			Official use only
		1 REFERENCE	
1.1	**Reference**	Leuschner P J, (2011). Acute Inhalation Toxicity Study of Copper Powder KU 7600 Standard Material in Rats. LPT Laboratory of Pharmacology and Toxicology GmbH & Co. Report No.: 27371 (Unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Confidential	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes – the study was carried out in accordance to the following test guidelines: OECD Guideline 436: Acute inhalation toxicity – Acute toxic class method (2009)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Copper flakes, coated with aliphatic acid	
3.1.1	Lot/Batch number	Batch No. 11G0015 Receipt No. 48194	
3.1.2	Specification	Particle size distribution (non-GLP determination): $d_{(0.1)}$ = 4.49 µm $d_{(0.5)}$ = 10.78 µm (median) $d_{(0.9)}$ = 21.42 µm	
3.1.2.1	**Description**	Reddish-brown (copper) solid powder.	
3.1.2.2	**Purity**	Confidential	
3.1.2.3	**Stability**	Expiry date: Min. May 2012	

3.2	**Test Animals**	
3.2.1	Species	*Rattus norvegicus.*
3.2.2	Strain	CD [stock: Crl:CD(SD)]
3.2.3	Source	Charles River Laboratories, Research Models and Services Germany GmbH, Germany
3.2.4	Sex	Male and Female
3.2.5	Age/weight at study initiation	Males: Approx. 7 weeks and 238-262 g Females: Approx. 9 weeks and 231-245 g
3.2.6	Number of animals per group	3 males (Group 1) and 3 females (Group 2).
3.2.7	Control animals	A concurrent negative (air) control group is not necessary according to OECD 436.
3.3	**Administration/ Exposure**	Inhalation (nose-only). Test animals were acclimatised to the test apparatus for approx. 1 hour on 2 days prior to testing. The restraining tubes did not impose undue physical, thermal or immobilization stress on the animals.
3.3.1	Post-exposure period	14 days
3.3.2	Concentrations	*1.24 ± 0.03 and 5.11 ± 0.02 mg* Copper flakes, coated with aliphatic acid /l air was measured at the animal's nose once every hour during exposure.
3.3.3	Particle size	MMAD (mass median aerodynamic diameter) ± GSD (geometric standard deviation) , measured in the exposure chambers: Group 1: 3.477 ± 2.61 µm Group 2: 3.477 ± 2.77 µm These are within the acceptable limits specified by OECD guideline 436.
3.3.4	Type or preparation of particles	Test material was used as supplied. The dust of the test material was generated with a rotating brush dust generator (RBG 1000, PALAS GmbH Partikel und Lasermesstechnik, 76229 Karlsruhe, Germany)
3.3.5	Type of exposure	Nose only
3.3.6	Vehicle	None
3.3.7	Concentration in vehicle	Not applicable
3.3.8	Duration of exposure	4 h
3.3.9	Controls	Not applicable

3.4	**Examinations**	Observations All the rats were observed for clinical signs at least once daily until all symptoms subsided, during and following exposure. Thereafter, all surviving animals were observed each working day during the subsequent 14 day post exposure period. Observations on deaths were made once daily to minimise loss of animals to the study. Cageside observations were also carried out for (but not limited to) indications of respiratory irritation, salivation, convulsions, tremors. diarrhoea, lethargy, sleep and coma. Body weights All the rats were weighed during the acclimatisation period, before the exposure on test day 1 and on test days 2, 4, 8 and 15. Changes in weight were calculated when survival exceeded one day. Terminal studies At the end of the 14 day observation period the animals were weighed and sacrificed and necropsy of all animals was carried out and all pathological changes were recorded. The lung weights of all animals were recorded. Organs of all animals were fixed in 10% buffered formalin (nose, i.e. head without brain, eyes and lower jaw) or 7% buffered formalin (larynx, trachea, lungs).
3.5	**Method of determination of LC_{50}**	Not necessary due to lack of mortality.
3.6	**Further remarks**	Assessment of respiratory tract irritation effects: The assessment of respiratory tract irritation effects were conducted according to the criteria set forth in the OECD proposal document ENV/JM/HCL(2004)9/REV and regulation (EC) 1272/2008, Annex I, Section 3.8.2.2.1: -There are currently no validated animal tests that deal specifically with respiratory tract irritation. However, useful information may be obtained from single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of toxicity (dyspnoea, rhinitis etc.) and histopathology (e.g. hyperaemia, oedema, minimal inflammation, and thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality

There were no mortalities in the Group 1 or Group 2 animals exposed to a 4-hour inhalation exposure to Copper flakes, coated with aliphatic acid

at concentrations 1.24 or 5.11 mg/l.

Clinical signs

All animals showed slight to moderate ataxia, slight to moderate tremor and slight to moderate dyspnoea (reduced frequency of respiration with increased volume) on test day 1 immediately after end of exposure until 3 hours or until test day 4 in all animals, respectively (3 of 3 male and 3 of 3 female animals, each).

On days 2 to 4 after exposure, all animals exposed to 5.11 mg/l concentration revealed reduced motility.

No evidence on respiratory tract irritation was observed in any of the animals.

4.2 Pathology

Gross Pathological Examination

Necropsy was carried out on all animals and all the gross pathological changes were recorded.

Dark or slight grey-stained discoloured lungs were observed in two male animals at the dose level of 1.24 mg/l air and in one male and one female at the dose level of 5.11 mg/l air.

4.3 Other

Body Weight

Significant reductions in body weight increase was exhibited by one of three female animals treated with 1.24 mg/l air and by two of three female animals treated with the higher concentration of 5.11 mg/l air at the end of the study (see below).

Females	**Increase in body weights over test period of 14 days**		
Group 1 (1.24 mg/l)	+2.9%	+7.1%	+10.0%;
Group 2 (5.11 mg/l)	+2.5%	+3.3%	+6.1%

Males	**Increase in body weights over test period of 14 days**		
Group 1 (1.24 mg/l)	+26.7%	+36.4%	+40.4%;
Group 2 (5.11 mg/l)	+22.3%	+24.2%	+36.0%

4.4 LC_{50}

>5.11 mg Copper flakes, coated with aliphatic acid /l air.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and

This study was conducted according to GLP, and to OECD Test

	methods	Guideline 436 (2009). *Two groups, one of 3 female and one of 3 male rats were exposed to a dry aerosol of* Copper flakes, coated with aliphatic acid for 4 hours at gravimetrically determined concentrations of 1.24 ± 0.03 or 5.11 ± 0.02 mg/l by nose-only inhalation. The animals were exposed using a dynamic nose-only exposure chamber and within the chamber, the generated aerosol particulates of the animals had mass median aerodynamic diameters (MMAD) of 3.744 µm and the Geometric Standard Deviations (GSD) of the MMAD were calculated as 2.61 or 2.77 (1.24 or 5.11 mg/l air respectively). All the rats were observed for mortality and clinical signs at least once daily until all symptoms subsided, during and following exposure. Thereafter, all surviving animals were observed each working day during the subsequent 14 day post exposure period.
5.2	**Results and discussion**	*No mortality was observed in rats exposed for 4 hours to 1.24 or 5.11 mg/l air of* Copper flakes, coated with aliphatic acid . The exposure did result in slight to moderate ataxia, slight to moderate tremor and slight to moderate dyspnoea in all animals, as well as reduced motility in all animals 2 to 4 days after exposure. These effects did not exceed moderate levels and are considered to be an overall sign of general toxicity common to dust exposure. No evidence of respiratory tract irritation was observed. One of three females treated with the low concentration (1.24 mg/l) and two of the three females treated at the high concentration (5.11 mg/l) showed a reduction in body weight increase compared to their starting weights by the end of the 14 day observation period. Gross pathological examinations revealed dark or slight grey-stained discoloured lungs in two male animals at the dose level of 1.24 mg/l air and in one male and one female at the dose level of 5.11 mg/l air. *The LC_{50} value for rats following 4 hours inhalation of* Copper flakes, coated with aliphatic acid was determined >5.11 mg/l air.
5.3	**Conclusion**	The acute inhalation LC_{50} (4 h) in male and female rats was found to be >5.11 mg/l. *Therefore,* Copper flakes, coated with aliphatic acid does not meet the criteria for classification for acute inhalation toxicity or for specific target organ toxicity according to the EC Regulation 1272/2008.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 6.1.3 Acute Toxicity

Annex Point IIA6.1 **Acute Inhalation Toxicity in the Rat**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	