Mortality	0	0	0	1	-	-
clinical signs	=	=	=	=	-	-
body weight	=	↓ [2]	↓ [2]	↓ [2]	+	+
food consumption	=	=	↓ [3]	↓ [3]	+	+
clinical chemistry	[4]	[4]	[4]	[4]	[4]	[4]
haematology	=	=	=	=	-	-
urinalysis	=	=	=	=	-	-
Thymus						
organ weight	=	↓ -46%	=	↓ -51%	-	+
gross pathology	=	=	=	=	-	-
microscopic pathology	=	=	=	=	-	-
Other						
gross pathology	0	0	0	1	-	+
Emaciated appearance						

[1] Based on all groups, i.e. groups 1-7.

[2] See 4.2

[3] See 4.3

[4] See Table A6_4-1. Results of clinical chemistry, haematology and urinalysis.

= No toxicologically significant change/incidence similar to control group.

Section A6.4.1-02 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in rats

Official use only

6 REFERENCE

6.1	**Reference**	[redacted] *2007, 90-Day dietary toxicity study with ACTICIDE® OIT (2-n-Octyl-4-isothiazolin-3-one) in the rat,* [redacted] *unpublished*
6.2	**Data protection**	*Yes*
6.2.1	Data owner	*THOR GmbH, Germany*
6.2.2		
6.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.

7 GUIDELINES AND QUALITY ASSURANCE

7.1	**Guideline study**	*Yes*

Section A6.4.1-02 **Subchronic toxicity (oral)**

Annex Point IIA6.4.1 *90 days dietary toxicity study in rats*

OECD Guideline no. 408, 1998
EPA Health Effects Test Guidelines (OPPTS 870.3100), 1998
EC Directive 2001/59/EC, Part B: No. L 225, 2001

7.2	**GLP**	*Yes*
7.3	**Deviations**	Yes 1. Inadvertently, no brain weight was recorded from animal no. 35 (group 4). Evaluation: sufficient organ weight data were available for adequate interpretation of the study results. 2. Inadvertently, no clinical signs were recorded on day 64 (groups 5-6). Evaluation: Sufficient clinical observations were performed for adequate interpretation of the study results.

8 MATERIALS AND METHODS

8.1	**Test material**	*As given in section 2*
8.1.1	Lot/Batch number	Acticide® OIT [redacted]
8.1.2	Specification	Technical grade
8.1.2.1	Description	Amber solid to liquid depending on ambient temperature
8.1.2.2	Purity	[redacted]
8.1.2.3	Stability	Stable
8.2	**Test Animals**	[redacted]
8.2.1	Species	rat
8.2.2	Strain	[redacted]
8.2.3	Source	[redacted]
8.2.4	Sex	Male and female
8.2.5	Age/weight at study initiation	Approximately 6 weeks
8.2.6	Number of animals per group	10
8.2.7	Control animals	Yes
8.3	**Administration/ Exposure**	Oral
8.3.1	Duration of treatment	At least 90 days
8.3.2	Frequency of exposure	daily
8.3.3	Postexposure period	none
8.3.4	**Oral**	

Section A6.4.1-02 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in rats

8.3.4.1 Type: in food

8.3.4.2 Dose:

Group	Dose Level ppm	Test article intake (mg test substance/kg body weight/day)[1]			
		males		females	
1	0	[redacted]	*0*	[redacted]	*0*
2	100	[redacted]	*6*	[redacted]	*8*
3	300	[redacted]	*19*	[redacted]	*23*
4	1000	[redacted]	*68*	[redacted]	*82*
5	0	[redacted]	*0*	[redacted]	*0*
6	3000	[redacted]	*210*	[redacted]	*257*

[1] Based on body weights and food intake corrected for food scatter (groups 1-4). [redacted]

food consumption per day: ad libitum

8.3.4.3 Vehicle: Not applicable

8.3.4.4 Concentration in vehicle: Not applicable

8.3.4.5 Total volume applied: Not applicable

8.3.4.6 Controls: plain diet

8.4 Examinations

8.4.1 Observations

8.4.1.1 Clinical signs: Yes, at least once daily

8.4.1.2 Mortality: Yes, at least twice daily.

8.4.2 Body weight: Yes, weekly and on the day preceding the first necropsy date

8.4.3 Food consumption: Yes, weekly.

Food scatter for groups 1-4 was determined on a daily basis from week 2 onwards. Actual food intake levels were corrected for this food scatter. Food intake of groups 1-4 in week 1 was based on the mean total food scatter determined per group and sex in week 2. For groups 5 and 6, food scatter could not be quantified due to the type of housing (i.e. Macrolon cages containing sawdust as bedding material).

8.4.4 Water consumption: no

8.4.5 Ophthalmoscopic examination: Yes

at Pre-test : All animals (including spare animals)

at week 13: Groups 1, 4, 5 and 6

8.4.6 Haematology: yes

Week 13: all animals.

Erythrocytes count, haemoglobin, Haematocrit, Mean corpuscular

		volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, Platelet count, Red cell distribution width, Total leucocytes count, Differential leucocyte count, Prothrombin time, Partial thromboplastin time
8.4.7	Clinical Chemisty	yes Week 13: all animals. Alanine aminotransferase, Alkaline phosphatase, Aspartate aminotransferase, Bilirubin, total, Chloride, Cholesterol, total, Creatinine, Glucose, Phosphorus, Protein, total, Protein, albumin Urea, Calcium, Potassium, Sodium
8.4.8	Urinalysis	No
8.5	**Sacrifice and pathology**	
8.5.1	Organ Weights	yes organs: Adrenal glands, Ovaries, Brain, Spleen, Epididymides, Testes, Heart, Thymus, Kidneys, Uterus, Liver
8.5.2	Gross and histopathology	Yes The following slides were examined by a pathologist: - all tissues and organs collected at the scheduled sacrifice from all animals of the control and the highest dose group (i.e. groups 1, 4, 5 and 6); - all gross lesions of all animals.

Identification marks: not processed	Pancreas
Adrenal glands	Peyer's patches (jejunum, ileum) if detectable
Aorta	Pituitary gland
Brain (cerebellum, mid-brain, cortex)	(Preputial gland)
Caecum	Prostate gland
Cervix	Rectum
(Clitoral gland)	Salivary glands - mandibular, sublingual
Colon	Sciatic nerve
Duodenum	(Seminal vesicles)
Epididymides	(Skeletal muscle)
(Eyes with optic nerve and Harderian gland)	(Skin)
Female mammary gland area	Spinal cord -cervical, midthoracic, lumbar
(Femur including joint)	Spleen
Heart	Sternum with bone marrow
Ileum	Stomach
Jejunum	Testes
Kidneys	Thymus

(Larynx)	Thyroid including parathyroid
(Lacrimal gland, exorbital)	(Tongue)
Liver	Trachea
Lung, infused with formalin	Urinary bladder
Lymph nodes - mandibular, mesenteric	Uterus
(Nasopharynx)	Vagina
Oesophagus	All gross lesions
Ovaries	

Tissues mentioned within brackets were not examined microscopically as there were no signs of toxicity or target organ involvement.

8.5.3 Other examinations: None.

8.5.4 Statistics: None.

8.6 Further remarks

Dose levels for groups 1-4 (0, 100, 300 and 1000 ppm) were based on results of a 14-day dietary range finding study with ACTICIDE® OIT [redacted]

In this 14-day range finder dose levels were selected to be 0, 2000, 6000 and 10.000 ppm. A dose level of 2000 ppm resulted in an irregular surface of the forestomach in most animals. At 6000 and 10.000 ppm all animals were sacrificed or died spontaneously.

Dose levels for groups 5-6 (0 and 3000 ppm) were added [redacted] since no clear effect level could be discerned based on the results of groups 1-4.

9 RESULTS AND DISCUSSION

9.1 Observations

9.1.1 Clinical signs: Hunched posture, abdominal swelling and/or piloerection were observed in most males and all females at 3000 ppm in weeks 1/2.

9.1.2 Mortality: One female at 3000 ppm was found dead on day 76. A cause of death could not be established histopathologically.

9.2 Body weight gain

Body weights/weight gain was reduced for males and females at 3000 ppm from week 1 of treatment onwards, achieving a level of statistical significance in all instances. Slightly reduced body weights and body weight gain were also recorded for females at 1000 ppm from week 5 onwards, but did not achieve a level of statistical significance. The total weight gain deficit was approximately 12% for the 1000 ppm group and approximately 15-20% for the 3000 ppm group, when compared to its concurrent control group.

9.3 Food consumption and compound intake

No toxicologically relevant changes in food intake before or after allowance for body weight were observed.

The average intake of active ingredient (OIT) achieved during the 13-week study period is given below.

Average OIT intake (mg OIT/kg body weight/day)*

Section A6.4.1-02 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in rats

100 ppm		300 ppm		1000 ppm		3000 ppm	
M	F	M	F	M	F	M	F
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2	*3*	*9*	*11*	*46*	*56*	*195*	*239*

* I.e. corrected for [redacted] for food scatter. [redacted] Values in italics represent values after correction for mean analytical accuracy.

9.4 Ophthalmoscopic examination

No abnormalities.

9.5 Blood analysis

9.5.1 Haematology

no effects

9.5.2 Clinical chemistry

no effects

9.5.3 Urinalysis

Not applicable.

9.6 Sacrifice and pathology

9.6.1 Organ weights

no effects

9.6.2 Gross and histopathology

Necropsy:

Irregular surface of the forestomach and/or a thickened limiting ride of the stomach was observed in all males and 8/10 females at 3000 ppm.

Histopathology

Hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach was recorded in all males at 3000 ppm (one male: minimal, eight males: slight and one male: moderate).

In nine females of group 6 hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach was recorded (one female: minimal, seven females: slight and one female: moderate).

9.7 Other

None.

10 APPLICANT'S SUMMARY AND CONCLUSION

10.1 Materials and methods

Based on a 14-day dietary range finding study [redacted], the dose levels for this 90-day dietary study were selected to be 0, 100, 300 and 1000 ppm. Based on the results obtained at these dose levels [redacted], additional groups at 0 and 3000 ppm were dosed.

The study was based on the following guidelines.

- EC Directive 67/548/EEC, B Repeated Dose (90 days) Toxicity (oral), 2001.
- OECD 408, Repeated Dose 90-day Oral Toxicity Study in Rodents, 1998.
- EPA 712-C-98-199, 90-Day Oral Toxicity in Rodents, 1998.

Section A6.4.1-02 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in rats

[redacted] Rats received the test substance by dietary intake for at least 90 days. One control group and three treated groups were tested, each consisting of 10 males and 10 females. Based on the results obtained at these dose levels and in consultation with the sponsor, additional groups at 0 or 3000 ppm were dosed.

The following parameters were evaluated:

Clinical signs, functional observations, body weight, food consumption and ophthalmoscopy. At termination: clinical pathology, macroscopy, organ weights and histopathology on a selection of tissues.

10.2 Results and discussion

Homogeneity and accuracy of diet preparations were considered to be acceptable. Stability of diets over 6 weeks at room temperature was confirmed.

The average intake of active ingredient (OIT) achieved during the 13-week study period was as follows (before and after correction for mean analytical accuracy):

Dietary inclusion level (ppm)	Mean analytical accuracy (% of target conc.)	Average OIT intake (mg OIT/kg body weight/day) [1]	
		males	females
100	40	[redacted] *2*	[redacted] *3*
300	49	[redacted] *9*	[redacted] *11*
1000	61	[redacted] *46*	[redacted] *56*
3000	93	[redacted] *195* [2]	[redacted] *239* [2]

[1] I.e. corrected [redacted] for food scatter. [redacted] Values in italics represent values after correction for mean analytical accuracy. [redacted]

[2] No quantitative assessment of food scatter could be performed due to the type of housing. Based on food scatter measurements at the 1000 ppm level, actual food intake and test article intake at the 3000 ppm level was considered to be at least 15% less than indicated in the table.

It was concluded that the lower recoveries were most likely due to reaction of OIT with sulfur containing compounds in the diet and/or effects of irreversible binding of OIT. Extraction was however complete with regard to the extractable OIT.

Histopathological assessment revealed hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach in most animals at 3000 ppm which correlated to thickening of the limiting ridge and irregular surface of the forestomach. These morphological changes were considered to represent a response to local irritation to test material residing in the forestomach. There were no histological changes apparent in tissues and organs other that the stomach.

The lower body weights (total weight gain deficit approximated 20%) and clinical signs consisting of hunched posture, abdominal swelling and/or piloerection at 3000 ppm were considered to be related to the stomach effects.

Section A6.4.1-02 **Subchronic toxicity (oral)**

Annex Point IIA6.4.1 *90 days dietary toxicity study in rats*

From the parameters assessed, no evidence for neurotoxic potential of the test substance was obtained.

No treatment-related mortality, and no effects on functional observations tests, food consumption, clinical pathology and organ weights occurred at any of the dose levels administered. Also, no clinical signs of toxicity, effects on body weight, macro- or microscopic abnormalities were apparent at dose levels up to 1000 ppm.

10.3 Conclusion

10.3.1	LO(A)EL	Local toxicity: 3000 ppm (critical effects: hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach *(all males and 9/10 females)*, thickening of the limiting ridge and irregular surface of the forestomach *(all males and 8/10 females)*.
10.3.2	NO(A)EL	Local toxicity: 1000 ppm (610 ppm based on overall analytical accuracy of the 1000 ppm diet preparations), corresponding to an actual intake of 68 and 82 mg active ingredient (OIT)/kg body weight/day for males and females respectively (46 and 56 mg active ingredient (OIT)/kg body weight/day for males and females respectively, based on overall analytical accuracy of the 1000 ppm diet preparations). Systemic toxicity: 3000 ppm (2790 ppm based on overall analytical accuracy of the 3000 ppm diet preparations), corresponding to an actual intake of 210 and 257 mg active ingredient (OIT)/kg body weight/day for males and females respectively (195 and 239 mg active ingredient (OIT)/kg body weight/day for males and females respectively, based on overall analytical accuracy of the 3000 ppm diet preparations).
10.3.3	Other	Evidence for primary systemic toxicity was absent at dose levels up to 3000 ppm.
10.3.4	Reliability	1
10.3.5	Deficiencies	Yes: any deviations from the protocol/test guideline were considered to have no adverse effect on the study integrity (see 2.3).

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*01/04/2009*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	*1*
Acceptability	*Acceptable*

Section A6.4.1-02 Subchronic toxicity (oral)

Annex Point IIA6.4.1

90 days dietary toxicity study in rats

Remarks	*In agreement with the applicants assessment*
	COMMENTS FROM ... *(specify)*
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_4 1. Results of clinical chemistry haematology and urinalysis~~

~~parameter changed~~	~~Unit~~	~~Controls~~			~~low dose~~			~~medium dose~~			~~high dose~~		
~~weeks after start of treatment~~													
~~males~~													
~~No effects~~													
~~females~~													
~~No effects~~													

Table A6_4-2. Results *(specify)* of repeated dose toxicity study

Parameter	**Control**		**low dose 100 ppm**		**medium dose 300 ppm**		**high dose 1000 ppm**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
number of animals examined	10	10	10	10	10	10	10	10
Mortality	0	0	0	0	0	0	0	0
clinical signs	0	0	0	0	0	0	0	0
body weight [2]	=	=	=	=	=	=	=	↓ (-18%)
food consumption	=	=	=	=	=	=	=	=
clinical chemistry	=	=	=	=	=	=	=	=
haematology	=	=	=	=	=	=	=	=
Stomach organ weight	NA	NA	NA	NA	NA	NA	NA	NA
gross pathology	=	=	=	=	=	=	=	=
microscopic pathology	=	=	=	=	=	=	=	=

[1] Based on all groups, i.e. groups 1-6.

[2] Total weight gain deficit over the study period compared to control weight gain given between parentheses.

NA Not applicable.

Table A6_4-2. ***(Continued)***

Parameter	Control		3000 ppm		dose-response +/- [1]	
	m	**f**	**m**	**f**	**m**	**f**
number of animals examined	10	10	10	10	10	10
Mortality	0	0	0	1	-	-
clinical signs Hunched posture, abdominal swelling and/or piloerection	0	0	7	10	+	+
body weight [2]	=	=	↓ (-14%)	↓ (-17%)	+	+
food consumption	=	=	=	=	-	-
clinical chemistry	=	=	=	=	-	-
haematology	=	=	=	=	-	-
Stomach organ weight	NA	NA	NA	NA	NA	NA
gross pathology Irregular surface of the forestomach and thickened limiting ride of the stomach	0	0	10	8	+	+
microscopic pathology Hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach	0	0	10	9	+	+

[1] Based on all groups, i.e. groups 1-6.

[2] Total weight gain deficit over the study period compared to control weight gain given between parentheses.

NA Not applicable.

Section A 6.4.2-01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

90-day dermal toxicity study in rats

Official use only

11 REFERENCE

11.1	**Reference**	[redacted] *1995, n-Octylisothiazolinone (OIT) 94% +/- 3%: 90-Day Dermal Subchronic Toxicity Study in the Rat,* [redacted] *unpublished*
11.2	**Data protection**	*Yes*
11.2.1	Data owner	*THOR GmbH, Germany*
11.2.2		
11.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.

Section A 6.4.2-01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5

90-day dermal toxicity study in rats

		12 GUIDELINES AND QUALITY ASSURANCE	
12.1	**Guideline study**	Yes. EPA 82-3 which equals OECD 411	
12.2	**GLP**	Yes	
12.3	**Deviations**	No	
		13 MATERIALS AND METHODS	
13.1	**Test material**	As given in section 2	
13.1.1	Lot/Batch number	[redacted]	
13.1.2	Specification	*Technical grade*	
13.1.2.1	Description	*Brown yellow liquid*	
13.1.2.2	Purity	[redacted]	X
13.1.2.3	Stability	[redacted]	
13.2	**Test Animals**		
13.2.1	Species	*Rat*	
13.2.2	Strain	[redacted]	
13.2.3	Source	[redacted]	
13.2.4	Sex	*Both*	
13.2.5	Age/weight at study initiation	*5-6 weeks* *Males: 115-159 g; Females 111-142 g*	
13.2.6	Number of animals per group	*10 per sex /group*	
13.2.7	Control animals	Yes	
13.3	**Administration/ Exposure**	Dermal	
13.3.1	Duration of treatment	90 days	
13.3.2	Frequency of exposure	Daily	
13.3.3	Postexposure period	No	
13.3.4	**Dermal**		

Section A 6.4.2-01 — **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 — *90-day dermal toxicity study in rats*

13.3.4.1	Area covered	5 cm x 7 cm on the back and the flanks of each animal
13.3.4.2	Occlusion	semiocclusive
13.3.4.3	Vehicle	Corn oil
13.3.4.4	Concentration in vehicle	1:200 (5 mg/kg bw /day); 1:40 (25 mg/kg bw /day); 1:8 (125 mg/kg bw /day)
13.3.4.5	Total volume applied	1 ml / kg bw
13.3.4.6	Duration of exposure	Other: 6 hours per day
13.3.4.7	Removal of test substance	water
13.3.4.8	Controls	vehicle
13.4	**Examinations**	
13.4.1	Observations	
13.4.1.1	Clinical signs	*Yes, once daily*
13.4.1.2	Mortality	*Yes, twice daily*
13.4.2	Body weight	*Yes, weekly*
13.4.3	Food consumption	*Yes, twice a week*
13.4.4	Water consumption	*No*
13.4.5	Ophthalmoscopic examination	*Yes, once pre-dose (all animals) and once during last week of treatment (controls and high dose animals)*
13.4.6	Haematology	Yes, from all animals at end of study; Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, prothrombin time, thromboplastin time
13.4.7	Clinical Chemisty	Yes, from all animals at end of study; Parameters: sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, lipids, glutamate dehydrogenase
13.4.8	Urinalysis	Yes, from all animals at end of study; Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood and other: leukocytes, ketones, bilirubin, urobilinogen, microscopy of centrifuged deposits: cells, organic inorganic components, casts.
13.5	**Sacrifice and pathology**	
13.5.1	Organ Weights	yes organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart and others: pituitary thyroid.
13.5.2	Gross and histopathology	yes high dose group and controls as well as intercurrent deaths organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus,

		oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female maammary gland, prostate, urinary bladder, gall bladder (mouse), lymph node,s peripheral nerve, bone marrow, skin, eyes or other
13.5.3	Other examinations	*Local skin reactions.* *For scale of evaluation see table A6.3-2 attached to this summary.*
13.5.4	Statistics	Test for homogeneity of variance: Lenvene's test, Bartlett's test (anova) Comparision: Dunnett's two-tailed t-test, Kruskal-Wallis test together with Wilcoxon rank-sum test SAS sofware package 6.04, TERASYS online data collection system
13.6	**Further remarks**	
		14 RESULTS AND DISCUSSION *(Describe findings. If appropriate, include table. Sample tables are given below.)*
14.1	**Observations**	
14.1.1	Clinical signs	*Apart from minimal to slight skin alterations in the intermediate and high dose group being described in section 4.5, there were no clinical changes during the experimental period that could be attributed to treatment with the test article.*
14.1.2	Mortality	*In males there were no mortalities throughout the treatment period.* *In females the following animals died during the treatment period:* *Group 1 - 0 mg/kg/day* *- 41 F, was found dead an day 13, replaced by 81 F* *Group 2 - 5 mg/kg/day* *- 57 F, was found dead after blood sampling an day 91* *Group 3 - 25 mg/kg/day* *- 64 F, was found dead after blood sampling an day 90;* *- 67 F, was found dead after blood sampling an day 91* *All mortalities are considered to be unrelated to treatment with the test material.*
14.2	**Body weight gain**	*There was an apparent effect on body weight gain in high dose males (125 mg/kg/day) from the fourth week of study onwards with statistically significant body weight data in weeks 9, 11 to 13 and at necropsy.* *In consequence, overall body weight change (week 1 to necropsy) was statistically significantly reduced for high dose males.* *There was no treatment-related effect on body weights at 5 and 25 mg/kg/day.* *There were no apparent treatment-related effects on body weight in female animals..*
14.3	**Food consumption and compound intake**	*There were no signs of treatment-related effects on overall food intake*
14.4	**Ophtalmoscopic examination**	*There were no treatment-related ocular changes.*
14.5	**Blood analysis**	

Section A 6.4.2-01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5

90-day dermal toxicity study in rats

14.5.1	Haematology	*Although statistical evaluations revealed a few minor significantly different changes, there were no treatment-related findings observed at the end of the experimental period.*
14.5.2	Clinical chemistry	*Although statistical evaluation revealed a few significantly different minimal changes, there were no treatment-related findings observed at the end of the experimental period.* *Markedly increased means for GLDH in control males were due to high levels of a single animal, which is considered to be of minor significance. Slightly increased statistically significant AST levels in high dose females are still in the range of our Background data and in the view of no adverse histopathological liver changes, these finding are felt to be of no toxicological significance.*
14.5.3	Urinalysis	*There were no treatment-related urine analysis findings at the end of the treatment period.*
14.6	**Sacrifice and pathology**	
14.6.1	Organ weights	*Although statistical evaluation revealed a few significantly different minor changes, there were no treatment-related organ weight changes in treated animals.*
14.6.2	Gross and histopathology	*MACROSCOPIC NECROPSY FINDINGS* *Apart from minimal to slight skin alterations in the intermediate and high dose group, there were no macroscopic lesions in any of the organs or tissues examined that could be ascribed to the test article.* *MICROSCOPIC NECROPSY FINDINGS* *The only microscopic treatment-related findings were lesions in the treated skin sites of high dose males and females. These lesions were squamous cell hyperplasia, sebaceous cell hyperplasia, folliculitis, dermatitis and hemorrhages.* *There were no histopathological lesions in the other organs and tissues suggestive of systemic target organ toxicity due to the test article.* *Tissues from low dose and intermediate dose groups were not examined histopathologically.*
14.7	**Other**	*LOCAL SKIN REACTIONS* *There were no cutaneous lesions in control animals being treated with corn oil and there were no cutaneous lesions in low dose animals receiving 5 mg/kg/day apart from a single occasion in a single female in week 12, when slight atonia was observed. This finding is considered to be of no toxicological significance.* *Administration of OIT [redacted] at a dose level of 25 mg/kg/day was relatively well tolerated and elicited only minimal local skin reactions. Overall mean scores (week 2 to 13) revealed the following values: erythema (grade: 0.0 to 0.2 M; 0.0 to 0.3 F; mean: 0.1 M; 0.2 F), edema (grade: 0.0 to 0.3 M; 0.0 to 0.2 F; mean: 0.1 M/F) and atonia (grade: 0.0 to 1.0 M; 0.0 to 0.9 F; mean 0.4 M; 0.3 F).* *A dose level of 125 mg/kg/day was not well tolerated and elicited slight to moderate skin lesions (overall mean scores, week 2 to 13) such as erythema (grade: 1.3 to 2.1 M; 1.3 to 2.0 F; mean: 1.8 M; 1.6 F), edema (grade: 1.0 to 2.0 M; 1.3 to 2.0 F; mean: 1.8 M/F), atonia (grade: 1.0 to 2.0 M; 1.2 to 2.0 F; mean: 1.8 M/F), desquamation (grade: 0.0 to 2.0 M; 0.0 to 1.4 F; mean: 1.3 M; 0.9 F) and fissures (grade: 0.1 to 0.8 M; 0.0 to 0.7 F; mean: 0.4 M; 0.2 F).*

Section A 6.4.2-01 — **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 — *90-day dermal toxicity study in rats*

Overall mean total scores (week 2 to 13) for the local reactions examined (erythema, edema, atonia, desquamation and fissures) are summarized as follows:

Mean total score

	G1	G2	G3	G4
males	*0.0*	*0.0*	*0.1*	*1.4*
females	*0.0*	*0.0*	*0.1*	*1.2*

In addition, scabbing (without exfoliation) was observed in the majority of all high dose animals throughout the experimental period.

15 APPLICANT'S SUMMARY AND CONCLUSION

15.1 Materials and methods

Per-guideline study with technical grade test item (96.4 % OIT).

15.2 Results and discussion

Treatment with the test article 0IT applied dermally to intact skin sites produced minimal local reactions at the application site in animals receiving 25 mg/kg/day and slight to moderate cutaneous lesions at the high dose levels of 125 mg/kg/day being characterized microscopically as cell hyperplasia, folliculitis, dermatitis and hemorrhages.

Furthermore, body weight gain of high dose males exclusively was distinctively reduced from week 4 of treatment onwards when compared to corresponding controls.

There were no noteworthy cutaneous reactions in male and female animals receiving the test material at a dose level of 5 mg/kg/day.

Several deaths that occurred throughout the experimental period in one control, one low dose and two intermediate dose females were due to experimental procedure and are considered to be unrelated to treatment with the test material.

15.3 Conclusion

0IT was very well tolerated at a dose level of 5 mg/kg/day when administered dermally to intact skin for at least 90 days.

Administration of the test article at a dose level of 25 mg/kg/day was relatively well tolerated and elicited only minor local skin reactions such as erythema, edema and atonia.

A dose level of 125 mg/kg/day was not well tolerated and elicited slight to moderate skin lesions being described macroscopically as erythema, edema, atonia, desquamation, fissures and scabbing without exfoliation. Microscopically these cutaneous lesions were defined as cell hyperplasia, folliculitis, dermatitis and hemorrhages.

In addition, high dose males exhibited an apparent adverse effect on body weight gain from the fourth week of study onwards.

15.3.1 LO(A)EL — *125 mg/kg/day based on slight to moderate local skin reactions: macroscopically: erythema, edema, atonia, desquamation, fissures and scabbing without exfoliation; microcsopically: cell hyperplasia, folliculitis, dermatitis and hemorrhages; decreased body weight gain in males from 4th week onwards)*

15.3.2 NO(A)EL — *25 mg/kg/day*

15.3.3 Other: local LOAEL — *25 mg/kg/day*

15.3.4 Reliability — *1*

15.3.5 Deficiencies — *No*

Section A 6.4.2-01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

90-day dermal toxicity study in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*17/04/2009*
Materials and Methods	[redacted]
Results and discussion	
Conclusion	
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*In agreement with the applicant's assessment.*
	COMMENTS FROM ... *(specify)*
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_3-1. Results of clinical chemistry haematology and urinalysis

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

parameter changed	*Unit*	*Controls*			*low dose*			*medium dose*			*high dose*		
weeks after start of treatment													
males													
females													

** p < 0,05*

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_3-2. Results *(cutaneous)* of repeated dose toxicity study

APPENDIX I

SCALE OF EVALUATION OF CUTANEOUS LESIONS

Erythema

0 - no erythema
1 - slight erythema (hardly visible)
2 - moderate erythema (well-defined)
3 - severe erythema (purplish-red)

Edema

0 - no edema
1 - slight edema (hardly visible to clearly visible with obvious swelling)
2 - moderate edema (swelling of approximately 1 mm)
3 - severe edema (swelling greater than 1 mm)

Atonia (without eschar formation)

0 - normal
1 - slight atonia (modification in elasticity)
2 - moderate atonia (slow return to normal)
3 - marked atonia (no elasticity)

Desquamation (without eschar formation)

0 - none
1 - slight desquamation
2 - moderate desquamation (crusts and scales)
3 - pronounced desquamation (marked scaling with bare areas)

Fissures

0 - none
1 - slight fissures (cracks in the epidermis)
2 - moderate fissures (cracks in the dermis)
3 - pronounced fissures (cracks with bleeding)

APPENDIX I (cont.)

Eschar formation

N - no
Y - yes

Exfoliation (eschar formation)

N - no
Y - yes

Table 6 Group Mean Local Skin Reaction [redacted]

Occasion week 1 of study

a) Male animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Eschar formation %	Incidence	0	0	0	0
Exfoliation %	Incidence	0	0	0	0

Table 6 Group Mean Local Skin Reaction [redacted]

Occasion week 1 of study

b) Female animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Eschar formation %	Incidence	0	0	0	0
Exfoliation %	Incidence	0	0	0	0

Table 7 Group Mean Local Skin Reaction [redacted]

Occasion week 2 of study

a) Male animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.1	2.0
	SD	0.0	0.0	0.3	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.1
	SD	0.0	0.0	0.0	0.3
	N	10	10	10	10
Eschar formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Table 7 Group Mean Local Skin Reaction [redacted]

Occasion week 2 of study

b) Female animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.1	2.0
	SD	0.0	0.0	0.3	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.3
	SD	0.0	0.0	0.0	0.5
	N	10	10	10	10
Eschar formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Table 11 Group Mean Local Skin Reaction [redacted]

Occasion week 6 of study

a) Male animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.1	1.7
	SD	0.0	0.0	0.3	0.5
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.1	1.4
	SD	0.0	0.0	0.3	0.5
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.3	1.3
	SD	0.0	0.0	0.5	0.5
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	0.8
	SD	0.0	0.0	0.0	0.4
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.7
	SD	0.0	0.0	0.0	0.5
	N	10	10	10	10
Escher formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Table 11 Group Mean Local Skin Reaction [redacted]

Occasion week 6 of study

b) Female animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.0	1.8
	SD	0.0	0.0	0.0	0.4
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.1	2.0
	SD	0.0	0.0	0.3	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	1.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.7
	SD	0.0	0.0	0.0	0.5
	N	10	10	10	10
Escher formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Table 18 Group Mean Local Skin Reaction [redacted]

Occasion week 13 of study

a) Male animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.2	2.1
	SD	0.0	0.0	0.4	0.3
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.2	2.0
	SD	0.0	0.0	0.4	0.0
	N	10	10	10	10
Atonia	Mean	0.0	0.0	1.0	2.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	1.3
	SD	0.0	0.0	0.0	0.5
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.3
	SD	0.0	0.0	0.0	0.5
	N	10	10	10	10
Escher formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Table 18 Group Mean Local Skin Reaction [redacted]

Occasion week 13 of study

b) Female animals

		Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 125 mg/kg/day
Erythema	Mean	0.0	0.0	0.2	1.9
	SD	0.0	0.0	0.4	0.3
	N	10	10	10	10
Edema	Mean	0.0	0.0	0.1	1.8
	SD	0.0	0.0	0.3	0.4
	N	10	10	10	10
Atonia	Mean	0.0	0.0	0.4	1.9
	SD	0.0	0.0	0.5	0.3
	N	10	10	10	10
Desquamation	Mean	0.0	0.0	0.0	1.0
	SD	0.0	0.0	0.0	0.0
	N	10	10	10	10
Fissures	Mean	0.0	0.0	0.0	0.2
	SD	0.0	0.0	0.0	0.4
	N	10	10	10	10
Escher formation %	Incidence	0	0	0	100
Exfoliation %	Incidence	0	0	0	0

Figure 1 [redacted]

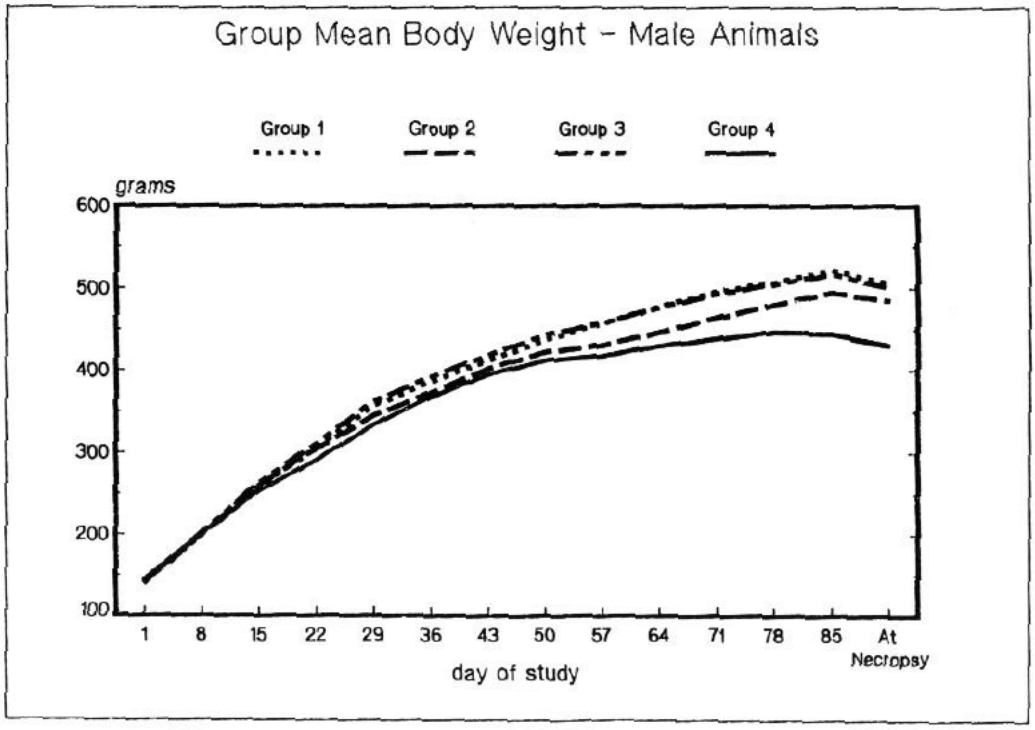
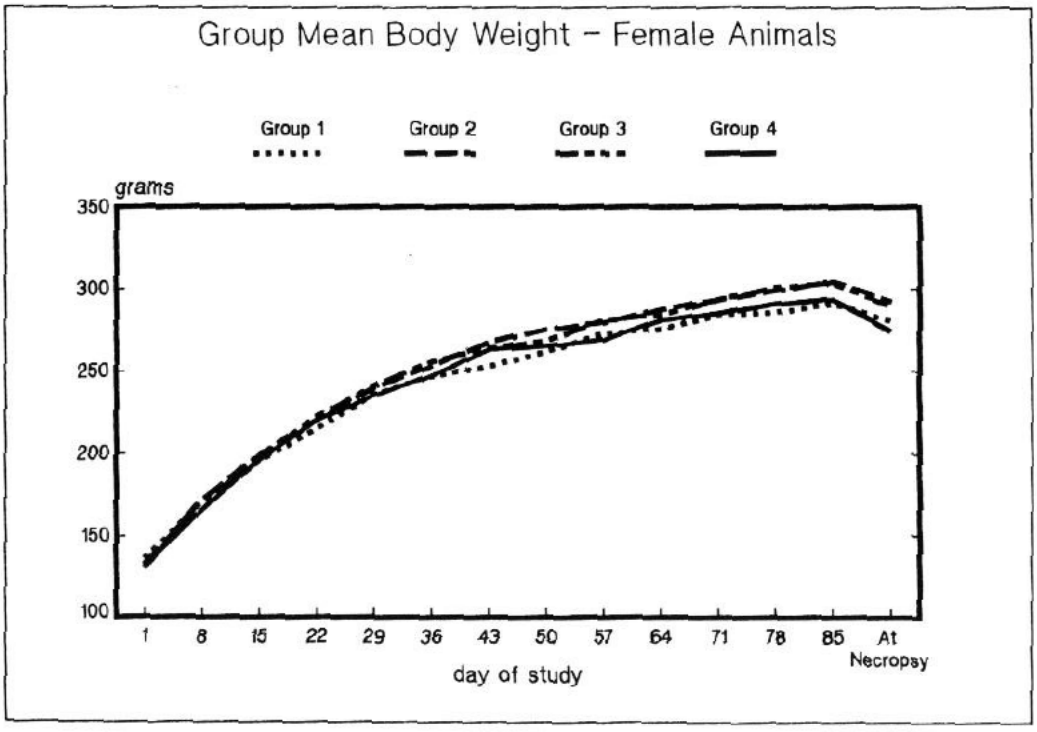


Figure 1 (cont.) [redacted]



Section A6.4.3 J **Annex Point A6.4**	**Subchronic inhalation toxicity test** 90 days inhalation study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The following situation applies to OIT during the manufacturing process and when used as preservative: • OIT has a very low vapour pressure of 0.0031 hPa at 20 °C (OECD 104; [redacted] "Determination of the Vapour Pressure of 2-Octyl-3(2H)-isothiazolone", 2002) • For the manufacturing of OIT and of b.p. containing OIT, a closed system technology applies. • For mixing and loading of OIT, due to the low vapour pressure, no inhalative exposure is expected. • Further, the manufacturer stipulates in the technical information for any OIT containing formulation that during handling and processing the formation of aerosols should be avoided. Since during the life cycle of OIT the inhalative route of exposure can be neglected, the information one would obtain from a 90 day inhalation study was considered to be not needed in order to determine the risk assigned. With view to animal welfare and to avoid unnecessary animal testing, particularly on mammals, it was therefore decided to abdicate such a study. A 90 day inhalation toxicity test is not necessary for risk assessment since a OEL is in force in several member states. This limit value has been used for risk assessment purpose (see Doc IIA and Doc IIC).	
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		

Section A6.4.3 J **Annex Point A6.4**	**Subchronic inhalation toxicity test** 90 days inhalation study
	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 A6.5-01 **Annex Point 6.5**	**Long term toxicity in rats**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [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 so far unknown potential for chronic toxicity.	
References	[redacted] 2007, 2-n-Octyl-4-isothiazolin-3-one - Justification for the non-submission of data: Chronic toxicity/Oncogenicity, [redacted], unpublished	
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	*17/04/2009*	
Evaluation of applicant's justification		
Conclusion	*acceptable*	
Remarks	*Further discussed in Doc IIA.*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

Section A6.5-01 Annex Point 6.5	Long term toxicity in rats
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 A6.5-02 — Long term repeated dose toxicity (oral feed)

Annex Point IIA6.5

18 months chronic toxicity/carcinogenicity study in mice

Official use only

		16 REFERENCE
16.1	**Reference**	[redacted] *1975, Eighteen month study on the carcinogenic potential of RH-893 in mice* [redacted] *unpublished.*
16.2	**Data protection**	*Yes*
16.2.1	Data owner	*THOR GmbH*
16.2.2		
16.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.
		17 GUIDELINES AND QUALITY ASSURANCE
17.1	**Guideline study**	No (pre-guideline)
17.2	**GLP**	No (pre-GLP)
17.3	**Deviations**	*Yes, numerous deficiencies with respect to recent guidelines*
		18 MATERIALS AND METHODS
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values depending on the true methodological parameters.
18.1	**Test material**	*Other: RH-893*
18.1.1	Lot/Batch number	*RH-893* [redacted]
18.1.2	Specification	*Technical grade*

Section A6.5-02 Long term repeated dose toxicity (oral feed)

Annex Point IIA6.5

18 months chronic toxicity/carcinogenicity study in mice

18.1.2.1	Description	*Dark liquide*
18.1.2.2	Purity	[redacted]
18.1.2.3	Stability	*Stable. Dietary admixtures were prepared weekly.*
18.2	**Test Animals**	Non-entry field
18.2.1	Species	*mice*
18.2.2	Strain	[redacted]
18.2.3	Source	[redacted]
18.2.4	Sex	*Both sex*
18.2.5	Age/weight at study initiation	*Age: 6 weeks* *Weight: 14.5 – 17.5 g in females; 17.0 – 20.5 g in males*
18.2.6	Number of animals per group	*125/sex/dose group*
18.2.7	Control animals	Yes (negative and two positive control groups): 2-Acetaminofluorene (AAF) in food and Dieethylnitrosamine (DEN) in drinking water
18.3	**Administration/ Exposure**	Oral
18.3.1	Duration of treatment	18 month (78 weeks)
18.3.2	Frequency of exposure	daily (feed admixture)
18.3.3	Postexposure period	No
18.3.4	**Oral**	
18.3.4.1	Type	in food
18.3.4.2	Concentration	0, 500, 1000 ppm in diet ad libitum substance uptake was not analysed (mg/kg bw)
18.3.4.3	Vehicle	Not applicable
18.3.4.4	Concentration in vehicle	Not analysed.
18.3.4.5	Total volume applied	Ad libitum
18.3.4.6	Controls	plain diet
18.4	**Examinations**	
18.4.1	Observations	
18.4.1.1	Clinical signs	*No data*
18.4.1.2	Mortality	*yes*
18.4.2	Body weight	*Yes, once a week in 25/sex/dose*
18.4.3	Food consumption	*Not examined but ad libitum*
18.4.4	Water consumption	*Not examined but ad libitum*

Section A6.5-02 — **Long term repeated dose toxicity (oral feed)**

Annex Point IIA6.5 — *18 months chronic toxicity/carcinogenicity study in mice*

18.4.5	Ophthalmoscopic examination	*no*
18.4.6	Haematology	no
18.4.7	Clinical Chemisty	no
18.4.8	Urinalysis	no
18.5	**Sacrifice and pathology**	
18.5.1	Organ Weights	Yes organs: liver
18.5.2	Gross and histopathology	Yes all dose groups (covers the usual tissues and not be a simple search of tumors) / reported only if effects organs: stomach, small and large intestines, liver, kidneys, spleen, lungs, female mammary gland, prostate, urinary bladder, skin, ovary, gonads.
18.5.3	Other examinations	*At 6 months, 25/sex/dose were sacrificed in view of findings (wheight depression, deaths) in positive control groups (Dimethylnitrosamine and 2-Aminofluorene.*
18.5.4	Statistics	Yes. Not described.
18.6	**Further remarks**	

19 RESULTS AND DISCUSSION

(Describe findings. If appropriate, include table. Sample tables are given below.)

19.1	**Observations**	
19.1.1	Clinical signs	*No data.*
19.1.2	Mortality	The survival of the mice through the 30th week was excellent for all groups with the exception of the DEN high dose group where survival was 50% and 64% respectively. At eighteen months, the survival remained excellent (greater than 96%) for negative control and RH-893 treatment groups; the survival rate of the mice fed diets containing AAF was good for approximately 50 weeks at which time they began dying in increasing numbers with survival rates of 37% in males and 1% females at 18 months.
19.2	**Body weight gain**	When compared to negative control mice, statistically significant lower body weights were observed in the mice receiving drinking water containing DEN and in the female mice fed diets containing AAF (600 ppm). These depressions in body weight were observed beginning the second week and present at the termination of the study at 78 weeks. Statistically significant lower body weights were observed in both males and females received 1000 ppm RH-893 during the first few weeks of the study. By week 15 of the study, these differences were insignificant and remained comparable to the control group throughout the remainder of the study. Sporadic statistically significant body weight differences were observed in the 500 ppm group during the study and were judged not to be compound related.
19.3	**Food consumption and compound intake**	*No data*

Section A6.5-02 Long term repeated dose toxicity (oral feed)

Annex Point IIA6.5

18 months chronic toxicity/carcinogenicity study in mice

19.4	**Ophtalmoscopic examination**	*No data*
19.5	**Blood analysis**	*No data*
19.5.1	Haematology	*No data*
19.5.2	Clinical chemistry	*No data*
19.5.3	Urinalysis	*No data*
19.6	**Sacrifice and pathology**	
19.6.1	Organ weights	Of mice sacrificed after 26 or 30 weeks of treatment, statistically significant increases in the liver/body weight ratios were observed in both male and female mice receiving drinking water containing 6-4 mg/kg/day DEN and females receiving drinking water containing 4 mg/kg/day DEN. Males receiving diets containing 500 ppm RH-893 showed a slight but statistically significant increase in the liver/body weight ratio while males receiving diets containing 1000 ppm RH-893 showed a slight but statistically significant lower liver/body weight ratio. At termination (78 weeks), the liver/body weight ratio was statistica11y significantly increased in male mice receiving diets containing 500 ppm RH 893 and female mice receiving diets containing 1000 ppm RH-893. These differences in liver/body weight ratio of the RH-893 treated mice are judged to be of no toxicological significance.
19.6.2	Gross and histopathology	Histopathologic examination of mice sacrificed after 26 or 30 weeks of treatment with DEN revealed "diffuse and nodular carcinoma" of the livers in most mice and bronchiectasis in many of the mice. Histopathologic examination of mice receiving RH-893 for 30 weeks demonstrated no pathologic or cytologic changes attributable to the administrationof RH-893. Of the non-neoplastic lesions observed, only hyperplasia of the urinary bladder in the male mice treated with AAF, is considered to be of toxicologic significance. A higher incidence of neoplastic lesions of the liver and urinary bladder was observed in both male and female mice fed diets containing AAF and is considered to be compound related. The incidence and types of neoplastic lesions in the RH-893 mice are those expected to occur spontaneously in this strain of mouse.
19.7	**Other**	-

20 APPLICANT'S SUMMARY AND CONCLUSION

20.1	**Materials and methods**	RH-893 [redacted] at concentrations of 0, 500 and 1000 ppm admixed with feed was fed to 125 [redacted] mice/sex/group [redacted] for 18 months. At 6 months, 25/sex/dose were sacrificed.
20.2	**Results and discussion**	Body weights were reduced at 1000 ppm in both sexes, especially early in the study. No adverse effect identified.

Section A6.5-02 Long term repeated dose toxicity (oral feed)

Annex Point IIA6.5

18 months chronic toxicity/carcinogenicity study in mice

20.3	**Conclusion**	No oncogenic response to exposure with up to 1000 ppm OIT.
20.3.1	LO(A)EL	No adverse effect identified.
20.3.2	NO(A)EL	1000 ppm OIT in diet
20.3.3	Other	
20.3.4	Reliability	*3*
20.3.5	Deficiencies	*Yes* *Stability of test substance in the diet was not analysed (recovery) and substance uptake was not analysed .- However, palatability of test diet was achieved.* *Examinations of several endpoints were not addressed. But fullterm survival and no-incidence with regard to typical endpoints in comparison to known carcinogenes were successfully demonstrated.*

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	*3*
Acceptability	*Acceptable*
Remarks	*In agreement with the applicant's assessment.*
	COMMENTS FROM ... *(specify)*
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 I: Eighteen Month Study on the Carcinogenic Potential of RH-893 in Mice

Summary of Findings

Group	Experimental Compound	Dose level	Sex	18 Month[b] Survivors/Initial	Body Wt. (g)		Liver/Body Wt. x 10^{-3}		No of Lesions/Number of mice examined		
		Diet (ppm)			6 Mo.	18 Mo.	6 Mo.	18 Mo.	Non-neoplastic	Neoplastic	Metastasis
1	negative	0	M	98/100	30.6	33.8	48.9	49.6	7/100	8/100	0/100
	control		F	96/103	28.1	34.1	49.1	48.7	13/100	2/100	0/100
2	RH-893	500	M	97/100	32.3	36.5	52.9*	52.3*	-	-	-
			F	99/100	28 5	33.7	50.9	49.2	-	-	-
3	RH-893	1000	M	97/100	30.4	34.0	46.4 *	50.6	1/48	4/48	0/48
			F	97/100	27.7	33 1	51.4	50.8*	2/48	5/48	0/48
4	AAF[d]	600	M	37/100	29.9	32.4	-	-	14/100	50/100	2/100
			F	1/100	24.9*	25.1[c]	-	-	8/98	117/98	4/98
		Daily Intake mg/kg/day									
5	DEN°	4	M	-	24.6*	-	52.3		-	-	-
			F	-	19.4*	-	71.6*		-	-	-
6	DEN[e]		M	-	20.4*	-	68.6*		-	-	-
			F	-	17.1*	-	101.0*		-	-	-

* significantly different from control $p < 0.05$

[a] Re-tabulated from: Appendix A, Tables

[b] Does not include mice sacrificed at 30weeks.

[c] Week 70 data.

[d] 2-Acetamidofluorene

[e] Diethyl nitrosamine (DEN) administered in drinking water.

Table II: Eighteen Month Study an the Carcinogenic Potential of RH-893 in Mice

Summary of Histopathologic Lesions

No. of Mice Examined	Male			Female		
	Neg. Control	AAF[a]	RH-893	Neg. Control	AAF	RH-893
	100	100	1000 ppm 48	100	98	1000 ppm 48
Non-neoplastic Lesions						
Hyperplasia bladder		.3				
Infections & granulomas	3			4	5	
Others	4	1	1	9	3	2
Total	7	14	1	13	8	2
Neoplastic Lesions						
Urinary bladder		15			17	
Liver	7	30	3		89	
Lymph node	1	3			3	3
Others		2	1		8	2
Total	8	50	4	2	117	5

[a] 2-Acetamidofluorene