Dossier(目次)

Substance: Diantimony trioxide

7.5.1 Repeated dose toxicity: oral
k_Hext_1999

UUID	IUC5-09bc6c36-b2a7-444f-bdef-debaf2d81055
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:31 CEST
Remarks	

Administrative Data

[]

Data waiving

Justification for data waiving

Study result type: experimental result — Study period

Reliability: 1 (reliable without restriction)

Rationale for reliability: Study was generated according to generally valid testing guidelines.

Data source

Reference

Reference type: publication

Author: Hext P.M., Pinot P.J. and Rimmel B.A. — Year: 1999

Title: Subchronic Feeding Study of Antimony Trioxide in Rats

Bibliographic source: J. Appl. Toxicol. 19, 205-209

Testing laboratory: Zeneca Central Toxicology Laboratory — Report no.

Owner company

Company study no. — Report date

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.8.1Toxicity to reproduction; s_hext_1999

Materials and methods

Test type

subchronic

Limit test

no

Test guideline

Qualifier

Guideline: other guideline: no guideline specified, but conducted according to OECD 408.

Deviations

Principles of method if other than guideline

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier: CAS number

Identity: 1309-64-4

Identifier: EC number

Identity: 215-175-0

Identifier: IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: solid
- Analytical purity: 99%
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: 12 male and 12 female Wistar rats of the Alpk:APSD strain
- Housing: in multiple rat racks at four or five per cage initially and then in fours after they had been assigned to experimental groups
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 1 - 2 weeks
- no other details on test animals are stated

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 +- 2°C
- Humidity (%): 55 +- 15%
- Air changes (per hr): at least 15 air changes per hour
- Photoperiod (hrs dark / hrs light): 12 hours per day fluorescent light

Administration / exposure

Route of administration

oral: feed

Vehicle

other: diets containing antimony trioxide

Details on oral exposure

VEHICLE
- Concentration in vehicle: diets contaning 0, 1000, 5000 or 20000 ppm antimony trioxide
- Purity: the homogeneity of the mixture was > 95%.
- no other details on exposure are reported

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The homogeneity of antimony trioxide in the diet was determined by analysis of samples from the low and high dose levels. Samples were taken from
three separate levels in the mixer. Variation between samples was <5%. The chemical stability of antimony trioxide in the diet has been determined at
these dose levels for a period of up to 98 days.

Duration of treatment / exposure

a 90-day duration study

Frequency of treatment

Doses/concentrations

0 ppm

Basis nominal in diet

1000 ppm

Basis nominal in diet

5000 ppm

Basis nominal in diet

20000 ppm

Basis nominal in diet

No. of animals per sex per dose

12 male and 12 female Wistar rats

Control animals

yes

Details on study design

Positive control

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: Cage-side observations were made daily, which included recording changes in clinical condition or behaviour.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: More detailed clinical observations were made each time the body weight was recorded.

BODY WEIGHT: Yes
- Time schedule for examinations: The body weight of each rat was recorded before exposure started and then once a week until termination.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption was recorded continously throughout the study for each cage of rats and calculated as a weekly mean.
- Received dose was calculated from the mean body weight for the inclusion rate of antimony trioxide.

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

HAEMATOLOGY: Yes
- Time schedule for collection of blood: At termination, blood samples were taken and analysed for haematology parameters.
- Anaesthetic used for blood collection: At termination of the study, rats were killed by an overdose of halothane and bled by cardiac puncture.
- Animals fasted: not stated
- Parameters checked: red cell count, haematrocrit, haemoglobin, mean cell volume, total and differential white cell count and platelet count.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: At termination, blood samples were taken and analysed for clinical chemistry parameters.
- Animals fasted: No data
- Parameters checked: urea, glucose, total protein, albumin, cholesterol, triglycerides, total bilirubin, creatinine, sodium, potassium, chloride, calcium and phosphorus. The activities of alkaline phosphatase, alanine aminotransferase, gamma-glutamyl transferase, creatinine kinase and aspartate aminotransferase were also assessed using automated methods.

URINALYSIS: Yes
- Time schedule for collection of urine: Urine samples were collected over a 16 hours period from rats housed individually in metabolism cages during the final week of study.
- Metabolism cages used for collection of urine: Yes
- Animals fasted: not stated
- Parameters checked : Volume, appearance, specific gravity and pH were measured for each urin sample, with semi-quantitative determinations of glucose, ketoses, bilirubin, protein and blood. An aliquote was centrifuged and the sediment was stained and examined.

Sacrifice and pathology

GROSS PATHOLOGY: Yes :
- Complete necropsies were performed on all rats.
- The adrenal glands, brain, kidneys, liver, epididymides and testes were weighed.
- All organs and tissues were examined for macroscopic lesions and fixed in 10% neutral buffered formalin or other appropriate fixative.

HISTOPATHOLOGY: Yes :
- All tissues from the controls and the top dose group were examined under the light microscope, together with any macroscopically abnormal tissue from the intermediate groups.

Other examinations

Statistics

All data were evaluated using analysis of variance and/or covariance for each specific parameter using GLM procedure in SAS.

Any other information on materials and methods incl. tables

Before the start of the study all rats were examined to ensure that they were physically normal and showed normal activity.

The eyes of all rats were examined before the experiment commenced. Those of the top dose level and control animals were examined during the week prior to termination using an indirect ophthalmoscope and a mydriate to dilute the pupil.

Results and discussions

Effect levels

Endpoint

	NOAEL
Effect level	1686 mg/kg bw/day
Sex	male
Basis for effect level / Remarks	
Endpoint	NOAEL
Effect level	1879 mg/kg bw/day
Sex	female
Basis for effect level / Remarks	

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

no effects

Food efficiency

Water consumption and compound intake (if drinking water study)

Ophthalmoscopic examination

no effects

Haematology

yes

Clinical chemistry

yes

Urinalysis

yes

Neurobehaviour

Organ weights

yes

Gross pathology

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

no effects

Details on results

CLINICAL SIGNS AND MORTALITY
- There was no substance-related effect on clinical signs of toxicity.

BODY WEIGHT AND WEIGHT GAIN
- There was no substance-related effect on body weight.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
- There was no substance-related effect on food intake.
- Food consumptions was similar for all groups.

FOOD EFFICIENCY

HAEMATOLOGY
- The red cell count was slightly elevated for males, with a small (not statistically significant) decrease in mean cell volume; other red cell parameters were unaffected.
- Females showed a slight decrease in mean cell volume and a small (not statistically significant) increase in red cell count.
- White cell count and platelet count were unaffected in either sex.

- Three of the twelve (25%) males in the high dose group had slight (n=2) or moderate (n=1) plasma cell infiltration in the cervical lymph node.
- This was not observed in treated females or in any control animal.

CLINICAL CHEMISTRY
- changes in some clinical chemistry parameters :
- Male animals in the high dose group showed a 30% increase in triglycerides (P<0.01) and a 12% decrease in alkaline phosphatase (P<0.05).
- Alkaline phosphatase was also decreased in female animals both at 5000 (24%) and 20000 (37%) ppm (P<0.01) and in a dose-dependent manner.
- Cholesterol and aspartate aminotransferase levels were significantly increased in females in the high dose group by 13 and 52%, respectively.

URINALYSIS
- Analysis of collected urine showed a significant increase in volume and an accompanying decrease in specific gravity for females at the highest
dose of antimony trioxide. Minor intergroup pH differnces in male were not dose related and were considered incidental.

ORGAN WEIGHTS
- A 10% increase in absolute and relative liver weight was observed in female and male animals in the high dose group compared with controls.
- There were no effects on other organ weights at any dose level.

GROSS PATHOLOGY
- There was no gross findings at necropsy considered to be related to feeding antimony trioxide.

HISTOPATHOLOGY: NON-NEOPLASTIC
- no histological changes in the liver were observed to support an adverse effect on liver
- There was a slight increase in cysts in the pituitary of both sexes in the high dose groups. This was not considered to be treatment related.

Remarks on results including tables and figures

All rats survived the dosing period in good condition. The few clinical signs that were recorded can be considered to be more of an adaptive nature than typical for age and strain. None of these were attributed by the authors of the study to the presence of antimony trioxide in the diet.

Table 1: Survival, weight gain and dose received for rats fed diets containing antimony trioxide.

		Mean body weight			
Dose (ppm)	Survival	Initial	Final	Body weight gain (g)	Calculated mean dose (mg/kg/day)
Males					
0	12 / 12	158 ± 16	488 ± 40	330	0
1000	12 / 12	159 ± 18	502 ± 64	343	84
5000	12 / 12	158 ± 19	487 ± 35	329	421
20000	12 / 12	159 ± 21	491 ± 28	332	1686
Females					
0	12 / 12	139 ± 11	269 ± 12	130	0
1000	12 / 12	138 ± 12	266 ± 13	128	97
5000	12 / 12	139 ± 12	267 ±200	128	494
20000	12 / 12	142 ± 15	279 ± 16	137	1879
		Values are means ± SD, based on 12 values; no significant differences at P0.05.			

Table 2: Intergroup comparison of urinary parameters

dose (ppm)	volume (ml)	specific gravity	pH
males			
0	7.4	1.040 ± 0.007	6.58 ± 0.51
1000	8.1	1.037 ± 0.005	6.92 ± 0.29*
5000	7.9	1.040 ± 0.006	6.75 ± 0.45
20000	7.7	1.038 ± 0.006	6.92 ± 0.29*
females			
0	3.8	1.047 ± 0.012	5.92 ± 0.29
1000	4.5	1.044 ± 0.012	6.00 ± 0.00
5000	4.3	1.052 ± 0.019	5.75 ± 0.62
20000	6.8	1.036 ± 0.006**	6.17 ± 0.39
Results are mean ± SD (12 values); *P0.05 and **P0.01 compared to control value.			

Table 3: Selected haematology parameters for rats fed diets containing antimony trioxide

Dose (ppm)	Red cell count ($x10^{-12}$/L)	Haemoglobin (g/dl)	Haematrocrit	mean cell volume (fl)	White cell count ($x10^{-9}$/L)	Platelet count ($x10^{-9}$/L)
males						
0	8.37 ± 0.33	15.2 ± 0.5	0.44 ± 0.02	50.2 ± 0.8	5.75 ± 1.13	801 ± 75
1000	8.78 ± 0.46	15.2 ± 0.6	0.44 ± 0.02	50.1 ± 1.1	6.48 ± 1.64	786 ± 107
5000	8.59 ± 0.43	14.8 ± 0.5	0.43 ± 0.02	49.7 ± 1.1	5.93 ± 1.08	792 ± 66
20000	9.09 ± 0.35*	15.5 ± 0.5	0.45 ± 0.02	49.2 ± 1.1	6.28 ± 1.42	802 ± 77

females						
0	8.13 ± 0.36	15.1 ± 0.7	0.43 ± 0.02	53.3 ± 1.6	4.82 ± 1.17	769 ± 113
1000	8.23 ± 0.33	15.2 ± 0.4	0.43 ± 0.02	52.5 ± 1.2	4.83 ± 1.31	742 ± 81
5000	8.25 ± 0.42	15.2 ± 0.8	0.43 ± 0.03	52.3 ± 1.3	4.59 ± 1.29	755 ± 81
20000	8.30 ± 0.30	15.3 ± 0.5	0.43 ± 0.01	52.2 ± 1.3*	5.17 ± 1.07	774 ± 83
Results are means ± SD (12 values); *P0.05 compared to control value						

Table 4: Selected clinical chemitry (plasma) parameters for rats fed diets containing antimony trioxide

Dose (ppm)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Alkaline phosphatase activity (IU/L)	Alanine aminotransferase activity (IU/L)	Aspartase aminotransferase (IU/L)
males					
0	2.23 ± 0.29	1.18 ± 0.24	202 ± 23	63.3 ± 11.2	91.7 ± 16.3
1000	2.20 ± 0.29	1.34 ± 0.22	220 ± 27	70.9 ± 13.5	96.9 ± 9.0
5000	2.29 ± 0.25	1.27 ± 0.20	197 ± 21	69.1 ± 10.4	99.6 ± 13.4
20000	2.39 ± 0.24	1.53 ± 0.40**	178 ± 42*	73.7 ± 34.8	112.2 ± 34.5
females					
0	1.97 ± 0.32	0.70 ± 0.19	136 ± 40	46.6 ± 8.5	91.5 ± 21.6
1000	2.02 ± 0.28	0.75 ± 0.18	122 ± 22	48.6 ± 11.6	104.8 ± 33.8
5000	2.13 ± 0.27	0.81 ± 0.16	105 ± 17**	51.8 ± 17.7	100.1 ± 28.8
20000	2.22 ± 0.17*	0.76 ± 0.24	87 ± 14**	66.8 ± 56.7	138.8 ± 90.0**
Results are means ± SD (12 values); *P0.05 and **P0.01 compared to control value.					

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Conclusions

Executive summary

Cross-reference to other study

k_Elliot_1998 (cytogenetic)

UUID	IUC5-99385ad7-7ad8-469d-9d51-62b1e9e9aed7
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:45 CEST
Remarks	

Administrative Data

[]

Data waiving

Justification for data waiving

Study result type: experimental result — Study period

Reliability: 1 (reliable without restriction)

Rationale for reliability: Study was generated according to valid testing guideline: OECD guideline 473

Data source

Reference

Reference type	publication
Author	Elliot b.M., Mackay J.M., Clay P. and Ashby J. — Year 1998
Title	An assessment of the genetic toxicology of antimony trioxide.
Bibliographic source	Mutation Research 415: 109-117
Testing laboratory	Central Toxicology Laboratory, Macclesfield, UK — Report no.
Owner company	
Company study no.	— Report date

Data access

data published

Data protection claimed

Cross-reference to same study

see endpoints: 7.6.1. Genetic toxicity in vitro and 7.6.2 Genetic toxicity in vivo
k_Elliot_1998 (bacteria)
k_Elliot_1998 (mammalian cells)
k_Elliot_1998 (DNA repair)
k_Elliot_1998 (bone marrow)

Materials and methods

Type of genotoxicity

other: cytogenetic

Type of study

other: human lymphocytes

Test guideline

Qualifier: according to

Guideline: OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)

Deviations

Principles of method if other than guideline

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier: CAS number

Identity: 1309-64-4

Identifier: EC number

Identity: 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: crystalline powder
- Analytical purity: 99.9%
- no other details on test material stated

Confidential details on test material

Method

Target gene

Species/strain

Species/strain other: human lymphocytes

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation with and without

Metabolic activation system

Test concentrations

10, 50, and 100 µg/ml (final concentrations) was added to duplicate cultures of cells.
The highest dose was limited to the solubility of the test compound.

Vehicle

- Vehicle(s)/solvent(s) used: DMSO
- Justification for choice of solvent/vehicle: no data

Controls

Negative controls

Solvent / vehicle controls

True negative controls

Positive controls yes

Positive control substance

Remarks

Details on test system and conditions

For the cultures containing S9-mix the test substance was removed after 3 hours of treatment and the cells given fresh media. In the absence of S9-mix the test substance were left in the cultures until harvest, except for the cultures taken at 92 hours, which had a medium change after 72 hours.
Colcemid was added to all the cultures 2 hours before harvest for preparation of metaphases on slides, which was performed at 68 and 92 hours after culture initiation. The slides were read blindly and 100 cells per culture were scored for metaphases.

METHOD OF APPLICATION: in medium

OTHER:
- The S9-mix was prepared from male Sprague-Dawley rates dosed daily by oral gavage for 3 days with a combined phenobarbital (80 mg/kg bw) and ß-naphthoflavone (100 mg/kg) corn oil solution. The cofactor was a solution of Na2HPO4, KCl, glucose-6-phosphate, NADP (Na salt) and MGCl2, pH 7.4.

- no other details on test system are reported

Evaluation criteria

Chromosomal aberrations as well as polyploidy and endoreduplication were recorded.

Statistics

The results were evaluated by statistical analysis using Fischer's one-sided exact test.

Any other information on materials and methods incl. tables

Results and discussions

Test results

Species/strain	other: human lymphocytes
Metabolic activation	with and without
Test system	
Genotoxicity	
Cytotoxicity	no
Vehicle controls valid	
Negative controls valid	
Positive controls valid	

Additional information on results

Remarks on results including tables and figures

There was no evidence of cytotoxicity measured by the mean mitotic index for the cultures treated with antimony trioxide compared to control.

No increase in the number of polyploidy and endoreduplicated cells was noted.

In lymphocytes from donor statistically significant dose dependent increase in the percentage aberrant cells (excluding cells with only gap-type aberrations) were seen at the 68 hour sampling time in cultures treated with antimony trioxide in the presence of S9-mix (p 0.05 at 50 µg/ml and p 0.01 at 100 µg/ml). No data from the 92-h sampling time is reported for this donor.

In lymphocytes from donor statistically significant increase in the percentage aberrant cells (excluding cells with only gap-type aberrations) was seen at the 68 hour sampling time in cultures treated with 100 µg antimony trioxide/ml with and without S9-mix (p 0.01). At the 92 hour sampling time, a statistically significant increase was seen at 100 µg/ml of antimony trioxide without S9-mix (p 0.05).

Table 1: Assessment of antimony trioxide in the in vitro cytogenetic assay in lymphocytes

Treatment	- S9-mix					
	68-hours sampling time			92-hours sampling time		
	mean% aberrant cells excluding gaps	aberrations/cell excluding gaps	mean % mitotic index	mean% aberrant cells excluding gaps	aberrations/cell excluding gaps	mean % mitotic index
Donor 1						
DMSO 10µl/ml	0.5	0.005	9.5			
Positive control +	32.0**	0.520	6.5			
Sb_2O_3 100 µg/ml	2.0	0.020	8.3			
Sb_2O_3 50 µg/ml	1.0	0.015	7.4			
Sb_2O_3 10 µg/ml	0.5	0.005	8.8			
Donor 2						
DMSO 10µl/ml	1.5	0.015	9.8	1.0	0.010	9.5
Positive control +	22.0**	0.280	3.5			
Sb_2O_3 100 µg/ml	12.5**	0.170	7.1	4.5*	0.045	7.4
Sb_2O_3 50 µg/ml	4.5	0.060	9.1			
Sb_2O_3 10 µg/ml	2.5	0.030	9.1			

Statistically significant increases in chromosomal damage at * p0.05 or ** p0.01 using Fishers exact test (one-sided); + mitomycin C (0.2 µg/ml) S9; cyclophosphamide (50 µg/ml) + S9.

Table 2: Assessment of antimony trioxide in the in vitro cytogenetic assay in lymphocytes

Treatment	+ S9-mix					
	68-hours sampling time			92-hours sampling time		
	mean% aberrant cells excluding gaps	aberrations/cell excluding gaps	mean % mitotic index	mean% aberrant cells excluding gaps	aberrations/cell excluding gaps	mean % mitotic index
Donor 1						
DMSO 10µl/ml	1.0	0.010	9.6			
Positive control +	34.0**	0.400	6.2			
Sb_2O_3 100 µg/ml	10.5**	0.135	8.8			
Sb_2O_3 50 µg/ml	4.5*	0.050	8.4			
Sb_2O_3 10 µg/ml	1.0	0.010	10.5			
Donor 2						
DMSO 10µl/ml	1.0	0.010	8.2	1.5	0.015	10.6
Positive control +	26.0**	0.260	4.3			
Sb_2O_3 100 µg/ml	9.5**	0.165	6.3	2.0	0.020	8.7

Sb_2O_3 50 µg/ml	1.0	0.010	8.7
Sb_2O_3 10 µg/ml	1.5	0.015	9.3

Statistically significant increases in chromosomal damage at * p0.05 or ** p0.01 using Fishers exact test (one-sided); + mitomycin C (0.2 µg/ml) S9; cyclophosphamide (50 µg/ml) + S9.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

s_Gebel_1997

UUID	IUC5-f693690a-c26a-4c85-8348-0a13faed6ba2
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:44 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Reference does not totally comply with the specific testing guideline: no detailed information about the test material, no positive control.		

Data source

Reference

Reference type	publication		
Author	Gebel T., Christensen S. and Dunkelberg H.	Year	1997
Title	Comparative and Environmental genotoxicity of Antimony and Arsenic		
Bibliographic source	Anticancer Research, 17: 2603-2608		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

Materials and methods

Type of genotoxicity

other: sister chromatid exchange (SCE)

Type of study

sister chromatid exchange assay in mammalian cells

Test guideline

Qualifier	
Guideline	other guideline: no guideline specified
Deviations	
Qualifier	equivalent or similar to
Guideline	OECD Guideline 479 (Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells)
Deviations	yes There are on detailed informations about the test material, and no positive control were used.

Principles of method if other than guideline

The potential of diantimony trioxide to induce sister chromatid exchanges (SCE) in vitro has been evaluated in human lymphocytes.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4
Identifier	

EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): diantimony trioxide
- Physical state: solid
- no other details on test material stated

Confidential details on test material

Method

Target gene

Species/strain

Species/strain lymphocytes: human lymphocytes

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

Cultures were treated with antimony trioxide at final concentrations of 0, 0.1, 0.5, 1, 2 and 5 µM,

corresponding to 0, 0.03, 0.15, 0.29, 0.58 and 1.5 µg/ml.

Vehicle

Controls

Negative controls yes pure DMSO

Solvent / vehicle controls

True negative controls

Positive controls no

Positive control substance

Remarks

Details on test system and conditions

The human lymphocytes were collected from healthy non-smoking donors, 25-35 years of age. The cells were stimulated with PHA and after 24 hours 5-bromo-2-deoxyuridine (BrdU) was added for 24 hours. Thereafter the cultures were treated with antimony trioxide for 24 hours.
In total, lymphocytes were cultured for 72 hours at 37°C. Diantimony trioxide (p.a. grade) was dissolved in distilled water and tested in concentrations up to a cytotoxic response in the culture, determined by the absence of dividing cells.
Colcemid was added 2 hours prior to harvest. Slides were prepared and coded.

Evaluation criteria

A total of 30 metaphases from each culture were scored for SCE. One hundred metaphases per slide were scored to determine cell proliferation.

Statistics

The results were statistically analysed in the two-sided Student's t-test.

Any other information on materials and methods incl. tables

Results and discussions

Test results

Species/strain

Metabolic activation

Test system

Genotoxicity

Cytotoxicity

Vehicle controls valid

Negative controls valid

Positive controls valid

Additional information on results

Diantimony trioxide induced a significant dose-dependent increase in the number of SCEs in lymphocytes in vitro from a minimum dose of 0.5 µM
(0 µM = 8.6 SCE/ metaphase, 0.1 µM = 10.0 SCE/ metaphase, 0.5 µM = 11.5 SCE/ metaphase ($p < 0.05$), 1 µM = 14.7 SCE/ metaphase($p < 0.001$),
2 µM = 25.2 SCE/ metaphase, 5 µM = cytotoxic (SCE/ metaphase)).

Remarks on results including tables and figures

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

s_Kuroda_1991

UUID	IUC5-0b9b98c2-310b-4462-9595-c092a236f85c
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:43 CEST
Remarks	

Administrative Data

[]

Data waiving

Justification for data waiving

Study result type: experimental result — Study period

Reliability: 2 (reliable with restrictions)

Rationale for reliability: Reference does not totally comply with the specific testing guideline. The antimony oxide was not completely dissolved in the vehicle (water), but the concentration of the compound in the fluid was determined.

Data source

Reference

Reference type: publication

Author: Kuroda K., Endo G., Okamoto A., Yoo Y.S. and Horiguchi S. — Year: 1991

Title: Genotoxicity of beryllium, gallium and antimony in short-trem assays

Bibliographic source: Mutation Research, 264:163-170

Testing laboratory — Report no.

Owner company

Company study no. — Report date

Data access

data published

Data protection claimed

Cross-reference to same study

see endpoint 7.6.1. Genetic toxicity in vitro
s_Kuroda_1991 (hamster)

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

Bacillus subtilis recombination assay

Test guideline

Qualifier

Guideline: other guideline: no guideline specified

Deviations

Qualifier: equivalent or similar to

Guideline: OECD Guideline 479 (Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells)

Deviations: yes Antimony trioxide was not completely dissolved.

Principles of method if other than guideline

The genotoxicity of metal salts, easily and slightly soluble in water, was surveyed with the rec Salmonella mutagenicity and SCE assays.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier: CAS number

Identity: 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: solid
- Analytical purity: 99.99%
- solubility in water: 17.1 µg/ml
- no other details on test material

Confidential details on test material

Method

Target gene

Species/strain

Species/strain bacteria, other: Bacillus subtilis M45(rec-) and H17(rec+)

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Species/strain S. typhimurium TA 100

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Species/strain S. typhimurium TA 98

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

50 mg antimony oxide was dissolved in 1 ml of destilled water and the solution was diluted serially 2-fold and used for the assay. The oxide was not completely dissolved . The concentration of the compound in the fluid was determined by ICP (inductively coupled plasma emission spectrometer).

Vehicle

Controls

Negative controls yes Kanamycin

Solvent / vehicle controls

True

negative controls

Positive controls yes Mitomycin C

Positive control substance

Remarks

Details on test system and conditions

The assays were conducted in the presence and absence of rat liver S9 mix (10%). The results were averages of duplicate plates. Survivals after the
preincubation step were counted on glucose minumum medium plates supplemented with histidine and biotin.
SCE (sister chromatid exchange) assays were carried out using V79 Chinese hamster cells obtaining from Flow Laboratories (USA). The cells were
grown in Eagle's MEM supplemented with kanamycin sulfate (100 µg/ml) and 7% fetal calf serum, at 37°C in a 5% CO2 atmosphere.

Cells (5 ml) were grown in a plastic petri dish for 24 hours. Then 100 or 50 µl of various concentrations of the compound in solution was added with BudR (1µg/ml) and incubated for 28 hours in the dark. For the last 2 hours, colcemid was added. The cells were treated with 0.075M KCl for 7 minutes, fixed with ethanol and acetic acid (3:1) and stained by modified FPG methode.

Evaluation criteria

SCE was scored in 20 well-stained metaphases containing 22 chromosomes.

Statistics

The statistical evaluation was done by Student's t-test.

Any other information on materials and methods incl. tables

Table 1: rec assay of the metal compound

metal compound	dose (µg/disk)	killing zone (mm)		M45-H17 (mm)
		M45	H17	
Sb_2O_3	0.3	13.5	11.0	2.5
	0.6	15.0	11.0	4.0
	1.1	15.5	11.0	4.5
Kanamycin	5	20.0	20.0	0
	10	23.0	23.0	0
(negative control)	20	24.5	24.0	0.5
Mitomycin C	0.05	24.0	16.0	8.0
	0.1	28.0	20.0	8.0
(positive control)	0.2	32.0	25.0	7.0
* Maximal dose was 30 µl of the supernatant fluids of the saturated solutions.				

Results and discussions

Test results

Species/strain S. typhimurium, other: TA100 and TA98

Metabolic activation

Test system

Genotoxicity negative

Cytotoxicity

Vehicle controls valid

Negative controls valid

Positive controls valid

Additional information on results

Rec assay:
In the rec assay, a strong positive rec effect (a difference in the diameter of the killing zones in the M45 plate and in the H17 plate larger than 4 mm)
was noted with Sb2O3.

Salmonella mutagenicity assay:
In the Salmonella mutagenicity assays, it is essential that the survivals in the plates are not reduced too much by the test compound. The minimal
inhibitory dose of the compound was determined by counting survivals of TA100 and TA98 in both the presence and absence of S9 mix after
preincubation.
Toxicity of antimony compounds tended to decrease in the presence of S9 mix. The assay was repeated 2 or 3 times. Antimony trioxide is not
mutagenic to Salmonella.

SCE assay:
In the SCE assay the antimony trioxide induced SCEs significantly; more than twice the number of the spontaneous SCEs at the highest

doses.

Remarks on results including tables and figures

Table 2: Minimal inhibitory dose of the metal compound in the Salmonella mutagenicity assay

metal compound	minimal inhibitory dose (µg/plate)[a]			
	TA100		TA98	
	-S9	+S9	-S9	+S9
Sb_2O_3[b]	> 1.71[c]	> 1.71[c]	> 1.71[c]	> 1.71[c]

[a] At these dose, the survivals after the preincubation step was less than 10% of solvent control (water 100µl/plate).

[b] Maximal dose was 100µl of the supernatant fluids of the saturated solutions per plate.

[c] At the maximum dose, the survivals after the preincubation step was greater than 10% of solvent control (water 100µl/plate).

Table 3: Salmonella mutagenicity assay of metal compound

metal compound	dose (µg/plate)	revertants per plate[a]			
		TA100		TA98	
		-S9 mix	+S9 mix	-S9 mix	+S9 mix
Sb_2O_3[b]	0.43	123	162	19	27
	0.86	110	169	26	24
	1.71	128	159	28	31
water	100 µl	129	177	27	25
Furylfuramide	0.01	510			
	0.1		937		
2-Amino-anthracene	1			416	
	0.5				357

[a] The results are average of duplicate plates

[b] Maximal dose was 100µl of the supernatant fluids of the saturated solutions per plate

Table 4: Metal compound positive in the SCE assay

metal compound	concentration (µg/ml)	SCEs/metaphase	
		expt. 1 (mean[a] ± SD)	expt. 2 (mean[a] ± SD)
Sb_2O_3[b]	0.09	6.0 ± 2.3	10.6 ± 3.7**
	0.17	8.2 ± 5.8**	9.0 ± 3.7**
	0.34	12.4 ± 6.6**	14.6 ± 6.3**
water	100µl	4.5 ± 2.2	6.3 ± 2.5
Micromycin C	0.01	46.8 ± 8.6**	56.0 ± 9.3**

[a]Mean of SCEs in 20 metaphases.

[b] Maximal concentration was 20µl of the supernatant fluids of the saturated solutions per ml of culture medium

*/** Significantly different from the value of solvent control (water), p0.05 and p0.1.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

7.6.2 Genetic toxicity in vivo
k_Elliot_1998 (bone marrow)

UUID IUC5-1328b55b-b566-4f81-af10-85e0f4450cea
Dossier UUID 0
Author ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date 2008-06-27 16:58:47 CEST
Remarks

Administrative Data

Data waiving
Justification for data waiving
Study result type experimental result Study period
Reliability 1 (reliable without restriction)
Rationale for reliability Study was generated according to valid testing guideline: OECD guideline 474

Data source

Reference

Reference type publication
Author Elliot b.M., Mackay J.M., Clay P. and Ashby J. Year 1998
Title An assessment of the genetic toxicology of antimony trioxide.
Bibliographic source Mutation Research 415: 109-117
Testing laboratory Centarl Toxicology Laboratory, Macclesfield Report no.
Owner company
Company study no. Report date

Data access

data published

Data protection claimed

Cross-reference to same study

see endpoints: 7.6.1. Genetic toxicity in vitro and 7.6.2 Genetic toxicity in vivo
k_Elliot_1998 (bacteria)
k_Elliot_1998 (mammalian cells)
k_Elliot_1998 (cytogenetic)
k_Elliot_1998 (DNA repair)

Materials and methods

Type of genotoxicity

other: bone marrow micronucleus assay

Type of study

micronucleus assay

Test guideline

Qualifier according to
Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)
Deviations

Principles of method if other than guideline

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number
Identity 1309-64-4
Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: crystalline powder
- Analytical purity: 99.9%
- no other details on test material stated

Confidential details on test material

Test animals

Species

mouse

Strain

CD-1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: male and female CD-1 mice supplied by Charles Rivwer Breeding Laboratories (Margate, UK)
- Age at study initiation: 5-11 weeks
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 19-23°C
- Humidity (%): 40-70%
- Photoperiod (hrs dark / hrs light): 12-hours light/dark cycle

- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Animals were given a single oral gavage dose of test material in 0.5% w/vhydroxypropylmethylcellulose in 0.1% w/v aqueous polysorbate 80.

Details on exposure

Duration of treatment / exposure

oral gavage doses were given for 21 days

Frequency of treatment

oral gavage doses were given daily to 5 males/ dose and time point

Post exposure period

Sampling was performed the day after the respective last dose was given.

Sampling times were 24 and 48 hours post dosing.

Doses / concentrations

400 mg/kg

Basis

667 mg/kg

Basis

1000 mg/kg

Basis

No. of animals per sex per dose

5 male and 5 female

Control animals

Positive control(s)

Cyclophosphamide was used as positive control.

Examinations

Tissues and cell types examined

polychromatic erythrocytes

Details of tissue and slide preparation

TREATMENT AND SAMPLING TIMES (in addition to information in specific fields):
- In the repeated dose study, oral gavage doses of 400, 667 and 1000 mg/kg were given daily to 5 males/ dose and time point for 7, 14 and 21 days. Sampling was performed the day after the respective last dose was given.

DETAILS OF SLIDE PREPARATION:
- Slides were scored blindly.

METHOD OF ANALYSIS:
Two thousand polychromatic erythrocytes were examined for micronuclei per animal.

- no other details of tissue and slide preperation are reported

Evaluation criteria

Statistics

The statistical analysis consisted of a one-sided Student's t-test on transformed data.

Any other information on materials and methods incl. tables

Animals were killed by an overdose of halothane, 24 or 48 hours after dosing. Bone marrow was sampled from femurs using a paintbrush smeared onto a microscope slide and stained with polychrome methylene blue and eosin. Slides were scored blindly. Two thousand polychromatic erythrocytes were examined for micronuclei per animal. One thousand erythrocytes were examined to determine the percentage of polychromatic erythrocytes.

Results and discussions

Test results

Sex male/female

Genotoxicity

Toxicity no effects

Vehicle controls valid

Negative controls valid

Positive controls valid

Additional information on results

A significant decrease in the percent polychromatic erythrocytes was only seen in females at the 24 h sampling time in the single dose study.
No statistically significant increase in the incidence of micronuclei was observed in the single or repeated dose study.

Remarks on results including tables and figures

Table 1: Assessment of antimony trioxide in the mouse bone marrow micronucleus assay: single dose, male

treatment	dose	males			
		mean incidence of MPE/1000 PE ± SD		mean % of polychromatic erythrocytes ± SD	
		24 hours	48 hours	24 hours	48 hours
Vehicle control	10 ml/kg	1.5 ± 0.6	0.2 ± 0.3	42.5 ± 8.9	37.6 ± 11.1
Cyclophosphamide	65 mg/kg	19.2 ± 5.2**		41.3 ± 5.2	
Antimony trioxide	5000 mg/kg	0.8 ± 0.6	0.6 ± 0.7	41.9 ± 5.7	34.6 ± 13.9

PE: polychromatic erythrocytes; MPE: micronucleated polychromatic erythrocytes; SD: standard deviation.

All means of MPE/1000 PE based on ten observations (two counts of 1000 PE per animal).

All means of % PE based on five observations (one count of 1000 erythrocytes per animal).

** statistically significant increase or decrease over controls (p0.01 in Students t-test (one-sided) on transformed data).

Table 2: Assessment of antimony trioxide in the mouse bone marrow micronucleus assay: single dose, female

treatment	dose	females			
		mean incidence of MPE/1000 PE ± SD		mean % of polychromatic erythrocytes ± SD	
		24 hours	48 hours	24 hours	48 hours
Vehicle control	10 ml/kg	0.8 ± 0.5	0.6 ± 1.1	41.4 ± 8.8	44.2 ± 5.1
Cyclophosphamide	65 mg/kg	16.2 ± 2.8**		43.0 ± 6.6	
Antimony trioxide	5000 mg/kg	1.4 ± 0.9	1.2 ± 0.9	26.7 ± 7.2**	39.9 ± 12.8

PE: polychromatic erythrocytes; MPE: micronucleated polychromatic erythrocytes; SD: standard deviation.

All means of MPE/1000 PE based on ten observations (two counts of 1000 PE per animal).

All means of % PE based on five observations (one count of 1000 erythrocytes per animal).

** statistically significant increase or decrease over controls (p0.01 in Students t-test (one-sided) on transformed data):

Table 3: Assessment of antimony trioxide in the mouse bone marrow micronucleus assay: repeat dose

Group	treatment	dose	mean incidence of MPE/1000 PE ± SD			mean % of polychromatic erythrocytes ± SD		
			day 8 sampling	days 15 sampling	days 22 sampling	day 8 sampling	days 15 sampling	days 22 sampling
1	Vehicle control	10 ml/kg/day	0.8 ± 0.7	0.3 ± 0.5	0.3 ± 0.3	30.2 ± 2.0	35.2 ± 9.3	34.8 ± 2.5
2	Antimony trioxide	400 ml/kg /day	0.7 ± 0.5	0.5 ± 0.6	0.4 ±0.4	31.8 ± 5.8	32.8 ± 3.4	31.6 ±7.3
3	Antimony trioxide	667 ml/kg/day	0.4 ± 0.4	0.4 ±0.4	0.2 ± 0.5	31.5 ± 3.1	32.2 ±4.0	32.0 ± 5.8
4	Antimony trioxide	1000 ml/kg/day	0.1 ± 0.2	0.1 ± 0.2	0.8 ± 0.8	34.7 ± 5.5	33.0 ± 2.3	34.0 ± 5.1
5	Cyclophosphamide	65 mg/kg	31.4 ± 3.9	30.1 ± 3.5	28.7 ± 3.2**	12.1 ± 5.6**	11.8 ± 2.2**	12.1 ± 2.8**

PE: polychromatic erythrocytes; MPE: micronucleated polychromatic erythrocytes; SD: standard deviation.

** Statistically significant increase in MPE or reduction in % PE, at p0.01 in the Students t-test (one-sided) on transformed data.

All MPE/1000 PE means based on 2000 PE per animal, all % PE means based on 1000 erythrocytes per animal.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

k_Whitewell (Covance Lab.)_2005/6

UUID	IUC5-6f5156a1-cfa3-49cf-945a-bd740db8f028
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:49 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	begin: 2005-11-04; end: 2006-03-07
Reliability	1 (reliable without restriction)		
Rationale for reliability	The study was generated according to valid testing guidelines: OECD guidelines 474 and 475		

Data source

Reference

Reference type	study report		
Author	Whitewell J.	Year	2006
Title	Evaluation of micronuclei and chromosome aberrations in the bone marrow of Sprague Dawley rats following a 21 day repeated exposure to antimony trioxide		
Bibliographic source			
Testing laboratory	Covance Laboratories Ltd	Report no.	2515/2-D6172
Owner company			
Company study no.		Report date	

Data access

data submitter is data owner

Data protection claimed

yes

Cross-reference to same study

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

Test guideline

Qualifier	according to
Guideline	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)
Deviations	
Qualifier	according to
Guideline	OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test)
Deviations	
Qualifier	
Guideline	other guideline: ICH Tripartite Harmonised Guideline on Genotoxicity: Specific Aspects of Regulatory Tests, 1995
Deviations	

Principles of method if other than guideline

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: white powder
- Analytical purity: 99,93%
- Impurities (identity and concentrations):
Pb: 346ppm
As: 341ppm
Fe: 9ppm
- Lot/batch No.: 29113
- Stability under test conditions: yes
- Storage condition of test material: at room temperature in the dark
- Average particle size: 0.91 µm
-Methode for particle size measurement: not stated
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: rats were obtained from Charles River UK Ltd, Margate, UK
- Age at study initiation: out-bred young adult
- Fasting period before study: Animals were not fasted prior to dosing.
- Housing: They were housed in groups of the same sex. Aspen wood chips were be used for bedding. Additionally, in order to enrich the environment and enhance the welfare of the animals, they were provided with wooden Aspen chew blocks.
- Diet (e.g. ad libitum): Diet (Special Diets Services Ltd, RM1.(E).SQC.) were provided ad libitum
- Water (e.g. ad libitum): Bottled water (public supply) were provided ad libitum.
- Acclimation period: Animals were acclimatised for at least 5 days.

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 19-25°C
- Humidity (%): 40-70%.
- Air changes (per hr): at least 15 fresh air changes per hour
- Photoperiod (hrs dark / hrs light): Holding rooms were illuminated continuously by fluorescent light for 12 hours out of each 24 hour cycle.

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Dosing preparations were made by suspending antimony trioxide in 0.5% (w/v) Hydroxypropylmethylcellulose + 0.1% (w/v) aqueous polysorbate
(0.5% HPMC + 0.1% polysorbate).

Details on exposure

Animals were dosed with the vehicle or test article for 21 consecutive days (approximately 24 hours apart). The positive control was given as a single
administration at 20 mg/kg, on the last day of dosing.

Duration of treatment / exposure

Animals were dosed with vehicle or test article for twenty one consecutive days.

Frequency of treatment

Animals were dosed with vehicle or test article once daily.

Post exposure period

Doses / concentrations

250 mg/kg
Basis

500 mg/kg
Basis

1000 mg/kg
Basis

No. of animals per sex per dose

6 male and 6 female per dose per day
Control animals

Positive control(s)

The negative (vehicle) control was 0.5% HPMC + 0.1% polysorbate.
Cyclophosphamide (CPA, Sigma Chemical Co, Poole, UK) was freshly dissolved in physiological saline at 2 mg/mL to serve as the positive control at a
final dose of 20 mg/kg.

Examinations

Tissues and cell types examined

Details of tissue and slide preparation

TREATMENT AND SAMPLING TIMES (in addition to information in specific fields):
Approximately two hours prior to the scheduled sample time, animals were injected intraperitoneally with colchicine (dose volume 10 mL/kg) to give a final concentration of 2 mg/kg, in order to arrest dividing cells in metaphase for the chromosome aberration endpoint. Two hours prior to harvest is considered sufficient time to achieve this without affecting background micronucleus frequencies due to spindle affects.

Test article and vehicle treated rats were killed 24 hours after the final administration. CPA-treated rats were killed 24 hours after the single dose. Rats were killed by asphyxiation with carbon dioxide (subsequently ensured by cervical dislocation) in the same order as they were dosed.

Both femurs from each animal were exposed, removed, cleaned of adherent tissue and the ends removed from the shank.
One bone was used for metaphase processing, the other for micronucleus preparations.

DETAILS OF SLIDE PREPARATION:
Mitotic index analysis:
Slides from animals treated with vehicle or test article were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis, based on 1000 cells scored per animal.

METHOD OF ANALYSIS:
Mitotic index analysis:
Slide analysis was performed by competent analysts trained in the applicable Covance Laboratories Harrogate (CLEH) standard operating procedures.

Analysis of results - Micronucleus:
Treatment of data
After completion of microscopic analysis and decoding of the data, the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes
(NCE) (expressed as %PCE) for each animal and the mean for each group was calculated. The individual and group mean frequency of micronucleated PCE ± standard deviation (%MNPCE) were also determined.
%PCE values were examined to see if there was any decrease in groups of treated animals that could be taken as evidence of bone marrow toxicity.
The frequencies of micronucleated PCE in vehicle control animals were compared with the historical negative control data to determine whether or not the assay was acceptable. For each group, inter-individual variation in the numbers of micronucleated PCE was estimated by means of a heterogeneity χ^2 test.
The numbers of micronucleated PCE in each treated group were then compared with the numbers in vehicle control groups by using a 2 x 2
contingency table to determine χ^2. Probability values of $P \leq 0.05$ were to be accepted as significant. A further statistical test (for linear trend) was used to evaluate possible dose-response relationships.

Evaluation criteria

Micronucleus:
The data were evaluated as to whether exposure to the test article was associated with:
1. a statistically significant increase in the frequency of micronucleated PCE occurring at one or more dose levels.
2. an incidence and distribution of micronucleated PCE at such a point that exceeded the laboratory's historical vehicle control data.
3. a dose-response trend in the proportion of micronucleated PCE (where more than two dose levels were analysed).

Metaphase analysis:
A test article was considered as positive in this assay if:
1. a statistically significant increase in the proportion of cells with structural aberrations occurred at one or more concentration and/or sample time, and
2. the proportion of cells with structural aberrations at such data points exceeded the normal range.

Statistics

Any other information on materials and methods incl. tables

Animals were treated in the main study as follows:

Treatment group	Dose administered (mg/kg/day)[a]	Dose volume (ml/kg)	Number of animals treated[b]
Vehicle	0	10	6M & 6F
Antimony trioxide	250	10	6M & 6F
Antimony trioxide	500	10	6M & 6F
Antimony trioxide	1000	10	6M & 6F
Positive control, CPA[c]	20	10	6M & 6F

[a] doses administered daily for 21 consecutive days, approximately 24 hours apart (except positive control)

[b] animals sampled 24 hours after final dose administration

[c] Cyclophosphamide; administered once only

Animals were observed daily for signs of ill health or overt toxicity. An individual record was maintained of the clinical condition of each animal.

Results and discussions

Test results

Sex male/female

Genotoxicity negative

Toxicity

Vehicle controls valid

Negative controls valid yes

Positive controls valid yes

Additional information on results

No clinical signs were observed in any control or test article treated groups.
Group mean body weight gains were reduced for test article treated animals as compared to concurrent vehicle controls (males and females) over the dosing period of the assay.

Mitotic index data did not indicate any test article related toxicity to the bone marrow.

Remarks on results including tables and figures

Overall remarks, attachments

Overall remarks

The data reported in this study were also published as follows: Kirkland D, Whitwell J, Deyo J and Serex T. Failure of antimony trioxide to induce micronuclei or chromosomal aberrations in rat bone-marrow after sub-chronic oral dosing. Mutation Research 2007; 627: 119-128.

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

It is concluded that antimony trioxide did not induce chromosome aberrations or micronuclei in the bone marrow cells of male and female rats when
tested at doses of 250, 500 and 1000 mg/kg/day over a continuous 21-day dosing regime.

Executive summary

Cross-reference to other study

k_Elliot_1998 (DNA repair)

UUID	IUC5-27b4e8d3-9a84-452b-98ab-a1ebf01fac9b
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:49 CEST
Remarks	

Administrative Data

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	1 (reliable without restriction)		
Rationale for reliability	Study was generated according to valid testing guideline: OECD guideline 486		

Data source

Reference

Reference type	publication		
Author	Elliot b.M., Mackay J.M., Clay P. and Ashby J.	Year	1998
Title	An assessment of the genetic toxicology of antimony trioxide.		
Bibliographic source	Mutation Research 415: 109-117		
Testing laboratory	Centarl Toxicology Laboratory, Macclesfield	Report no.	
Owner company			
Company study no.		Report date	

Data access

Data protection claimed

Cross-reference to same study

see endpoints: 7.6.1. Genetic toxicity in vitro and 7.6.2 Genetic toxicity in vivo
k_Elliot_1998 (bacteria)
k_Elliot_1998 (mammalian cells)
k_Elliot_1998 (cytogenetic)
k_Elliot_1998 (bone marrow)

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

unscheduled DNA synthesis

Test guideline

Qualifier	according to
Guideline	OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo)
Deviations	

Principles of method if other than guideline

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4
Identifier	EC number
Identity	215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: crystalline powder
- Analytical purity: 99.9%
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

other: Alderley Park AlPk:ApfSD

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Source: male and female CD-1 mice supplied by Charles Rivwer Breeding Laboratories (Margate, UK)
- Age at study initiation: 5-11 weeks
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 19-23°C
- Humidity (%): 40-70%
- Photoperiod (hrs dark / hrs light): 12-hours light/dark cycle

- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Dose of test material were given in 0.5% w/v hydroxypropylmethylcellulose in 0.1% w/v aqueous polysorbate 80.

Details on exposure

Duration of treatment / exposure

Frequency of treatment

single dose

Post exposure period

At 2 or 16 hours after administration hepatocytes were isolated.

Doses / concentrations

3200 mg/kg

Basis

5000 mg/kg

Basis

No. of animals per sex per dose

five male rats per dose

Control animals

Positive control(s)

1,2-dimethylhydrazine (DMH) served as positive control.

Examinations

Tissues and cell types examined

hepatocytes

Details of tissue and slide preparation

At 2 or 16 hours after administration hepatocytes were isolated following collagenase perfusion and incubated with 3H-thymindine for 4 hours followed by a cold chase overnight. The slides were coated in Ilford K2 emulsion and left for 14 days at 4°C before developing.

Slides were coded and scored blind. An image analysis system was used to score the nuclear and cytoplasmatic grain, assessing 60 cells per animal.

Evaluation criteria

Statistics

Any other information on materials and methods incl. tables

Results and discussions

Test results

Sex	male
Genotoxicity	
Toxicity	no effects
Vehicle controls valid	
Negative controls valid	
Positive controls valid	

Additional information on results

There was no increase in net nuclear grains or percentage of cells in repair at either sampling time.

Remarks on results including tables and figures

Table 1: Assessment of antimony trioxide in the rat liver DNA repair (UDS) assay

Treatment	No. of animals	Mean N ± SD	Mean C ± SD	Mean (N-C) ± SD	Mean % cells in repair
2 hours					
HPMC (10 ml/kg)	2	3.2 ± 2.0	5.4 ± 3.3	-2.1 ± 1.3	0
Antimony trioxide (3200 mg/kg)	5	2.8 ± 0.8	5.3 ± 1.8	-2.5 ± 1.0	0
Antimony trioxide (5000 mg/kg)	5	3.1 ± 0.8	6.2 ± 2.3	-3.1 ± 1.5	0
DMH * 2HCl (30 mg/kg)	2	20.6 ± 7.2	4.1 ± 2.1	16.5 ± 9.2	93
16 hours					
HPMC (10 ml/kg)	2	3.3 ± 2.3	5.3 ± 3.7	-2.0 ± 1.4	0
Antimony trioxide (3200 mg/kg)	5	2.4 ± 1.2	4.6 ± 2.9	-2.2 ± 1.7	0
Antimony trioxide (5000 mg/kg)	5	3.6 ± 1.7	6.6 ± 3.2	-3.0 ± 1.5	0
DMH * 2HCl (30 mg/kg)	2	22.3 ± 3.9	5.9 ± 5.3	16.4 ± 1.4	93

N ± SD: mean nuclear grain count ± standard deviation; C ± SD: mean cytoplasmic grain count ± standard deviation; (N-C) ± SD: mean net nuclear grain count ± standard deviation.

All cell in repair has an N-C value of e 5.

DMH: 1,2-dimethylhydrazine

Overall remarks, attachments

Overall remarks

Attached background material

Attached document	
Remarks	

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

s_Gurnani_1992

UUID	IUC5-4f0c9428-5e8c-424a-a9a9-ff823a186788
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:47 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	3 (not reliable)		
Rationale for reliability	Documentation is insufficient for assessment: no positive control was used, it is not stated as to whether the slides were coded and scored blindly, detailed information on test material are missing.		

Data source

Reference

Reference type	publication		
Author	Gurnani N., Sharma A. and Talukder G.	Year	1992
Title	Comparison of the clastogenic effects of antimony trioxide on mice in vivo following acute and chronic exposure.		
Bibliographic source	BioMetals, 5: 47-50		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

other: effects on mice in vivo in bone marrow and germ cells

Test guideline

Qualifier	
Guideline	other guideline: no guideline specified
Deviations	

Qualifier	equivalent or similar to
Guideline	OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test)
Deviations	yes There were no positve or negative controls used. Additional detailed information on test material are missing.

Principles of method if other than guideline

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: solid
- no other details on test material stated

Confidential details on test material

Test animals

Species

mouse

Strain

Swiss

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Swiss albino mice were raised at the Departmental Animal house.
- Age at study initiation: 8 weeks
- Weight at study initiation: average mass of 25-30 g
- Housing: They were housed in polycarbonate cages.
- Diet (e.g. ad libitum): standard pellet diet (Gold Mohur feed manufactured by Lipton India Limited)
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 +/- 3°C
- Humidity (%): 50 +/- 15%
- Photoperiod (hrs dark / hrs light): 12 hours day period

- no other details on test animals and environmental conditions are reported

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
Aqueous suspensions of Sb_2O_3 were administered orally.
Concurrent controls recieved only distilled water.

- no other details on exposure stated

Duration of treatment / exposure

For chronic exposure the doses were administered daily for 21 days.

Frequency of treatment

For acute exposure, the observations were made after 6, 12, 18 and 24 hours.
For chronic exposure, the doses were administered daily and observations were made on days 7, 14 and 21.

Post exposure period

In all cases, 1.5 hours before sacrifice, the animal was injected intraperitoneally with 4 mg/kg colchicine and killed by cervical dislocation.

Doses / concentrations

400 mg/kg body mass

Basis

666.67 mg/kg body mass

Basis

1000 mg/kg body mass

Basis

No. of animals per sex per dose

five animals per dose

Control animals

yes, concurrent no treatment
Positive control(s)

Examinations

Tissues and cell types examined

Details of tissue and slide preparation

CRITERIA FOR DOSE SELECTION:
The doses used were calculated as a proportion of the oral LD50 (> 20000 mg/kg body mass, according to Merck Index).

TREATMENT AND SAMPLING TIMES (in addition to information in specific fields):
Bone marrow of femurs was removed by flushing with 1% sodium citrate solution, incubated at 37°C for 20 minutes centrifuged and fixed in cold
ethanol/glacial acetic acid (3:1).

DETAILS OF SLIDE PREPARATION:
Bone marrow samples were washed twice in fixative and slides were prepared by flame drying, coded and stained in diluted Giemsa.

METHOD OF ANALYSIS:
A total of 100 metaphase plates from each animal were scored, making 500 cells for each experimental set. Different types of chromosomal
aberration, chromatid gaps, chromatid breaks, centric fusions and polyploidy were recorded seperately.

OTHER:
Evaluation criteria

Statistics

Data from short-term acute exposure were analyzed by the t-test to find out the differences in frequencies of chromosomal aberrations between the sexes and between the doses used.
In order to compare the effects of the duration after exposure and sex, if any, on the action of Sb2O3, a two-way analysis of variance test was used.
The results of the chronic exposure were analyzed by one-tailed trend test.
Two-way ANOVA, followed by Ducan's multiple range test was used to analyze any significant differences between the different doses and sampling
times on the effect of the compound.

Any other information on materials and methods incl. tables

Results and discussions

Test results

Sex

Genotoxicity

Toxicity

Vehicle controls valid

Negative controls valid

Positive controls valid

Additional information on results

Antimony trioxide did not induce chromosomal aberrations following single acute exposure. No statistically significant difference could be recorded between the treated and the normal control mice, of either sex, with respect to the frequency of chromosomal aberrations or mitotic index at 6, 12,
18 and 24 hours after exposure.

At the chronic exposure, the highest dose (100 mg/kg body mass) was lethal on day 20 of treatment. Frequency of aberrations (without gaps)
increased proportionately with dose administered to a highly significant level ($p<0.001$) for the first 14 days. Longer exposure (21 days) was lethal.
A similar value was seen for the frequency of breaks induced.

Remarks on results including tables and figures

Table 1: Data on bone marrow chromosomal aberrations in male mice

duration (days)	dose (mg/kg bw)	log dose	No. of animals taken	total chromosomal aberrations						frequency of aberrations (%)		break/cell
				G	G	B	B	RR	Polyploids	including gap	without gap	

7	control	2.40	5	6	0	5	0	0	2	2.6 ± 0.894	1.4 ± 1.140	0.01
	400	2.60	5	8	1	9	0	0	2	4.2 ± 1.095	2.2 ± 0.447	0.018
	666.67	2.82	5	11	0	11	0	0	6	5.6 ± 0.547	3.4 ± 0.547	0.022
	1000	3	5	21	0	19	0	9	11	13.8 ± 0.447	9.6 ± 1.140	0.074
trend test P value										*** 6.715	*** 4.88	***5.45
14	control	2.40	5	6	0	5	0	0	3	2.8 ± 0.447	1.6 ± 0.547	0.01
	400	2.60	5	9	0	11	0	0	5	5 ± 0.707	3.2 ± 0.447	0.022
	666.67	2.82	5	17	0	13	0	0	7	7.4 ± 0.547	4 ± 0	0.026
	1000	3	5	26	2	23	0	10	8	16.2 ± 0.447	10.2 ± 0.836	0.086
trend test P value										*** 5.50	*** 4.72	*** 7.5
21	control	2.40	5	6	0	5	0	0	3	2.8 ± 0.447	1.6 ± 0.547	0.01
	400	2.60	5	8	0	13	0	0	10	6.2 ± 0.836	4.6 ± 0.547	0.026
	666.67	2.82	5	19	0	16	0	2	4	8.6 ± 0.894	4.8 ± 0.836	0.04
	1000	3	5	-	-	-	-	-	-	-	-	-
trend test P value										-1.18	-2.82	-0.39

Abberations:

G and G: chromatid and isochromatid gap

B and B: chromatid and isochromatid break

The frequency of aberrations was calculated as the percentage of total chromosomal aberration ± SD of the mean among five animals per set.

The trend test P values were determined by a one-tailed trend test.

*** indicates significantly different at p0.001.

Table 1 shows the relationship between the chromosomal aberrations induced by different doses following chronic exposure for long periods. Table 2 gives the analysis of variance (ANOVA) to compare the relative frequency of chromosomal aberrations induced by two doses (lower and middle) and three durations of exposure to the chemical.

Table 2: Two-way ANOVA for chromosomal aberrations in male mice

dose (mg/kg bw) [factor B]	frequency of aberrations (%) after exposure duration [factor A] of			
	7 days	14 days	21 days	Ł
0	2.6	2.8	2.8	8.2
400	4.2	5	6.2	15.4
666.67	5.6	7.4	8.6	21.6
Ł	12.4	15.2	17.6	45.2

source of variation	degrees of freedom	sum of squares	mean sum of squares	F_s
factor A: duration (column)	2	4.52	2.26	4.33
factor B dose (row)	2	29.99	14.99	28.72*
error	4	2.09	0.52	

The test was only done for the lowest and middle doses as the highest dose was lethal.

* indicates significant, level of significance P=0.05

Overall remarks, attachments

Overall remarks

The purity of the antimony trioxide was not stated in the publication. However, according to personal communication with one of the authors the antimony trioxide was of analytical grade and purchased from Merck . It has been verified by Merck that the only antimony trioxide that has been sold by Merck in is of a minimum of 99% purity. Therefore it could not be verified, but anticipated that the antimony trioxide used in this study was of acceptable purity.

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Interpretation of results

Conclusions

Executive summary

Cross-reference to other study

s_Watt_1983_rats

UUID	IUC5-7d437508-9dfe-43e3-a00c-9c8d94955d5c
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:51 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	3 (not reliable)		
Rationale for reliability	This study does not meet important criteria of current standard methods: the main points of criticism are that the exposure duration is 12 months thus deviating from the OECD guideline on chronic toxicity/carcinogenicity (which suggests an exposure period of 24 months in rats), and the analytical verification of exposure concentrations and particle sizes of the test aerosols are inadequate, and therefore do not allow the derivation of correct NOAELS/LOAELS. However, the significant increase in incidence of scirrhous carcinomas in the lungs of animals exposed to diantiony trioxide by inhalation may be considered a reliable finding.		

Data source

Reference

Reference type	other: dissertation		
Author	Watt WD	Year	1983
Title	Chronic inhalation toxicity of antimony trioxide: Validation of the threshold limit value		
Bibliographic source	1983; 1, pp 1-133. Wayne State University, Detroit, Michigan		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.7 Carcinogenicity, S_Watt_ 1983_swine

Materials and methods

Limit test

no

Test guideline

Qualifier	
Guideline	other guideline: no guideline specified
Deviations	

Principles of method if other than guideline

The study was an attempt to evaluate the inhalation toxicty of antimony trioxide dust by exposing female rats and miniature swine to concentrations of antimony trioxide at levels relatively close to the threshold limit value.
In a chronic inhalation toxicity study, the carcinogenicity of antimony trioxide was investigated in female CDF Fisher rats and parallel in swine (see Chapter 7.7 S_Watt_1983_swine). 148 female rats from the Charles River Laboratories , 14 weeks of age were exposed to 0, 1.9 and 5.0 mg antimony trioxide /m3 for 6 h/day, 5 days/week for one year in whole body exposure chambers. Surviving animals were kept up to 15 months post-exposure for observation. Prior to and after approximately 3, 6, 9 and 12 months of exposure and 12 to 15 months post-exposure animals were sacrificed and evaluated for evidence of toxicity.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: solid, dust
- Analytical purity: 99.4
- Impurities (identity and concentrations): 0.02 % arsenic, 0.20 % lead
- particle size: averaged 0.40 µ with geometric deviation of 2.13 (high concentration)
0.44 µ with geometric deviation of 2.23 (low concentration)
-Method for particle size measurement: Atomic Absorption Spectrometry; dust sized by scanning electron photomicrograph and measureing Ferretsdiameter
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Fischer 344

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories
- Age at study initiation: 14 weeks
- Housing: in pairs in holding cages (in a room adjacent to the exposure room)
- Diet: Purina Rat Chow (Ralston Purins Co.)
- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

inhalation: dust

Type of inhalation exposure (if applicable)

whole body

Vehicle

unchanged (no vehicle)

Details on exposure

- dust dissemination: by the use of a modified hammer mill; paticles seperated and agitated by the whirling blades were lifted in an air stream
- air changes in exposure room: 7.7 changes /hr (high dose)
25.1 changes /hr (low dose)
16.9 changes /hr (control)
- air sample taken within the exposure chambers at the same level as the suspended rat cages - not taken in the cages

TEST MATERIAL
- aerodynamic particle size: 15 µ diameter or smaller

- no other details on exposure are reported

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Air samples were taken within the exposure chambers at the same level as the suspended rat cagesduring exposure period
Only particles with mean aerodynamic diameter of 15 µm or less would pass into the chamber.

Duration of treatment / exposure

approx. 1 year exposure period

Frequency of treatment

6 hours per day, 5 days per week

Post exposure period

up to 15 months

Doses / concentrations

4.2 ± 3.2 mg Sb/m3 (high dose chamber)

Basis analytical conc. averaged dose; corresponding to 5.0 ± 3.8 mg Sb2O3/m3

1.6 ± 1.5 mg Sb/m3 (low dose chamber)

Basis analytical conc. averaged dose, corresponding to 1.9 ± 1.8 mg Sb2O3/m3

No. of animals per sex per dose

approx. 49 rats per sex per dose (148 rats divided in 3 groups)

Control animals

yes, sham-exposed

Details on study design

Positive control

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: prior to exposure, after 3, 6 and 9 month of exposure, and at the end of exposure

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule:

BODY WEIGHT: Yes
- Time schedule for examinations:periodically before, throughout and after exposure

ORGAN WEIGHT: Yes
- heart, lung, liver, spleen and kidney

HAEMATOLOGY: Yes , blood taken from orbital sinus
- Anaesthetic used for blood collection: Yes (identity): with Surital or V-Pento
- Parameters examined: differential count, red blood cell count, white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin volume

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood:
- Parameters examined: alkaline phosphatase, glutamate oxalacetate transaminase, glutamate pyrovate transaminase, lactic dehydrogenase,
hydroxybutyrate dehydrogenase, creatine phosphokinase, total protein, albumin, globulin, albumin-globulin ratio, blood urea nitrogen, creatine, bilirubin, sodium, potassium, glucose, cholesterol levels

- no other observations are reported

Sacrifice and pathology

- sacrifice of randomly chosen rats (exsanguinated) prior to and after 3,6,9 and 12 months of exposure and 12 and 15 months post-exposure
GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes

Other examinations

Statistics

Any other information on materials and methods incl. tables

- 3 dose groups: low dose, high dose and control

Results and discussions

Effect levels

Endpoint	LOAEC
Effect type	carcinogenicity
Effect level	5 mg/m³ air
Sex	female
Basis for effect level / Remarks	A LOAEC of 5.0 mg/m3 is suggested based on the development of scirrhous carcinomas and the NOAEC is set to 1.9 mg/m3. However, this level is considered unreliable because of inadequate concentration verification.
Endpoint	NOAEC
Effect type	carcinogenicity
Effect level	1.9 mg/m³ air

Sex female

Basis for effect level / Remarks

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption and compound intake (if feeding study)

Food efficiency

Water consumption and compound intake (if drinking water study)

Ophthalmoscopic examination

Haematology

yes

Clinical chemistry

Urinalysis

Neurobehaviour

Organ weights

yes

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

yes

Details on results

CLINICAL SIGNS AND MORTALITY
- all survived

BODY WEIGHT AND WEIGHT GAIN
- rats from high and low dose group show greater weight gains than the control group
- since some of the weight differences occur before the start of exposure, the extent of contribution of antimony can not be determined

HAEMATOLOGY
- slight increasein eosinophils at 6 month exposure at high dose rats (in contrast to control group)

CLINICAL CHEMISTRY
- increase of blood urea nitrogen values in high dose group in contrast to low dose group and in low dose group in contrast to control group

(values not statistically significant, but th strong pattern suggest an exposure relationship)

ORGAN WEIGHTS
- no differences between the weights of major organs except the lungs:
- combined heart-lung weight showed the pattern of high dose group being heavier than the low dose group and the low dose group being heavier
than the control group at 3 and 6 months exposure (pattern remains consistent troughout the exposure period for lung weight alone)
(stastistical significance: high dose : control group at 9 month and 12 month of exposure; low dose : control group at 12 months)
- at one year post-exposure all groups are equal in lung weights

GROSS PATHOLOGY:
- lungs of exposed animals were mottled; the mottling increased with dose level and lenght of exposure; the mottling is a manifestation of foci of
fibrosis

HISTOPATHOLOGY: NON-NEOPLASTIC
- responses to antimony exposure observed in the lungs:
- in most cases the incidence and/or severity of the response increased with exposure time and level
- in high and low dose rats
- consists of focal fibrosis (most important response), adenomateous hyperplasia,multinucleated giant cells, cholesterol clefts, pneumonocyte hyperplasia, pigmented
macrophages (statistically significant for high and low dose groups in most cases from the end of exposure period to the end of post-exposure)

HISTOPATHOLOGY: NEOPLASTIC (if applicable)
- responses to antimony exposure observed in the lungs
- in most cases the incidence and/or severity of the response increased with exposure time and level
- majority of lung neoplasms are in the high dose rats at 2 month and one year post-exposure (neoplasms are either scirrhous carcinomas,

squamous cell carcinomas or bronchioalveolar adenomas)
- no evidence of metastasis

- no other findings are reported

Remarks on results including tables and figures

- the results show evidence of antimony trioxide related toxicity
- in addition to the lung parameters only blood urea nitrogen and body weights show exposure related alteration
- the lung is the main target organ of antimony trioxide inhalation toxicity: focal fibrosis indicates a substantial toxic effect and significant development of primary lung neoplasms occured
- this study shows that exposure to antimony trioxide significantly increase the incidence of pulmonary scirrhous carcinomas in female rats, 2 to 15 months after 1 year inhalation of 5.0 ± 3.8 mg antimony trioxide/m3. The incidence in the group sacrified after 12 month was 44 % (15/34).
- a LOAEC of 5.0 mg antimony trioxide/m3 is suggested based on scirrhous carcinoma.
- the NOAEC is set to 1.9 mg antimony trioxide/m3
Exposure to Sb2O3 at 4.2 mg/m3 causes development of primary lung neoplasms. Focal fibrosis develops in rats from exposure to either 4.2 mg/m3 (as Sb) or 1.6 mg/ m3 (as Sb) of antimony trioxide.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Relevance of carcinogenic effects / potential

Conclusions

Executive summary

Cross-reference to other study

s_Groth_1986a

UUID	IUC5-5960d26e-fac3-4260-b67e-d7ef7f6014ef
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:52 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Method comparable to current standards and detailed documentation, but major deficiencies: only one dose per compound was investigated, confounding exposure to arsenic, very unsteady exposure levels during the study. The exposure duration is 12 months and thus deviates from the OECD guideline on chronic toxicity/carcinogenicity, which suggests an exposure period of 24 months in rats.		

Data source

Reference

Reference type	publication		
Author	Groth DH, Stettler LE, Burg JR, Busey WM, Grant GC and Wong L	Year	1986
Title	Carcinogenic effects of antimony trioxide and antimony ore concentrate in rats		
Bibliographic source	J Toxicol Environ Health 1986a; 18: 607-626		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

this study is also described in chapter 7.5.3 repeated dose toxicity

Materials and methods

Limit test

no

Test guideline

Qualifier	
Guideline	other guideline: no guidline specified
Deviations	

Principles of method if other than guideline

In the study, the carcinogenic effects of antimony trioxide and antimony ore (Sb_2S_3) were evaluated in Wistar rats, 90 males and 90 females per group
The animals, 8 months of age, were exposed by inhalation to antimony trioxide 45 mg/m3, antimony ore 36-40 mg/m3 or filtered air (controls) in exposure chambers, 7 h/day, 5 days/week for up to 52 weeks. At 6, 9, and 12 months after initiating exposures 5 animals/sex/group were sacrificed and autopsied, the remainder of the animals were sacrificed 18-20 weeks post-exposure.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4
Identifier	EC number
Identity	

215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): 1. Antimony trioxide (Sb2O3)
2.Antimony ore concentrate (Sb ore, principally Sb2S3)
- Substance type: pure active substrate (antimony trioxide)
- Physical state: solid
- Analytical purity: SB2O3: 80 % Sb, Sb ore conc.: 46 %
- Impurities (identity and concentrations >0.01%): 1. in Sb2O3: 0.23 % Pb, 0.21 % Sn, 0.014% Ce, 0.004% As, 0.0066% Al, 0.0044% Cd
2. in Sb ore conc.: 0.25% Pb, 0.16% Sn, 0.003% Au, 0.0792% As, 0.053% Zn, 0.06% Cu, 0.334% Fe,
0.0359% Mg, 0.483% Al
- particle size: Mass median diameter = 1.23 (Sb2O3)
2.22 (Sb ore)
-Method for particle size measurement: with scanning electron microscope equipped with an automated image analyzer
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Breeding Labs, Wilmington, Mass.
- Age at study initiation: 8 - 10 weeks
- Age at start exposure: approx. 8 months
- Housing: 6 Rocjester-type, 3.45 m3, stainless steel exposure chambers (2 males/cage and 3 females/cage)
- Diet : pelleted commercial rat diet ad libitum, except during exposure
- Water: tap water ad libitum
- Acclimation period: approx. 6 months
- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

inhalation: dust

Type of inhalation exposure (if applicable)

whole body

Vehicle

unchanged (no vehicle)

Details on exposure

EXPOSURE DESCRIPTION:
· Exposure apparatus: Wright dust feeder
· air supply passed through an absolute filter (99.97% - 99.99% retention of particles > 3µm in diameter)
· mass median aerodynamic diameter = 2.80 µm (Sb2O3) and = 4.78 µm (Sb ore)
· air temperature maintined at 75 °C F
· humidity was monitored
· airflow: 12 -15 air changes / hour
· chambers operated under slight negative pressure
· concentration of dust measured 3x /day during exposure period

- no other details on exposure stated

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Duration of treatment / exposure

up to 52 weeks / 12 months

Frequency of treatment

7 h/d and 5 d/wk

Post exposure period

5 months

Doses / concentrations

45.0 mg/m3 mean daily TWA Sb2O3

Basis analytical conc. in chamber 3E; Range of TWAs: 0-118.5 mg/m3

46.0 mg/m3 mean daily TWA Sb2O3

Basis analytical conc. in chamber 3W; Range of TWAs: 0-191.1 mg/m3

36.0 mg/m3 mean daily TWA Sb ore

Basis analytical conc. in chamber 2E; Range of TWAs: 0-83.2 mg/m3

40.1 mg/m3 mean daily TWA Sb ore

Basis analytical conc. in chamber 2W; Range of TWAs: 0-91.1 mg/m3

No. of animals per sex per dose

90 animals per sex per group:
- 2 groups Sb2O3 exposed
- 2 groups Sb ore exposed
- 1 control group

Control animals

yes

Details on study design

- Dose selection rationale: it is the middle of the range of concentrations to which the workers have been exposed
- no other details on study design are reported

Positive control

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: twice a day, except weekends and holidays: once a day

BODY WEIGHT: Yes
- Time schedule for examinations: 1 day before exposure and at week 1,2,3 and 4, and monthle thereafter

- no other observations are stated

Sacrifice and pathology

Sacrifice: at 6, 9 and 12 months after initating exposure: 5 male and 5 female rats from each group
at 71- 73 weeks after initating exposure all animals were sacrified
GROSS PATHOLOGY: Yes, all organs
HISTOPATHOLOGY:
Yes, sections of the following tissues from all sacrified animals were prepared for microscopy
- liver, kidney, pankreas, spleen, adrenal, thyroid, pituitary, bladder, brain, eye, bone marrow, skin, mesenteric and tracheobronchial lymph nodes,
stomach, ascending and descending colon, lungs from each rat
- testicle and prostate from male rats
- mammary gland, ovary, uterus and cervix from female rats
- abnormal tissues

at the serial sacrifices (6,9 and 12 mo) samples were taken for analysis of concentation of Sb and other trace elements:
- portion of liver, lungs, kidneys, brain, spleens and blood of 5 males / 5 females in each group

Other examinations

Statistics

- Dunnett's multiple-comparison procedure (for weights)
- Kaplan-Meier method (Kaplan and Meier, 1958) for survival distribution

Any other information on materials and methods incl. tables

- there were difficulties in generating the target concentration (50 mg/m3), hence significante fluczuations appeared (very low concentrations in the first 2 months in chamber 2W and 2 E, reaching of 50 mg/m3 mean approx. after month for the first time in all chambers)

Results and discussions

Effect levels

Endpoint	LOAEC
Effect type	carcinogenicity
Effect level	45 mg/m³ air
Sex	female
Basis for effect level / Remarks	neoplasms

Observations

Clinical signs and mortality

yes

Body weight and weight gain

yes

Food consumption and compound intake (if feeding study)

Food efficiency

Water consumption and compound intake (if drinking water study)

Ophthalmoscopic examination

no effects

Haematology

no effects

Clinical chemistry

Urinalysis

Neurobehaviour

Organ weights

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

yes

Details on results

CLINICAL SIGNS AND MORTALITY
- hemorrage around the ears in all rats during first 2 mo (caused by metal ear tags)
- sporadic eye bleeding and hematuria in all groups (more frequently in the Sb2O3 and Sb ore group)

- survival showed no differences between control and exposure group

BODY WEIGHT AND WEIGHT GAIN
- the Sb2O3 males weighed significantly less than control males from wk 26 - wk 50 (6,2 % max. difference)
- the Sb ore female rats weighed less than the controls from wk 26 - wk 50 (6,4 % max. difference)

GROSS PATHOLOGY:
- at final sacrifice, the lungs of all animals of the both exposure groups contained slightly elevated, confluent, white and yellow foci on the pleural
surfaces of all lobes
- no tumor was identified at autopsy

HISTOPATHOLOGY: NON-NEOPLASTIC
1. Sb2O3 females:
- at 6 mo, lungs contained particles evenly scattered throughout all lobes and in more than 90% of the alveoli
- dense particle aggregates about the size of macrophages were present in about 10 % of the alveoli
- alveolar wll thickened
- cuboidal and columnar cell metaplasia occurred in some foci
- up to 12 mo exposure the symptomes increased (density of particles, amount of protein)
- at 12 mo, the first lung neoplasms were seen: one bronchiolalceolar adenoma and one squamous-cell carcinoma
- at 16/17 mo, the density of particles, amount of protein decreased, but the extent of interstitial fibrosis increased

2. Sb ore females:
- histopathology of the lungs was qualitively similar to that of the Sb2O3 exposed females, but fewer particles and less protein was found
- the extent of interstitial fibrosis and cell metaplasia was the same

3. Sb2O3 males:
-at six month: lungs had the same amount of interstitial thickening than the females', but less alveolar protein
- at 12 month: same severity of interstitial thickening as the females, in some areas appearing of amyloids
- the metaplasia was less extensive than in females
- at 16 mo: the metaplasia was less severe than in females; amount of alveolar protein was less thanat 12 mo and much less than in the females

4. Sb ore males:

- the alteration of the lung were similar to those seen with Sb2O3

5. controls:
- no significant patholigical alterations seen in any of the lungs
- occasional foci containing lymphocytes, typical of chronic pneumonia in a few rats

HISTOPATHOLOGY: NEOPLASTIC (if applicable)
- no lung tumor in control rats and in the male rats exposed to eithe compound
- Sb2O3 and Sb ore exposed femals developed lung neo plasms
- the first neoplasm at a rat which dies after 41 weeks of exposure to Sb ore
- the first Sb2O3 exposed rat showed lung tumor after 53 weeks
- incidence of lung tumor for Sb2O3 exposed rats = 27 % and for Sb ore rats = 25%
- tumor types: sqamous cell carcinomas, bronchioloalveolar adenomas, bronchiooalveolar carcinomas, scirrhous carcinomas
- typical tumors for this strain were observed in all groups

OTHER FINDINGS
After 9 month of exposure:
- the concentration of Sb in the lungs of male rats (38,300 µg Sb/g) exposed to Sb2O3 was significantly greater thanthat in female rats (25,600 µg Sb/g) exposed to Sb2O3
- the lungs of both males and femals exposed to Sb2O3 contained more Sb than the lungs of males (5.4 times more) and females (5.7 times more)
exposed to Sb ore
- lungs of male rats exposed to Sb2O3 contained more arsenic (213 µg/g) than lungs of female rats exposed to Sb2O3 (150 µg/g)
- the lungs of both males and femals exposed to Sb2O3 contained more arsenic than the lungs of males (21 times more) and females (10.8 times
more) exposed to Sb ore
- all other tissues from female rats contained higher arsenic concentration than the males' (in each group; except the femurs)

- no other findings are reported

Remarks on results including tables and figures

- no treatment related mortality, no lung tumours in control of either sex or in male exposed to either compound.
- incidence of various lung neoplasms significantly increased in female rats exposed to 45 mg Sb2O3/m3
- if only the animals examined after 53 weeks (12 month) are included in the study, the incidence of lung neoplasma was 19/59 (32%)
- no tumours found in males despite higher Sb concentration in the lungs
- it can be speculated that female rats are more susceptible to the induction of lung cancer by Sb2O3 and Sb ore
- the tumour response does not appear to be a function of the lung tissue concentration of Sb
Antimony trioxide had the potential to cause significant increase in lung cancer in female rats under the present conditions, but the underlying mechanism is not clear.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Relevance of carcinogenic effects / potential

Conclusions

Executive summary

Cross-reference to other study

k_Newton_1994_chronic

UUID IUC5-d75959e7-3ff0-4cae-ba56-b2fd09a22d0d
Dossier UUID 0
Author ebrcO2 / EBRC Consulting GmbH / Hannover / Germany
Date 2008-06-27 16:58:52 CEST
Remarks

Administrative Data

[]

Data waiving

Justification for data waiving

Study result type experimental result Study period no data

Reliability 2 (reliable with restrictions)

Rationale for reliability This study is of restricted reliability because the duration of one year deviates from the duration recommended by OECD 451/453 (two years), and also because chronic interstitial inflammation of minimal to moderate severity was observed in the lungs of several control and treated animals.

Data source

Reference

Reference type publication

Author Newton PE, Bolte HF, Daly IW, Pillsbury BD, Terrill JB, Drew RT, Ben-Dyke R, Sheldon AW and Rubin LF Year 1994

Title Subchronic and chronic inhalation toxicity of antimony trioxide in the rat

Bibliographic source Fundam Appl Toxicol 1994; 22: 561-576

Testing laboratory Bio/dynamics Inc. Report no.

Owner company

Company study no. Report date

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.1.1, Basic toxicokinetics, s_Newton_1994
chapter 7.5.3, Repeated dose toxicity:inhalation
chapter 7.7, Carcinogenicity, k_Newton_1994_subchronic

Materials and methods

Limit test

no

Test guideline

Qualifier equivalent or similar to

Guideline OECD Guideline 451 (Carcinogenicity Studies)

Deviations

Principles of method if other than guideline

This study did not follow the conventional 2-year exposure design , but was based upon the oncogenetic results of both the Watt (1983) and
Groth (1986) studies which were one year of exposure followed by an observation period. As both studies produced cancer, the question was not whether Sb2O3 was carcinogen but what was the dose-response relationship in both genders.

GLP compliance

yes self certified

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide
- Substance type: pure active substance
- Physical state: solid, dust
- Analytical purity: 99.68 ± 0.10 % (mean ± SD)
- particle size: "equal sized, not milled"
- Method for particle size measurement: scanning election microscopy; equivalent area diameter
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Breeding Laboratory (Kingsten, NY)
- Age at study initiation: approx. 8 weeks
- Weight at study initiation: 140 - 169 g (males) and 99 - 122g (females)
- Housing: · 4 stainless-steel and glas chambers (Hartford, Aberdeen, MD) with pyramental tops and bottoms (vol. = 6000 l each)
· each chamber held two 80 -animal racks; each rack had 16 stainless-stell,open mesh cages on each of five levels (housed individually)
· no excreta pans betweens levels during exposure period
· each animals' location was rotated weekly
- Diet: Purina Laboratory Chow No. 5001 (Ralston Purina Co., St. Louis, MO), ad libitumexcept during actual exposures
- Acclimation period: 12 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 23.33
- Humidity (%): 40 - 60
- Rate of air: 1880 - 2510 l / min (operated dynamically)
- Air changes (per min): 2.4 - 3.2 min
- equilibration time: 11.0 - 14.7 min
- Photoperiod (hrs dark / hrs light): 12 12-hr light/dark cycl
- chamber pressures: slight negative (- 0.5 cm H2O)

Administration / exposure

Route of administration

inhalation: dust

Type of inhalation exposure (if applicable)

whole body

Vehicle

unchanged (no vehicle)

Details on exposure

TEST ATMOSPHERE (if not tabulated)
- MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.): 3.05 ± 0.21 µ / 1.57 ± 0.06

GENERATION OF TEST ATMOSPHERE
- Exposure: Chamber airflow entered tangentially into the turrent at the top of the chamber
- Method of conditioning air: · using 2 fluidizing bed generators (Model 3400 and 9310; TSI, Inc., St. paul, MN)
· powdered test material was metered from a reservoir into fluidized bed
· no other bed material used in any of the generators
· resultant dust-ladened streams were delivered from the top of the fluidizing beds to the inlet turrets of chambers

- no other details on exposure stated

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

- Recording of airflow, temperautre and relative huminity: hourly
- samples obtained from breathing zone

Duration of treatment / exposure

52 weeks

Frequency of treatment

6 hr/day and 5 days / week

Post exposure period

12 month

Doses / concentrations

0 mg/m3

Basis analytical conc.

0.06 ± 0.04 (mean ± SD) mg/m3

Basis analytical conc.

0.51 ± 0.13 (mean ± SD) mg/m3

Basis analytical conc.

4.50 ± 1.33 (mean ± SD) mg/m3

Basis analytical conc.

No. of animals per sex per dose

65 animals per sex per group

Control animals

yes, sham-exposed

Details on study design

- Dose selection rationale: results of the subchronic study
- Rationale for animal assignment: based upon availability of historical data, hardiness and resistance of the strain to diseases, negative experiences
with other strain in previous study (Groth et al., 1986)
- no other details on study design stated

Positive control

none

Examinations

Observations and examinations performed and frequency

EXPOSURE ATMOSPHERE ANALYSIS:
- Recording of airflow, temperautre and relative huminity: hourly
- samples obtained from breathing zone
- sample collected on Whatman glass microfiber membrane filters (Gelman, type GF/A) held in close-faced filter holders (Gelman, No. 4338)
- samples analysed gravimetrically and analytically (atomic absorbtion)
- sample periods: 90 min samples, 1 all-day sample
- particle size distribution measurements: · periods: approx. quarterly during the study
· aparatus: scanning electron microscopy and quantitive image analyses (prestudy)
factory-calibrated TSI Aerodynamic Particle sizer (Model 3300)

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: weekly

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: weekly

BODY WEIGHT: Yes
- Time schedule for examinations: twice pretest, weekly for the first 13 weeks, monthly thereafter and at termination (fasting)

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes / No / No data
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes / No / No data

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: pretest and on the day before their schedule termination
- Dose groups that were examined: all animals

HAEMATOLOGY: Yes
- Time schedule for collection of blood: after 12, 18 and 24 months
- Anaesthetic used for blood collection: Yes, under light ethyl ether anesthesia
- Animals fasted: Yes
- How many animals: 5 animals per sex per group
- Parameters checked in table [No.1] were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: after 1, 2, 4 ,8 and 13 weeks of exposure
- Animals fasted: Yes
- How many animals: 5 animals per sex per group
- Parameters checked in table [No.?] were examined.

- no other observations are performed

Sacrifice and pathology

SACRIFICE::

- 5 animals per sex and per group were terminated after: 6 and 12 months of exposure and 6 months postexposure
- all survivors were terminated at 24 months (12 months postexposure)
- blood samples obtained at all termination intervals
- fecal samples were collected at the 18- and 24- month terminations

GROSS PATHOLOGY: Yes (see table No. 2) in all animals
HISTOPATHOLOGY: Yes (see table No. 3) in all animals

Other examinations

- in the lungs, the right lobes were sampled and saggital sections, including major bronchi, processed for microscopic examination
- left lung lobes were taken for SB2O3 analyses at each termination (frozen till analyses)

Statistics

- parametric data were analyzed using an analysis of variance
- statistically differences analyzed using Tukey's (equal population) or Scheffe's (unequal population) test of multiple comparison
- survival analyzed using the method of Thomas et al. (1977)

Any other information on materials and methods incl. tables

Table 1: Performed clinical laboraty tests

Clincal laboratory tests	
Hematological indices	Hemoglobin
	Hematocrit
	Erythrocyte count
	Mean corpuscular hemoglobin
	Mean corpuscular volume
	Mean corpuscular hemoglobin concertration
	Total leukocyte count
	Differential leukocyte counts
	Erythrocytemorphology
Serum biochemical evaluations	Aspartate aminotransferase
	Alanine aminotransferase
	Alkaline phosphatase
	Blood urea nitrogen
	Fasting glucose
	Total protein
	Total chloride
	Sodium determination
	Potassium determination

Table 2: Performed gross necropsy

Gross necropsy examination	External surface
	All orifices
	Cranial cravity
	Carcass
	External and sectioned surfaces of brain and spinal cord
	Nasal cavity
	Paranasal sinuses
	Thoracic, abdominal and pelvic cavaties and their viscera
	Cervical tissues and organs

Table 3: Performed histopathology

Tissues preserved in neutral-buffered 10 % formalin for histopathological evaluation	Adrenal gland
	Aorta
	Sternum
	Brain
	Esophagus
	Eyes
	Heart
	Cecum
	Colon
	Duodenum
	Ileum
	Jejunum
	Kidneys
	Liver
	Right lung lobes
	Lymph lobes
	Mammary gland
	Larynx
	Nasal turbinates (cut into 4 sections)
	Nerve
	Ovaries
	Pancreas
	Pituitary

	Prostate and salivary glands
	Seminal vesicles
	Skin
	Spinal chord
	Spleen
	Stomach
	Testes with epididymides, thymus, thyroid/parathyroid glands
	Trachea
	Urinary bladder
	Uterus
	Vagina
	Any tissues with gross lesions
Histopathological examined tissues from all animals (after poutine processing and hematoxyl-eosin staining)	Heart*
	Nasal turbinates
	Larynx
	Trachea
	Lung*
	Peribrinchial lymph node

* stained with Masson's Trichrome stain and examined singly

Results and discussions

Effect levels

Endpoint	NOAEC
Effect type	carcinogenicity
Effect level	> 4.5 mg/m³ air (analytical)
Sex	male/female
Basis for effect level / Remarks	neoplasms during chronic exposure

Observations

Clinical signs and mortality

yes

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

not examined

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

yes

Haematology

yes

Clinical chemistry

no data

Urinalysis

no data

Neurobehaviour

no data

Organ weights

no effects

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

no effects

Details on results

CLINICAL SIGNS AND MORTALITY
- 56 % survival of males and 48 % survival of females, but no differences between the differente groups

BODY WEIGHT AND WEIGHT GAIN
- no significant differences among the groups in body weight gain

OPHTHALMOSCOPIC EXAMINATION
- corneal irregularities but nearly equally in all groups and there not considered to be treatment related
- dose- related chromodacryrrhea in malesand also in femals (can be secondary to dental abnormality - what was not examined)
- exposure-related increase in ocular opacities (see Table 1) - more severe effects in females

HAEMATOLOGY
- elevated total leukocyte counts and atypicallymphocytes in some animals in all groups at terminal euthanization (indicates leukemia)
- (leukemia is a common finding in aged Fischer 344 rats)
- Sb2O3 was present in the red blood cells, but not in the plasma (Table 4)

ORGAN WEIGHTS
- absolute and relative lung weights were unaffected by the exposures at all concentrations

GROSS PATHOLOGY
- pinpoint black foci in the animals exposed to Sb2O3, most frequently during postexposure period (foci are believed to be aggregates of macrophages ladened with the test material), see Table 2 and 3
- microscopic findings considered to be related to exposure to Sb2O3 were seen in the lungs and peribrinchial lymph nodes during 1-year exposure (table 2) and the 1-year observation periods (table 3)
- chronic interstitial inflammation was observed in the lungs of numerous animals during exposure and observation periods
- interstitial fibrosis, granulomatous inflammation and bronchiolar/alveolar hyperplasia occurred either primarily or exclusively in a small number
of animals during observation period
- pulmonary carcinomas were seen in only 3 animals (2 males, one each from the 0 and the 4.5 mg/m3 group) and 1 female (from the 0.51 mg/m3
group); these carcinomas were not considered to be exposure related

HISTOPATHOLOGY: NON-NEOPLASTIC
- near steady-state lung burden levels appeard to have been reached in all groups by 6 months after exposure

HISTOPATHOLOGY: NEOPLASTIC (if applicable)
- no Sb2O3 related neoplasms

HISTORICAL CONTROL DATA (if applicable)

OTHER FINDINGS
- small, but dose-related amounts of Sb2O3 were found in the feces at 18 months but not at 24 months (Table 4)
Sb2O3 pulmonary clearance rates fit by a single exponential curve: a lung containing approx. 2 mg of Sb2O3 , pulmonary clearance was decreased about 80% with an increase in clearance halftimeof 2 to 10 month. Under these exposure conditions of this study Sb2O3 was not a carcinogen.

- no other findings are reported

Remarks on results including tables and figures

Table 1: Incidence (percentage) of opacities seen in Fischer 344 rats during in-life observations, ophthalmoscopic evaluations, and microscopic evaluations

Exposure (mg/m3)	In-life observations	Ophthalmoscopic* evaluation	Necropsy evaluation	Microscopic** evaluation
Males				
0	14	11	18	14
0.06	7	15	7	N/A
0.51	7	21	10	N/A
4.50	11	18	14	11
Females				
0	0	13	4	13
0.06	15	40	20	N/A
0.51	12	36	12	N/A
4.50	20	47	20	33
Combined				
0	8	12	12	13
0.06	11	26	13	N/A
0.51	10	29	11	N/A
4.50	16	33	17	22

* Includes focal posterior polar cataract, posterior subcapsular cataract, and complete cataract.
** Includes moderate or severe lenticular degeneration
N/A = not analyzed

Table 2: Nonneoplastic micoscopic findings seen after a 1-year inhalation exposure to Sb2O3

Organ/tissue examined (lungs)		Males				Females			
	Group:	I	II	III	IV	I	II	III	IV
	Number examined:	13	13	12	13	16	13	11	14
Interstitium: chronic inflamemation	1>	1	0	1	0	1	1	0	2
	2>	8	7	10	7	8	6	8	10
	3>	1	1	0	5	1	4	2	2
	TL	10	8	11	12	10	11	10	14
Granulomatous inflammation	1>	0	0	0	1	1	0	0	0
	TL	0	0	0	1	1	0	0	0
Interstitium: fibrosis	TL	0	0	0	0	0	0	0	0
	TL	0	0	0	0	0	0	0	0
Bronchiolar/alveolar hyperplasia	1>	5	7	4	0	4	8	4	0
	2>	1	4	5	8	2	2	4	9
	3>	0	0	0	5	0	0	0	5
Alveolar/intraalveolar macrophages	TL	6	11	9	13	6	10	8	14
Alveolar/intraalveolar macrophages:	1>	0	13	7	0	0	13	5	0
	2>	0	0	5	1	0	0	6	3
	3>	0	0	0	12	0	0	0	11
foreign particulate material	TL	0	13	12	13	0	13	11	14
Macrophages in the perivascular/peribronchiolar	1>	0	2	6	0	0	6	4	0
	2>	0	0	0	5	0	0	0	6
Aggregates of lymphoid cells:	3>	0	0	0	2	0	0	0	1
foreign particulate material	TL	0	2	6	7	0	6	4	7
Peribronchial lymph node macrophages:	1>	0	3	5	2	0	0	6	3
	2>	0	0	0	3	0	0	0	2
	3>	0	0	0	8	0	0	0	8
foreign particulate material	TL	0	3	5	13	0	0	6	13

Table 3: Nonneoplastic Microscopic Findings seen after a 1 -Year chronic exposure seen during the 1 -year observation period

Organ/tissue examined (lungs)		Males				Females			
	Group:	I	II	III	IV	I	II	III	IV
	Number examined:	52	52	53	52	49	52	54	50
Interstitium: chronic inflamemation	1>	4	7	12	0	3	12	14	1
	2>	19	27	24	14	24	23	23	29
	3>	8	3	0	32	6	5	11	18
	4>	1	0	0	2	0	0	0	0
	TL	32	37	36	48	33	40	48	48
Granulomatous inflammation	1>	2	1	4	4	2	0	2	2
	2>	1	0	0	3	0	2	2	1
	3>	0	1	1	0	0	0	1	0
	TL	3	2	5	7	2	2	5	3
Interstitium: fibrosis	1>	0	0	1	0	0	0	0	2
	2>	0	0	0	1	0	1	1	2
	3>	0	0	0	1	0	0	0	0
	TL	0	0	1	2	0	1	1	4
Bronchiolar/alveolar hyperplasia	1>	2	0	0	2	0	0	0	1
	2>	1	1	2	1	1	0	0	4
	3>	0	0	0	1	0	0	0	1
	TL	3	1	2	4	1	0	0	6
Alveolar/intaalveolar macrophages	1>	22	28	36	0	15	21	15	0
	2>	7	14	9	9	9	17	30	22
	3>	2	2	1	41	4	2	3	28
	4>	0	0	0	2	0	0	0	0
	TL	31	44	46	52	28	40	48	50
Alveolar/intraalveolar macrophages:	1>	0	15	30	0	0	24	30	0
	2>	0	0	8	12	0	0	19	8
	3>	0	0	0	39	0	0	0	40
foreign particulate material	TL	0	15	38	51	0	24	49	48
Macrophages in the perivascular/peribronchiolar	1>	0	22	36	0	0	31	40	2
	2>	0	0	10	36	0	0	7	35
aggregates of lymphoid cells:	3>	0	0	0	11	0	0	0	10
foreign particulate material	TL	0	22	46	47	0	31	47	47
Peribronchial lymph node macrophages:	1>	0	6	15	2	0	6	15	0
	2>	0	0	19	6	0	0	13	8
	3>	0	0	0	31	0	0	1	31
foreign particulate material	TL	0	6	34	39	0	6	29	39

Table 4: Red blood cell and fecal levels of Sb2O3 during a 1-year chronic exposure followed by a 1-year observation period

	Red blood cell (µg/g) (months)				Feces (µg/g) (months)	
Group	6	12	18	24	18	24
	Males					
I	ND	ND	0.17 ± 0.39	ND	ND	ND
II	0.53 ± 0.31	1.09 ± 0.21	0.86 ± 0.68	ND	0.18 ± 0.40	ND

(0.055mg/m3)						
III (0.51mg/m3)	5.07 ± 0.29	7.55 ± 0.60	3.93 ± 0.25	2.53 ± 0.27	3.59 ± 1.65	ND
IV (4.5 mg/m3)	34.5 ± 3.8	70.7 ± 6.3	38.6 ± 4.8	30.5 ± 7.5	4.39 ± 0.63	ND
Females						
I	ND	ND	ND	ND	ND	ND
II (0.055mg/m3)	0.74 ± 0.06	1.48 ± 0.10	0.81 ± 0.30	ND	0.17 ± 0.37	ND
III (0.51mg/m3)	5.69 ± 0.62	9.94 ± 1.32	6.53 ± 0.90	3.39 ± 0.28	0.16 ± 0.22	ND
IV (4.5 mg/m3)	75.6 ± 8.4	121 ± 10.6	74.6 ± 18.3	36.6 ± 15.5	1.39 ± 0.35	ND

ND = none detected

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Relevance of carcinogenic effects / potential

Conclusions

Executive summary

Cross-reference to other study

- Groth DH, Stettler LE, Burg JR, Busey WM, Grant GC and Wong L. Carcinogenic effects of antimony trioxide and antimony ore concentrate in rats.
J Toxicol Environ Health 1986a; 18: 607-626.
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Cancer. Edited by Goldsmith DF, Winn DM and Shy CM 1986b; pp 243-253. Praeger, New York.
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University, Detroit, Michigan.

k_Omura_2002_mice

UUID	IUC5-2b5efd9c-2800-4e3d-903b-9c231c54d3bf
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:55 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Acceptable, well-documented study report, which meets the basic scientific principles. Comparable to guideline study.		

Data source

Reference

Reference type	publication		
Author	Omura M, Tanaka A, Hirata M and Inoue N	Year	2002
Title	Testicular toxicity evaluation of two antimony compounds, antimony trioxide and antimony potassium tartrate, in rats and mice		
Bibliographic source	Environ Health Prev Med 2002; 7: 15-18		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.8.1 toxicity to reproduction: K_Omura_2002_rats

Materials and methods

Test type

Limit test

no

Test guideline

Qualifier	according to
Guideline	other guideline: Guidelines for Animals Experiments in the Faculty of Medicine, Kyushu University
Deviations	
Qualifier	according to
Guideline	other guideline: Law No. 105 of the Government of Japan
Deviations	
Qualifier	according to
Guideline	other guideline: Notification No. 6 of the Government of Japan
Deviations	

Principles of method if other than guideline

Comparison of testicular toxicities of two antimony compounds (ATO and APT) in rats and mice. ATO is a slightly water soluble substance and APT is a highly water soluble substance.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): ATO (antimony trioxide)
- Name of second test material (as cited in study report): APT (antimony potassium tartrate)
- Physical state: solid powder
- Analytical purity: >99.999% (ATO)
>99.5 % (APT)
- no other details on test material stated

Confidential details on test material

Test animals

Species

mouse

Strain

CD-1

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Kyudo Co., Ltd., Tosu, Japan
- Age at study initiation: (P) 7 wks
- Housing: air-conditioned conventtional room
- Acclimation period: approx. 1 week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 24 - 26°C
- Humidity (%): 40 - 80%
- Photoperiod (hrs dark / hrs light): 12 h light / 12 h dark

- no other details on test animals and environmental conditions

Administration / exposure

Route of administration

oral: gavage

Type of inhalation exposure (if applicable)

Vehicle

water

Details on exposure

VEHICLE
- Justification for use and choice of vehicle (if other than water): distilled water
- no other details on exposure are stated

Details on mating procedure

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Duration of treatment / exposure

4 weeks of administration

Frequency of treatment

- administration by gavage: 5 days per week
- daily doses of the compounds were 27.4 (APT) , 12.0 (ATO) and 1200 (ATO) mg/kg bw (corresponding daily doses of antimony were 10, 10 and
1000 mg/kg bw)

Details on study schedule

Doses / concentrations

27.4 mg APT/kg bw = 10 mg antimony/kg bw
Basis nominal conc.

12.0 mg ATO/kg bw = 10 mg antimony/kg bw
Basis nominal conc.

1200 mg ATO/kg bw = 1000 mg antimony/kg bw
Basis nominal conc.

No. of animals per sex per dose

10 male mice per dose

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale:
· In the preliminary study mice showed high tolerance for 10-day administration of APT at 274 mg/kg bw.
· because of the longer adminstration period (4 weeks) in present study, daily dose was 27.4 mg APT/kg bw (= 10 mg antimony/kg bw)
· daily dose of ATO in the low-ATO group was 12.0 mg/kg bw which is equivalent to 10 mg antimony/kg bw
· as ATO is slighly soluble in water and expected to be less bioavailable than APT the daily high-ATO dose was 1200 mg/kg bw

- no other details on study design stated

Positive control

none

Examinations

Parental animals: Observations and examinations

BODY WEIGHT: Yes
- Time schedule for examinations: at least one time at the termination of four-week administration
- no other details on examinations are reported

Estrous cyclicity (Parental animals)

Sperm parameters (Parental animals)

Parameters examined in [P] male parental generations:
- evaluating number, motility and morphology of sperm in the cauda epididymis

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: no
- no other details on litter observations are stated

Postmortem examinations (Parental animals)

SACRIFICE
- Male animals: All surviving animals were killed one day after final administration by inhalation of CO2

GROSS NECROPSY
- no data

HISTOPATHOLOGY / ORGAN WEIGHTS
- testes, epididymides, ventral prostate and seminal vesicle were removed and weighed (seminal vesicle weighed without fluid)
- evaluating histopathologic changes in testis (all round and ovoid cross-sections of seminiferous tubule in one transverse were examined)
- following histopathologic changes were examined: · disorganization and exfoliationof the seminiferous epithelium
· degeneration of germ cells
· vacuolization of the epithelium
· sperm retention in the epithelium
· delayed spermation

Postmortem examinations (Offspring)

Statistics

- statistical differences were analyzed using Fisher's least significant difference procedure (after one-way analysis of variance)
- differences were considered significant when p value was below 0.05

Reproductive indices

Offspring viability indices

Any other information on materials and methods incl. tables

- after acclimation period, animals were randomly divided into 4 groups (at 8 weeks of age):

1. the APT group

2. the low-ATO group

3. the high-ATO group

4. the control group

- distilled water (vehicle) was administered to the control group in the same manner as to the test groups

Results and discussions

Effect levels

Endpoint	NOAEL for testicular toxicity
Generation	
Sex	male
Effect level	> 1200 mg/kg bw/day
Basis for effect level / Remarks	

Observations: parental animals

Clinical signs (parental animals)

yes

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

no data

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

no effects

Reproductive performance (parental animals)

not examined

Organ weights (parental animals)

no effects

Gross pathology (parental animals)

no data

Histopathology (parental animals)

yes

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
- two mice in the high ATO-group and one control mouse died due to accidents at administration

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
- the two antimoy compounds did not affect body weight gain
- for body weights at the end of 4-week administration see Table 1

REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)
- the two antimoy compounds did not affect the count, motility or morphology of caudal sperm (see Table 2)

ORGAN WEIGHTS (PARENTAL ANIMALS)
-seminal vesicle weight was slightly decreased in mice in both ATO groups
- the two antimoy compounds did not affect any other organ weights
- for weights of testis, epididymis, ventral postate and seminal vesicle at the end of 4-week administration see Table 1

HISTOPATHOLOGY (PARENTAL ANIMALS).
- 1 of 10 mice in the low-ATO group showed exfoliation of the seminiferous ephelium (frequency in this mouse > 50%)
- no mice in the high_ATO group showed an obvious increase in frequency of exfoliation
- APT did not cause any changes

- no other findings are reported

Observations: offspring

Viability (offspring)

Clinical signs (offspring)

Body weight (offspring)

Sexual maturation (offspring)

Organ weights (offspring)

Gross pathology (offspring)

Histopathology (offspring)

Details on results (offspring)

Remarks on results including tables and figures

- the increased frequency of epithelial exfoliation, which was observed in 1 mouse in the low-ATO group, is considered as not administration related as no mice or rat of the high-ATO group showed any increase in the frequency
- no apparent effects of the 2 antimony compounds on sperm parameters or weights of reproduction organs or accessory sex organs were observed
- repeated administration of ATO at a dose of 1200 mg/kg bw for a period of 4 weeks was not toxic to testes

Table 1: Body weight, reproductive organs weights and accessory sex organs weights in rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO).

	Control	APT*	low ATO*	high ATO*
Rats	n = 8	n = 8	n = 8	n = 7
Body weight (g)	402.2±18.7	388.3±25.5	395.6±32.6	399.2±21.9
Testis weight (g)	1.641±0.082	1.703±0.077	1.744±0.161	1.625±0.140
Epididymis weight (g)	0.504±0.042	0.541±0.019	0.540±0.042	0.537±0.042
Ventral prostate weight (g/100 g bw)	0.133±0.018	0.136±0.020	0.124±0.026	0.133±0.008
Seminal vesicle weight (g/ 100 g bw)	0.139±0.019	0.140±0.013	0.142±0.016	0.141±0.017
Mice	n = 9	n = 10	n = 10	n = 8
Body weight (g)	35.0±0.8	33.7±3.0	34.2±2.9	33.4±2.2
Testis weight (g)	0.249±0.032	0.245±0.034	0.259±0.039	0.238±0.027
Epididymis weight (g)	0.104±0.012	0.099±0.008	0.100±0.009	0.096±0.011
Ventral prostate weight (g/100 g bw)	0.048±0.016	0.078±0.033	0.069±0.033	0.086±0.075
Seminal vesicle weight (g/ 100 g bw)	0.879±0.233	0.880±0.137	0.829±0.167	0.662±0.230

bw = body weight

* daily administration dose was 27.4 mg, 12.0 mg and 1200 mg/kg bw in the APT, low ATo and the high ATO group, respectively. The antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice.

Table 2: Sperm count, sperm motility and sperm morphology of rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO).

	Control	APT*	low ATO*	high ATO*
Rats	n = 8	n = 8	n = 8	n = 7
Sperm count ($x10^6$/cauda epididymidis)	133.1±21.7	130.6±16.6	142.2±35.1	140.9±18.7
% motile sperm	83.3±6.6	77.9±9.6	72.4±11.0	77.2±10.6
% progressively motile sperm	60.3±19.6	48.8±10.7	44.5±17.7	52.2±12.4
% sperm head abnormality	0.9±0.3	0.8±0.6	0.7±0.5	0.8±0.3
% sperm tail abnormality	0.5±0.4	0.4±0.3	0.3±0.2	0.3±0.2
% sperm without tail	2.0±0.7	2.0±0.6	1.8±0.8	2.4±1.2
Mice	n = 9	n = 10	n = 10	n = 8
Sperm count ($x10^6$/cauda epididymidis)	31.1±8.6	28.7±8.2	30.2±5.3	28.1±7.4
% sperm head abnormality	2.9±2.4	1.4±1.0	1.9±1.9	2.8±1.9
% sperm tail abnormality	0	0.1±0.1	0.0±0.1	0.1±0.3
% sperm without tail	2.9±2.4	2.7±1.9	2.9±3.1	4.3±5.3

* daily administration dose was 27.4 mg, 12.0 mg and 1200 mg/kg bw in the APT, low ATo and the high ATO group, respectively. The antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice

- sperm motility data of mice was not included in this report as the intval between the removal of the epididymis and sperm motality analysis was too long and the data was therefore thought to be unreliable.

Neither ATO nor APT is toxic to the testes of mice. It is possible that the concentrations of these antimony compounds in the tested did not

become sufficiently high for their enotoxicities to result in damage of germ cells.
The results indicate that water solubility of the antimony compounds affects their toxicity to testes.

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Conclusions

Executive summary

Cross-reference to other study

k_Omura_2002_rats

UUID	IUC5-8dcb472d-8b36-4e30-8aaa-7fd9248b711e
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:56 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Acceptable, well-documented study report, which meets the basic scientific principles. Comparable to guideline study.		

Data source

Reference

Reference type	publication		
Author	Omura M, Tanaka A, Hirata M and Inoue N	Year	2002
Title	Testicular toxicity evaluation of two antimony compounds, antimony trioxide and antimony potassium tartrate, in rats and mice		
Bibliographic source	Environ Health Prev Med 2002; 7: 15-18		
Testing laboratory		Report no.	
Owner company			
Company study no.		Report date	

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.8.1 toxicity to reproduction: K_Omura_2002_mice

Materials and methods

Test type

Limit test

no

Test guideline

Qualifier	according to
Guideline	other guideline: Guidelines for Animals Experiments in the Faculty of Medicine, Kyushu University
Deviations	
Qualifier	according to
Guideline	other guideline: Law No. 105 of the Government of Japan
Deviations	
Qualifier	according to
Guideline	other guideline: Notification No. 6 of the Government of Japan
Deviations	

Principles of method if other than guideline

Comparison of testicular toxicities of two antimony compounds (ATO and APT) in rats nad mice. ATO is a slightly water-soluble and APT is ahigh water-solublesubstrate.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 1309-64-4

Identifier EC number

Identity 215-175-0

Identifier IUPAC name

Identity dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): ATO (antimony trioxide)
- Name of second test material (as cited in study report): APT (antimony potassium tartrate)
- Physical state: solid powder
- Analytical purity: >99.999% (ATO)
>99.5 % (APT)
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Kyudo Co., Ltd., Tosu, Japan
- Age at study initiation: (P) 6 wks
- Housing: SPF room
- Acclimation period: approx. 2 weeks

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 - 25°C
- Humidity (%): 50 - 60%
- Photoperiod (hrs dark / hrs light): 12 h light / 12 h dark

- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

oral: gavage

Type of inhalation exposure (if applicable)

Vehicle

water

Details on exposure

VEHICLE
- Justification for use and choice of vehicle (if other than water): distilled water
- no other details on exposure are reported

Details on mating procedure

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Duration of treatment / exposure

4 weeks of administration

Frequency of treatment

- administration by gavage: 3 days per week
- daily doses of the compounds were 27.4 (APT) , 12.0 (ATO) and 1200 (ATO) mg/kg bw (corresponding daily doses of antimony were 10, 10 and
1000 mg/kg bw)

Details on study schedule

Doses / concentrations

27.4 mg APT/kg bw = 10 mg antimony/kg bw

Basis nominal conc.

12.0 mg ATO/kg bw = 10 mg antimony/kg bw

Basis nominal conc.

1200 mg ATO/kg bw = 1000 mg antimony/kg bw

Basis nominal conc.

No. of animals per sex per dose

8 male rats per dose

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale:
· In the preliminary study mice showed high tolerance for 10-day administration of APT at 274 mg/kg bw.
· because of the longer adminstration period (4 weeks) in present study, daily dose was 27.4 mg APT/kg bw (= 10 mg antimony/kg bw)
· daily dose of ATO in the low-ATO group was 12.0 mg/kg bw which is equivalent to 10 mg antimony/kg bw
· as ATO is slighly soluble in water and expected to be less bioavailable than APT the daily high-ATO dose was 1200 mg/kg bw

- no other details on study design are stated

Positive control

none

Examinations

Parental animals: Observations and examinations

BODY WEIGHT: Yes
- Time schedule for examinations: at least one time at the termination of four-week administration
- no other details on examinations are stated

Estrous cyclicity (Parental animals)

Sperm parameters (Parental animals)

Parameters examined in [P] male parental generations:
- evaluating number, motility and morphology of sperm in the cauda epididymis

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: no
- no other details on litter observation stated

Postmortem examinations (Parental animals)

SACRIFICE
- Male animals: All surviving animals were killed one day after final administration by inhalation of CO_2

GROSS NECROPSY
- no data

HISTOPATHOLOGY / ORGAN WEIGHTS
- testes, epididymides, ventral prostate and seminal vesicle were removed and weighed (seminal vesicle weighed without fluid)
- evaluating histopathologic changes in testis (all round and ovoid cross-sections of seminiferous tubule in one transverse were examined)
- following histopathologic changes were examined: · disorganization and exfoliationof the seminiferous epithelium
· degeneration of germ cells
· vacuolization of the epithelium
· sperm retention in the epithelium
· delayed spermation

Postmortem examinations (Offspring)

Statistics

- statistical differences were analyzed using Fisher's least significant difference procedure (after one-way analysis of variance)
- differences were considered significant when p value was below 0.05

Reproductive indices

Offspring viability indices

Any other information on materials and methods incl. tables

- after acclimation period, animals were randomly divided into 4 groups (at 8 weeks of age):

1. the APT group

2. the low-ATO group

3. the high-ATO group

4. the control group

- distilled water (vehicle) was administered to the control group in the same manner as to the test groups

Results and discussions

Effect levels

Endpoint	NOAEL for testicular toxicity
Generation	
Sex	male
Effect level	> 1200 mg/kg bw/day
Basis for effect level / Remarks	

Observations: parental animals

Clinical signs (parental animals)

yes

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

no data

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

no effects

Reproductive performance (parental animals)

not examined

Organ weights (parental animals)

no effects

Gross pathology (parental animals)

no data

Histopathology (parental animals)

yes

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
- one rat in the high ATO-group died due to accidents at administration

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
- the two antimoy compounds did not affect body weight gain
- for body weights at the end of 4-week administration see Table 1

REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)
- the two antimoy compounds did not affect the count, motility or morphology of caudal sperm (see Table 2)

ORGAN WEIGHTS (PARENTAL ANIMALS)
- the two antimoy compounds did not affect any of the evaluated organ weights
- for weights of testis, epididymis, ventral postate and seminal vesicle at the end of 4-week administration see Table 1

HISTOPATHOLOGY (PARENTAL ANIMALS)
- delayed spermation (1 of 8 rats in the low-ATO group and 1 of 7 rats in the high-ATO group, frequency was < 1%)
- APT did not cause any changes

- no other findings are reported

Observations: offspring

Viability (offspring)

Clinical signs (offspring)

Body weight (offspring)

Sexual maturation (offspring)

Organ weights (offspring)

Gross pathology (offspring)

Histopathology (offspring)

Details on results (offspring)

Remarks on results including tables and figures

- no apparent effects of the 2 antimony compounds on sperm parameters or weights of reproduction organs or accessory sex organs were observed

- repeated administration of ATO at a dose of 1200 mg/kg bw for a period of 4 weeks was not toxic to testes

Table 1: Body weight, reproductive organs weights and accessory sex organs weights in rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO)

.	Control	APT*	low ATO*	high ATO*
Rats	n = 8	n = 8	n = 8	n = 7
Body weight (g)	402.2±18.7	388.3±25.5	395.6±32.6	399.2±21.9
Testis weight (g)	1.641±0.082	1.703±0.077	1.744±0.161	1.625±0.140
Epididymis weight (g)	0.504±0.042	0.541±0.019	0.540±0.042	0.537±0.042
Ventral prostate weight (g/100 g bw)	0.133±0.018	0.136±0.020	0.124±0.026	0.133±0.008
Seminal vesicle weight (g/ 100 g bw)	0.139±0.019	0.140±0.013	0.142±0.016	0.141±0.017
.				
Mice	n = 9	n = 10	n = 10	n = 8
Body weight (g)	35.0±0.8	33.7±3.0	34.2±2.9	33.4±2.2
Testis weight (g)	0.249±0.032	0.245±0.034	0.259±0.039	0.238±0.027
Epididymis weight (g)	0.104±0.012	0.099±0.008	0.100±0.009	0.096±0.011
Ventral prostate weight (g/100 g bw)	0.048±0.016	0.078±0.033	0.069±0.033	0.086±0.075
Seminal vesicle weight (g/ 100 g bw)	0.879±0.233	0.880±0.137	0.829±0.167	0.662±0.230

bw = body weight

* daily administration dose was 27.4 mg, 12.0 mg and 1200 mg/kg bw in the APT, low ATo and the high ATO group, respectively. The antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice.

Table 2: Sperm count, sperm motility and sperm morphology of rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO).

.	Control	APT*	low ATO*	high ATO*
Rats	n = 8	n = 8	n = 8	n = 7
Sperm count ($x10^6$/cauda epididymidis)	133.1±21.7	130.6±16.6	142.2±35.1	140.9±18.7
% motile sperm	83.3±6.6	77.9±9.6	72.4±11.0	77.2±10.6
% progressively motile sperm	60.3±19.6	48.8±10.7	44.5±17.7	52.2±12.4
% sperm head abnormality	0.9±0.3	0.8±0.6	0.7±0.5	0.8±0.3
% sperm tail abnormality	0.5±0.4	0.4±0.3	0.3±0.2	0.3±0.2
% sperm without tail	2.0±0.7	2.0±0.6	1.8±0.8	2.4±1.2
.				
Mice	n = 9	n = 10	n = 10	n = 8
Sperm count ($x10^6$/cauda epididymidis)	31.1±8.6	28.7±8.2	30.2±5.3	28.1±7.4
% sperm head abnormality	2.9±2.4	1.4±1.0	1.9±1.9	2.8±1.9
% sperm tail abnormality	0	0.1±0.1	0.0±0.1	0.1±0.3
% sperm without tail	2.9±2.4	2.7±1.9	2.9±3.1	4.3±5.3

* daily administration dose was 27.4 mg, 12.0 mg and 1200 mg/kg bw in the APT, low ATo and the high ATO group, respectively. The

antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice. Neither ATO nor APT is toxic to the testes of rats. It is possible that the concentrations of these antimony compounds in the tested did not become sufficiently high for their enotoxicities to result in damage of germ cells.

The results indicate that water solubility of the antimony compounds affects their toxicity to testes .

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Conclusions

Executive summary

Cross-reference to other study

s_Hext_1999

UUID	IUC5-15eb72d5-61f5-4387-80e0-a6672ffbe98c
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:55 CEST
Remarks	

Administrative Data

[]

Data waiving

Justification for data waiving

Study result type: experimental result — Study period

Reliability: 1 (reliable without restriction).

Rationale for reliability: guideline conform, well conducted study.

Data source

Reference

Reference type	publication
Author	Hext PM, Pinto PJ and Rimmel BA — Year 1999
Title	Subchronic feeding study of antimony trioxide in rats
Bibliographic source	Appl Toxicol 1999; 19: 205-209
Testing laboratory	— Report no.
Owner company	
Company study no.	— Report date

Data access

data published

Data protection claimed

Cross-reference to same study

chapter 7.5.1 Repeated dose toxicity:oral; K_hext_1999

Materials and methods

Test type

Limit test

Test guideline

Qualifier

Guideline

Deviations

Principles of method if other than guideline

- use of a protocol that conforms to modern guidelines

GLP compliance

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4
Identifier	EC number
Identity	215-175-0
Identifier	IUPAC name
Identity	dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxde
- Physical state: solid, powder
- Analytical purity: 99 %
- no other details on test material stated

Confidential details on test material

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: rodent breeding unit at Zeneca Pharmaceuticals, Cheshire, UK
- Housing: in multiple rat racks at 4-5 per cage initially and then in fours after being assigned to experimental groups
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21±2°C
- Humidity (%): 55±15%
- Air changes (per hr): 15
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light (fluorescent)

- no other details on test animals and environmental conditions stated

Administration / exposure

Route of administration

oral: feed

Type of inhalation exposure (if applicable)

Vehicle

Details on exposure

DIET PREPARATION
- Mixing appropriate amounts with (Type of food): CT1 diet (Special Diets Sevices Ltd.)
- no other details on exposure are stated

Details on mating procedure

Analytical verification of doses or concentrations

Details on analytical verification of doses or concentrations

Duration of treatment / exposure

90 days

Frequency of treatment

continously

Details on study schedule

Doses / concentrations

84 (males) / 97 (females) mg/kg bw*day

Basis nominal in diet calculated mean daily dose of Sb2O3

412 (males) / 494 (females) mg/kg bw*day

Basis nominal in diet calculated mean daily dose of Sb2O3

1686 (males) / 1879 (females) mg/kg bw*day

Basis nominal in diet calculated mean daily dose of Sb2O3

No. of animals per sex per dose

12M/ 12F
Control animals

yes
Further details on study design

- Dose selection rationale: based on previous studies described in literature and on a preliminary study performed in the same laboratory
Positive control

Examinations
Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: daily

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: daily

BODY WEIGHT: Yes
- Time schedule for examinations: weekly

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- samples of the dietary levels were taken throughout the study and analyzed for antimony trioxide

OTHER:
- at termination blood samples were taken and analysed for haematology and clinical chemistry parameters
- urin samples were collected over a 16-h period during the final week of study

- no other details on examinations are reported
Estrous cyclicity (Parental animals)

Sperm parameters (Parental animals)

Litter observations

Postmortem examinations (Parental animals)

SACRIFICE
- rats killed by an overdose of halothane and bled by cardiac puncture
- Male animals: All surviving animals
- Maternal animals: All surviving animals

GROSS NECROPSY
- complete necropsy performed on all rats
- light microscopic examinations of all tissues from the controls and the high dose group
- all organs and tissues from all rats were examined for macroscopic lesions

HISTOPATHOLOGY / ORGAN WEIGHTS
- adrenal glands, brain, kidneys, liver, epididymides and testes were weighed

-blood samples were taken and analysed for haematology and clinical chemistry parameters
Postmortem examinations (Offspring)

Statistics

- all data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS.
Reproductive indices

Offspring viability indices

Any other information on materials and methods incl. tables

- Dose-groups: 0, 1000, 5000, 20000 ppm

- measurements for haematology: red cell count, haemocrit, haemoglobin, mean cell volume, total and differential white cell count, platelet count

- measurements for biochemistry (on plasma from blood samples): urea, glucose, total protein, albumin, cholesterol, triglycerides, total bilirubin, creatine, sodium, potassium, chloride, calcium, phosphorus

- measurements of: activities of alkaline phosphatase, alanine amonitransferase, gamma-glutamyl transferase, creatinekinase, aspartate aminotransferase

Results and discussions

Effect levels

Endpoint	NOAEL
Generation	P
Sex	male/female
Effect level	1879 mg/kg bw/day
Basis for effect level / Remarks	for histopathological changes in reproductive organs

Observations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

no effects

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

not examined

Reproductive performance (parental animals)

not examined

Organ weights (parental animals)

yes

Gross pathology (parental animals)

yes

Histopathology (parental animals)

no effects

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
- No substance-related effect

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
- No substance-related effect

TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)
- No substance-related effect

ORGAN WEIGHTS (PARENTAL ANIMALS)
- 10% increase in liver weight in F and M animals in high dose

GROSS PATHOLOGY (PARENTAL ANIMALS)
- Slight increase in cysts in the pituitary of both sexes in the high dose groups
- 3/12 males in the high dose group slight (n=2) or moderate (n=1) plasma cell infiltration in the cervical lymph node (Not observed in treated
females or in any control animal)

HISTOPATHOLOGY (PARENTAL ANIMALS)
- No histological changes in liver

OTHER FINDINGS (PARENTAL ANIMALS)
- urine analysis: significant increase in volume and decrease in specific gravity for females at the highest dose
- haematology: no statistically significant findings
- clinical chemistry: · Males: increase in triglycerides (P<0.01) and decrease in alkaline phosphatase (P<0.05)
· Females: decrease in alkaline phosphatase at 5000 and 20000 ppm (P<0.01), cholesterol and aspartate aminotransferase levels were increased
· alkaline phosphatase activity was decreased in both sexes at highest dose (more prominent in females); similar but lesser decrease was seen in females at 5000 ppm

- no other findings are reported

Observations: offspring

Viability (offspring)

Clinical signs (offspring)

Body weight (offspring)

Sexual maturation (offspring)

Organ weights (offspring)

Gross pathology (offspring)

Histopathology (offspring)

Details on results (offspring)

Remarks on results including tables and figures

- the reduced alkaline phosphatse activity in both sexes may be an indication of a minimaleffect on nutrition

- possible effect on the liver (perturbations in lipid levels, elevated plasma aspartate and alanine aminotransferase levels, small increase in weight)

- no histopathologic changes

- no significant systematiceffects of antimony trioxide

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Conclusions

Executive summary

Cross-reference to other study

k_Schroeder (MPI)_2003

UUID	IUC5-48be25f9-b1fb-4cab-b3c0-38b65bb1aff6
Dossier UUID	0
Author	ebrc02 / EBRC Consulting GmbH / Hannover / Germany
Date	2008-06-27 16:58:58 CEST
Remarks	

Administrative Data

[]

Data waiving			
Justification for data waiving			
Study result type	experimental result	Study period	begin: 2002-12-20; end: 2003-03-30
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Comparable to guideline study (OECD guideline 414) with minor deviations: the analytically verified exposure levels were close in this study, and may therefore not fully qualify as three different dose groups.		

Data source

Reference

Reference type	study report		
Author	Schroeder R.E.	Year	2003
Title	An inhalation developmental toxicity study in rats with antimony trioxide.		
Bibliographic source			
Testing laboratory	MPI research, Inc. 54943 North Main Street, Mattawan, Michigan	Report no.	952-002
Owner company			
Company study no.		Report date	2003-11-17

Data access

data submitter is data owner

Data protection claimed

yes

Cross-reference to same study

-chapter 7.5.3 Repeated dose toxicity: inhalation

Materials and methods

Limit test

Test guideline

Qualifier	according to
Guideline	OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Deviations	

Principles of method if other than guideline

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	1309-64-4
Identifier	EC number
Identity	215-175-0
Identifier	IUPAC name
Identity	dioxodistiboxane

Details on test material

- Name of test material (as cited in study report): antimony trioxide

- Substance type: pure active substance
- Physical state: white powder
- Analytical purity: 99.8%
- Impurities (identity and concentrations):
Pb: 596ppm
As: 356ppm
Fe: 13ppm
- Expiration date of the lot/batch: 16598
- Stability under test conditions: stable for study duration
- Storage condition of test material: room temperature
- MMAD: 1.59 µm to 1.82 µm
- Method for particle size measurement: TSI Aerodynamic Particle Sizer

- no other details on test material are stated

Confidential details on test material

Test animals

Species

rat

Strain

Sprague-Dawley

Details on test animals and environmental conditions

TEST ANIMALS
- Source: male and female Sprangue-Dawley rats received from Charles River Laboratories, Portage, Michigan
- Age at study initiation: approximately nine weeks at arrivel and ten weeks at study initiation
- Weight at study initiation: 193 to 270 g
- Housing: Throughout the study, all rats were kept in an environmentally controlled room. From acclimation until euthanasia, the rats were individually housed in suspended, sainless steel, wire-mesh type cages, except during mating when the females were housed in similar cages with
males (1:1).
- Diet (e.g. ad libitum): ad libitum during non-exposure periods
- Water (e.g. ad libitum): ad libitum, using an automatic watering system
- Acclimation period: 1 week
-other: Only female rats with positive evidence of mating were selected for study and weighted an day 0 of gestation prior to test article exposure.
104 mated female rats were assigned to the treated or control groups.

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 18.3 - 21.1 °C
- Humidity (%): 35 - 70% (in exposure chamber: 2 to 14%)
- Photoperiod (hrs dark / hrs light): fluorescent lighting was provided for approximately 12 hours per days.

- no other details on test animals and environmental conditions are stated

Administration / exposure

Route of administration

other: dust aerosolatmosphere

Type of inhalation exposure (if applicable)

nose only

Vehicle

Details on exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: The exposure were conducted in a 63 L stainless steel and acrylic nose-only exposure chamber with a stainless steel baffle.
The treated animals were exposed to the article approximately six hours per day, from day 0 to day 19 of gestation at concentrations of 2.6, 4.4 and
6.3 mg/M3.

- Method of holding animals in test chamber: For the exposure, each animal was removed from the home cage and placed in a nose-only restraint
tube. The conicol end of the tube was inserted into the chamber prior to generation of test atmosphere. Food and water were not available to the
animals during the exposure period.

- Source and rate of air:
- Method of conditioning air:

- System of generating particulates/aerosols: Dust aerosol atmosphere of the test article were generated using a Wright Dust feeder (WDF) as the
primary device in the genaeration system.

- Temperature, humidity, pressure in air chamber: The chamber was maintained to the maximum extent possible at a mean temperature between
18 to 24°C and a relative humidity between 3 to 7%.

- Air flow rate: a chamber airflow of at least 0.6 L per minute per animal supplied by the generation system and an oxygen level at or above 19%.

- Air change rate: at least 10 chamber air changes per hour

- Method of particle size determination: One particle size distribution was performed at least once during each exposure using the TSI Aerodynamic
Particle Sizer (PS), to determine the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of any aerosol present.

- no other details are reported

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Nominal Concentration:
- The amount of test article delivered by the generation system during the exposure was divided by the total volume of air passing through the chamber to give the nominal concentartion.

Analytical Concentration:
- Chamber atmosphere samples dor determination of the test article exposure level were collected. The samples were withdrawn from the exposure chamber through metricel membrane filters mounted on an open-faced filter holder. The gravimetric concentrations were calculated with the use of atomic absorption spectroscopy.

Details on mating procedure

- Impregnation procedure: cohoused
- If cohoused:
- M/F ratio per cage: 1:1
- no other details are stated

Duration of treatment / exposure

Animals were treated from fertilization (day 0 of gestation) to day 19.

Frequency of treatment

6 hours per day

Duration of test

20 days

Doses / concentrations

2.6 mg/M*3

Basis

4.4 mg/M*3

Basis

6.3 mg/M*3

Basis

No. of animals per sex per dose

Control animals

yes, concurrent no treatment

Further details on study design

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: All rats were observed twice each day, seven days a week, for morbidity, mortality, signs of injury and availability of food and water.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: Daily from days 0 through 20 gestation, each rat was removed from the cage and given a detailed clinical examination. During the
treatment period, these examinations were conducted as animals were removed from the exposure chambers. The first group of animals examined
was randomized each day of the exposure period.

BODY WEIGHT: Yes
- Time schedule for examinations: Individual body weight were recorded on day 0, 3, 6, 9, 12, 15, 18 and 20 of gestation. Individual body weight
change was calculated for the following gestation day intervals: 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20 and 0-20. Adjusted body weight (day 20
gestation body weight minus the gravid uterine weight) and adjusted body weight change (day 0-20 of gestation) were also calculated. Body weights
recorded at arrival are not reported, but are maintained in the study file.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes
- Food consumption was recorded on the corresponing body weight days and calculated for the following intervals: 0-3, 3-6, 6-9, 9-12, 12-15,
15-18, 18-20 and 0-20 of gestation.

POST-MORTEM EXAMINATIONS: Yes (Clinical Pathology)
- Blood samples (5 ml/sample) were collected via cardiac puncture, after carbon dioxide inhalation, from 10 randomly selected pregnant animals per group on day 20 of gestation. The samples were collected into tubes containing EDTA anticoagulant and separated into red blood cells (RBC) and
plasma components. The RBC component was refrigerated at 2-8°C and shipped for analysis of concentrations of bound antimony. The plasma
samples were stored frozen (-20°C) until it was determined that these analyses were not required.

- Organs examined: A complete necropsy was performed on all dams under procedures approved by a veterinary pathologist. Special emphasis was
placed on structural abnormalities or pathologic changes that may have influenced the pregnancy. The lungs, nasopharyngeal tissue and gross
lesions from the dams were saved in 10% neutral buffered formalin. After weighting, the lungs were infused via the trachea with formalin. The carcasses were then discarded.
The lung and brain were weighted. The brain weights were used to calculate lung/brain weight ratios, but the brains were not saved.

- no other details on examinations are reported

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes
- Other:
Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. If no foci were seen, the female was considered not pregnant and all data was excluded from statistical analysis.

Fetal examinations

Each implant was characterized as either a viable or nonviable fetus, or either an early or late resorption. Viable fetuses responded to touch while
nonviable fetuses did not and showed no sign of autolysis. Early resorptions were characterized as implantation sites consisting of tissues but no
recognizable fetal characteristics, while late resorptions displayed recognizable fetal characteristics, but undergoimg the process of autolysis.

Statistics

Group Pair-wise Comparisons
Fisher's Exact Test
Arcsin-Square-Root Transformation
Descriptive Statistics
Covariate Analysis

Indices

Historical control data

Any other information on materials and methods incl. tables

Table 1: 104 female rats were assigned to the treated or control groups

group assignment			
group number	target exposure level (mg/M^3)	actual analytical exposure level (mg/M^3)	number of mated female rats
1	0	0	26
2	1.5	2.6	26
3	3.0	4.4	26
4	6.0	6.3	26

[a] represents mean of daily analytical exposure levels over the entire study

[b] the mean analytical exposure levels are used in the presentation of all summary tables and appended individual data within the report

Each female rat was assigned an animal number and implanted with a microchip bearing a unique identification number. For the exposure, each animal was removed from the home cage and placed in a nose-only restraint tube. The conicol end of the tube was inserted into the chamber prior to generation of test atmosphere. Food and water were not available to the animals during the exposure period.

Results and discussions

Effect levels

Endpoint LOAEC

Effect type	maternal toxicity
Effect level	2.6 mg/m³ air
Basis for effect level / Remarks	This LOAEL was based on an increase in lung weights both absolute and relative to brain weights at all exposure levels evaluated.
Endpoint	NOAEC
Effect type	developmental toxicity
Effect level	6.3 mg/m³ air
Basis for effect level / Remarks	

Maternal toxic effects

yes

Details on maternal toxic effects

- All animals survived to scheduled euthanasia on day 20 of gestation.
- No effect of treatment with antimony trioxide at an exposure level up to and inclusive of 6.3 mg/M*3 was evident from the detailed clinical examinations and from gestation body weight or body weight gains.
- No adverse effect of treatment with antimony trioxide at exposure level up to and inclusive 6.3 mg/M*3 was evident from gestation food consumption data.
- The red blood cell antimony levels were statistically higher than controls in each of the treated groups. The response was not clearly dose-
responsive but the highest levels of antimony (5.591 µg/g vs 0.128 in controls) were seen in the highest exposure group (6.3 mg/M*3). This

indicates that there is systemic exposure of the test article, and therefore is likely that the fetuses are being exposed.
- No effect of treatment with antimony trioxide at an exposure level up to and inclusive of 6.3 mg/M*3 was evident from maternal macroscopic
findings.
- A dose- related increase in lung weights, absolute and relative to brain weights, was seen in the antimony trioxide-treated groups. These differences in lung weights from controls were statistically significant and considered indicative of a treatment-related response. Absolute
lung weights were 24.2%, 31.1% and 38.6% heavier than control in the 2.6, 4.4 and 6.3 mg/M*3 groups. Lung weights relative to brain weights were 20.2%, 26.3% and 34.8% heavier.
- Test article-related miroscopic findings were observed in the lungs of all animals evalueted at all exposure levels. The primary test article-related
microscopic change was a diffuse accumulation of pigmented alveolar macrophages which likely reflected phagocytosis and accumulation on the test article particulate matter. These types of findings are common with exposure to particulate matter.
- Pregnancy rates were compareable between the control and antimony trioxide-treated groups. No effects of treatment with antimony trioxide at an exposure level up to and inclusive of 6.3 mg/M*3 was evident from uterine implantation data. Gravid uterine weights, adjusted GD 20 body weights,
and adjusted body weight change GD 0-20 for zhe treated groups were compareable to controls. No effect of treatment was evident from these data.

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

- No effect of treatment with antimony trioxide at an exposure level up to and inclusive of 6.3 mg/M*3 was evident from fetal body weights. Mean fetal body weights distinguished by sex and for both sexes combined in the treated groups were comparable to controls.
- Mean fetal sex ratios (% male fetuses per litter) in the treated groups ranged from 48.0 to 49.8 and were compareable to controls (48.9).
- No effect of treatment with antimony trioxide at an exposure level up to and inclusive of 6.3 mg/M*3 was evident from fetal crown-rump distance.
- No malformations or developmental variations were seen in control or treated fetuses during the external examinations.
- Anophthalmia (absence of the eye) was seen in a single fetus in the 6.3 mg/M*3 group. While this malformation has not been seen in recent
historical control data for this laboratory, its low incidence in occurrence in this study was considered spontaneous and unrelated to treatment. No
other visceral malformations or developmental variations were seen among these fetuses.
- No skeletal malformations were seen among the control and treated fetuses.

Remarks on results including tables and figures

Table 2: The gravimetric chamber mean exposure levels

chamber atmosphere monitoring			
group	target concentration (mg/m³)	mean chamber concentration (mg/m³) ± SD	mean nominal concentration (mg/m³) ± SD
1	0	0 ± NA	NA
2	1.5	2.6 ± 2.43	54.3 ± 40.08
3	3.0	4.4 ± 3.88	40.1 ± 25.15
4	6.0	6.3 ± 4.18	48.2 ± 21.85
SD : Standard Deviation NA : Not Applicable			

Table 3: Temperature, relative humidity and chamber airflow were monitored continuously and recorded approx. every 30 minutes during exposure. Chamber airflow data recorded in the study data are summarized below:

chamber environment conditions

group	temperature (°C)		relative humidity (%)		chamber airflow (L/min)	
	mean	SD	mean	SD	mean	SD
1	21	1.3	7	2.1	34.0	0.00
2	22	1.3	3	0.5	25.1	2.78
3	22	1.5	5	1.4	39.4	2.71
4	22	1.6	4	1.5	45.5	3.89
SD : Standard Deviation						

Table 4: Particle size distribution measurement

particle size distribution data		
group	mean MMAD (µM) ± SD	mean GSD ± SD
1	NA	NA
2	1.74 ± 0.405	1.744 ± 0.3189
3	1.82 ± 0.582	1.713 ± 0.3780
4	1.59 ± 0.151	1.714 ± 0.2363
MMAD : mass median aerodynamic diameter GSD : geometric standard deviation SD : standard deviation NA : not applicable		

Table 5: Maternal microscopic observations; test article-related microscopic findings are summarized in the table below

Test article-related microscopic findings Terminal Females					
Exposure level: mg/M³		0	2.6	4.4	6.3
Number examined		10	10	10	10
Lung		(10)	(10)	(10)	(10)
Hyperplasia, type II cell,	minimal	0	5	4	5
	mild	0	2	4	3
	moderate	0	2	0	2
		0	1	0	0
Inflammation, acute	minimal	0	7	4	6
	mild	0	4	4	4
		0	3	0	2
Macrophages, pigmented alveolar,	minimal	0	10	10	10
	mild	0	2	1	0
	moderate	0	5	9	3
		0	3	0	7

Overall remarks, attachments

Overall remarks

Attached background material

Attached document

Remarks

Attached full study report

Applicant's summary and conclusion

Conclusions

Executive summary

Cross-reference to other study