Committee for Risk Assessment

RAC

Annex 4

Opinion

on gallium arsenide in relation to toxicity to reproduction

EC Number: 215-114-8
CAS Number:1303-00-0

ECHA/RAC/A77-O-0000001412-86-11/A4

Adopted

23 July 2013

Additional Information Report for a Substance
Under Harmonised Classification & Labelling Process

Endpoint: Fertility

Gallium arsenide

EC Number: 215-114-8

CAS Number: 1303-00-0

Authors:

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Contents

1. Identity of the Substance

Substance name: Gallium arsenide

EC Number: 215-114-8

CAS Number: 1303-00-0

2. Toxicity for Reproduction

2.1 Animal Data

Animal Data Study No 1

Animal Data Study No:	1
Study Reference:	Colomina MT, Llobet JM, Sirvent JJ, Domingo JL, Corbella J. Evaluation of the reproductive toxicity of gallium nitrate in mice. Food Chem. Toxicol 31, 1993, 847-851.
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	1993
Reliability:	2 , reliable with restriction

Rationale for Reliability incl. Deficiencies:

- Probably no GLP;
- some details like stability of test solutions, administered volume not specified;
- some parameters like food consumption, body weight not or only selectively reported;
- overall deficiencies not relevant to conclusion

Relevance of the Study:

High subcutaneous doses of gallium nitrate to male mice (up to 96 mg/kg bodyweight per day for 14 days) produced no indications of an impairment of fertility.

The study is relevant because it demonstrates that even parenterally administered gallium (as soluble nitrate) at high dose levels does not affect sperm counts and motility as well as reproductive performance in male mice. Therefore, it is highly unlikely that the small amounts of gallium bioavailable from the poorly soluble gallium arsenide plays a role in the “fertility” effects reported in mice of the subchronic inhalation study of the NTP (NTP 2000). The study is reliable and adequate for the specific aspects of an inherent hazard of gallium ions on male reproduction.

Detailed Study Summary and Results:

Animal Data Study No 1

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	Gallium nitrate
EC number:	236-815-5
CAS number:	13494-90-1; 69365-72-6 (Hydrate)
Degree of purity:	99.9%
Impurities:	N.A.
Batch number:	N.A.

Animal Data Study No 1

Test Animals	
Species/strain/sex:	Swiss mice, male
Number of animals per sex per dose:	▪ 8 males for mating; ▪ 12 males for pathology assessment and examination of spermatozoa; ▪ 16 females for mating
Age and weight of animals at the study initiation:	8 weeks; 33-34 g
Administration/Exposure	
Route of administration	subcutaneous
Doses/concentration levels, rationale for dose level selection:	0 (controls), 24, 48 and 96 mg/kg/day every other day for 14 days before mating
Duration of study and duration	about 28 days;
Frequency of exposure:	once daily every other day for 14 days
Control group and treatment	8/12 males, 16 females
Post exposure observation period:	up to 14 days
Vehicle:	identification, concentration and volume used, justification of choice of vehicle (if other than water): 0.9% saline; 0.15 ml per 30g mouse
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	solution, N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	0, 24, 48, 96 mg/kg bw
If other route of administration than the oral route is chosen provide a justification:	The route was justified with the route used in human therapy.

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	M/F ratios per cage: 1:2; 4 days; copulatory plug;
Premating exposure period for males and females (P and F1):	14 days for males; none for females
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	females: • no. of pregnant mice • implantations • live fetuses per litter • early resorptions • late resorptions • dead fetuses per litter males: • body weight • absolute and relative testis weight • absolute and relative epididymis weight • spermatid and spermatozoal count • sperm motility • sperm morphology • tubular diameter • histopathology of testis and epididymis
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	Clinical observations performed but frequency not specified; uterus, testis and epididymis
Statistical methods used:	• Fisher exact test for pregnancy rates; • Duncan's multiple range test for sperm counts, body and organ weights; • Wilcoxon scores and Kruskal-Wallis test for sperm motility and morphology

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

Fertility and reproductive performance in the gallium nitrate-treated groups did not differ significantly from controls. No significant changes were observed in the relative testes and epididymides weights. Sperm counts and the percentage of motile cells were similar between treated and untreated animals. Histopathological examination of the testes and epididymides did not reveal any changes at any dose of gallium nitrate. The no-observed-adverse effect level was 96 mg/kg body weight.

Parameters to report (as appropriate, depending on test type)

a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	see above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	no effects on body weight, body weight gain, clinical signs; food consumption not measured
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	females: No. of pregnant mice, implantations, live fetuses per litter, early resorptions, late resorptions and dead fetuses per litter not significantly affected; males: Body weight, absolute and relative testis weight, relative epididymis weight, spermatid and spermatozoal count, sperm motility, sperm morphology, tubular diameter, histopathology of testis and epididymis, absolute epididymis weight partly significantly but not dose-dependently lower at 48 and 96 mg/kg due to lower body weight.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

The bioavailability and assumed accumulation of gallium ions after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.

Animal Data Study No 2

Animal Data Study No:	2
Study Reference:	Gosney JR (1984). Effects of hypobaric hypoxia on the Leydig cell population of the testis of the rat. J. Endocrinol. 103: 59-62
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	1984
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GLP;
- some parameters not reported;
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that exposure of rats under hypobaric hypoxia affects testicular weight and volume. By histopathology degeneration and sloughing of spermatogenic cells was observed in occasional tubules. The volume of the testis occupied by Leydig cells was reduced.

Detailed Study Summary and Results:

Animal Data Study No 2

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	N.A. (exposure to air)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Animal Data Study No 2

Test Animals	
Species/strain/sex:	rat / Wistar / male
Number of animals per sex per dose:	10 males per group
Age and weight of animals at the study initiation:	specified as „young adult“
Administration/Exposure	
Route of administration	Exposure to air in a hypobaric chamber
Doses/concentration levels, rationale for dose level selection:	380 mm Hg (corresponding to 5500 m altitude)
Duration of study and duration	The reduced pressure was gradually achieved over a period of 1 week and thereafter maintained for 28 days.
Frequency of exposure:	N.A.
Control group and treatment	760 mm Hg
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Animal Data Study No 2

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- body weight, testicular weights, testicular volume, - Leydig cell volume, seminiferous tubule volume, - histopathology of testes, lungs and heart.
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	
Statistical methods used:	Student's t-test

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

The study demonstrates that experimental exposure to severe continuous hypobaric hypoxia (380 mmHg for 28 days) has deleterious effects on areas of seminiferous epithelium of the testis of male rats, and also induces a decrease in testicular size and an absolute decrease in their Leydig cell complement.

Parameters to report (as appropriate, depending on test type)

a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	- body weight, testicular weights, testicular volume, - Leydig cell volume, seminiferous tubule volume, - histopathology of testes, lungs and heart.
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	The mean weight of both groups at the commencement of the experiment was 220g and the final weight of the control and hypoxic group were 341g and 234g respectively.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	After 28 days at 380 mmHg testes were of smaller weight (and volume) in comparison with matched controls (1.220 vs. 1.553 g; $P < 0.005$). Histological examination revealed degeneration and sloughing of spermatogenic cells in occasional tubules. Quantitative methods also revealed a marked difference in the volume of the testis occupied by Leydig cells. These were of a significantly smaller total volume in the hypoxic animals (0.046 vs. 0.083 ml; $P < 0.001$) and this could not be accounted for merely by the smaller overall testicular volume.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

This study in rats, points to the association between hypoxaemic conditions produced by hypobaric hypoxia and testicular effects.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anemia induced by lung toxicity. This study is further supplementary information supporting this opinion.

Animal Data Study No 3

Animal Data Study No:	3
Study Reference:	Gasco M, Rubio J, Chung A, Villegas L, Gonzales GF (2003). Effect of high altitude exposure on spermatogenesis and epididymal sperm count in male rats. Andrologia 35: 368-374
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	2003
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- probably no GLP;
- some parameters, body weight not or only selectively reported;
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that exposure of rats under hypobaric conditions at high altitude affects testicular, epididymal and seminal vesicle weights. Spermatogenesis and epididymal sperm counts are also altered.

Detailed Study Summary and Results:

Animal Data Study No 3

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	N.A. (exposure to air)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Animal Data Study No 3

Test Animals	
Species/strain/sex:	rat / Holtzman / male
Number of animals per sex per dose:	6 males per group
Age and weight of animals at the study initiation:	3 month
Administration/Exposure	
Route of administration	Exposure to ambient air at 4340 m altitude in Cerro de Pasco (Peru)
Doses/concentration levels, rationale for dose level selection:	N.A.
Duration of study and duration	3, 7, 14, 21, 28, 35 and 42 days
Frequency of exposure:	continuous exposure
Control group and treatment	exposure to ambient air at sea level
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Animal Data Study No 3

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	weight of testes, epididymis and seminal vesicles length of stage of seminiferous cycles homogenization-resistant epididymal sperm from nonperfused rats were enumerated
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	Testes and epididymis and seminal vesicles were trimmed free of fat and fascia and weighed. Seminiferous tubules were prepared for transillumination assessment. The length of stage of rat seminiferous cycles was assessed by transillumination under an inverted stereomicroscope at 40•magnification. For each rat, a total length of 1000 mm was assessed. The stages assessed were as follows: I, I–III, IV–V, VI, VII, VIII, IX–XI, XII, XIII–XIV. Mean length of each seminiferous stage(s) from control group (exposed at sea level) was considered as 1 and the length of stages in each high altitude exposed rat was assessed relatively to the control group. Epididymal sperm count Homogenization-resistant epididymal sperm from nonperfused rats were enumerated. Homogenization was performed in 5 ml saline (NaCl 0.9%). Modifications included refrigeration at 4 C for 24 h of homogenized epididymal preparation to allow the release of sperm from the walls.
Statistical methods used:	Student's t-test

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
Exposure of male rats to an altitude of 4340 m for 3, 7, 14, 21, 28, 35 and 42 days resulted in a modification of the pattern of the seminiferous tubule stages.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	At day 3, stages I, IV-V, VI, VII and IX-XI were relatively shorter at high altitude than at sea level. At day 7, stages VIII, IX-XI, XII and XIII-XIV were reduced. At day 14, stages VII, VIII and IX-XI were reduced. At day 21 and 28, stages VIII, XII and XIII-XIV were significantly increased at high altitude. At day 35 an increase in stage XIII-XIV was observed. At day 42, stages II-III, IX-XI and XII were significantly increased at high altitude. Epididymal sperm count was significantly reduced at day 7 of exposure to high altitude and maintained low levels with respect to sea level up to 42 days.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
In conclusion, high altitude exposure affects spermatogenesis, particularly onset of mitosis and spermiation. This in turn affects epididymal sperm count. Thus, this study in rats again points to an association between hypoxaemic conditions produced by hypobaric hypoxia and testicular effects. The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.	

Animal Data Study No:	4
Study Reference:	Farias JG, Bustos-Obregón E, Orellana R, Bucarey JL, Quiroz E, Reyes JG (2005). Effects of chronic hypobaric hypoxia on testis histology and round spermatid oxidative metabolism. Andrologia. 37: 47-52.
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	2005
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- probably no GLP
- some experimental details, such as description of how hypobaric conditions have been achieved, missing;
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that chronic or intermittent exposure of rats under hypobaric conditions simulating high altitude affects testicular weight and histopathology and causes metabolic changes in round spermatids.

In order to evaluate the effects of the exposure to continuous chronic hypobaric hypoxia (CCHH) and intermittent chronic hypobaric hypoxia (ICHH) on testis histology and on oxidative metabolism of spermatogenic cells (SC), male rats were exposed to a 4600-m simulated altitude (PO2: 89.6 mmHg).

Detailed Study Summary and Results:

Animal Data Study No 4

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	N.A. (exposure to hypobaric air)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Animal Data Study No 4

Test Animals	
Species/strain/sex:	rats / Wistar / male
Number of animals per sex per dose:	6 males per group
Age and weight of animals at the study initiation:	age not specified, 342 ± 14 g
Administration/Exposure	
Route of administration	exposure to hypobaric / normobaric air in a hypobaric chamber
Doses/concentration levels, rationale for dose level selection:	exposed to a 4600-m simulated altitude (PO_2: 89.6 mmHg).
Duration of study and duration	60 days
Frequency of exposure:	ICHH rats were exposed to hypobaric hypoxia for 96 h followed by a normobaric condition for 96 h. CCCH rats remained in hypobaric conditions, but were transferred to normobaric conditions every 96 h.
Control group and treatment	sea-level normobaric conditions, housed in conditions comparable with those of the hypoxic groups
Post exposure observation period:	See above
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Animal Data Study No 4

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	See above
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- testes weight - classification of spermatogenisis stages - cell morphology of round spermatids (RS) - functional state of RS mitochondria
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	The testes from each animal were weighed and one testis was fixed in Bouin fixative solution for 24 h. Haematoxylin and eosin stained testes were analyzed to classify the different stages of spermatogenesis. Representative areas of the samples were micro photographed and were measured and analysed using the Image Tool software v 3.0. Rat spermatogenic cells were prepared. Round spermatids (RS) were identified both by their size, as well as by the typical aspect of their nucleus. The integrity of the cell membrane (cell viability) was estimated by incubating the cell suspension with 4 µM ethidium bromide and examination under fluorescence microscopy. Shape changes of RS were determined. These changes are dependent on the energy state of the cell. RS were observed by transmission light microscopy. To evaluate the functional state of the mitochondria, the oxygen consumption of RS was monitored.
Statistical methods used:	- Software: GraphPad Prism v 2.01 (San Diego, CA, USA) - data: one-way anova followed by a Dunnett test - shape changes: a Kruskal– Wallis test (nonparametric)

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
The exposure of male rats to CCHH and ICHH induced evident changes in testicular morphology and loss of spermatogenic cells, in all stages of the spermatogenic cycle. This post-meiotic spermatogenic cell loss in the testis correlated well with metabolic changes in round spermatids that evidenced a strong metabolic stress in these cells.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	After 60 days, ICHH and CCHH groups presented a significant decrease in testicular mass, an increase in interstitial space, a decrease in height of the seminiferous epithelium, depletion of cellular elements, vacuolization in epithelial cells and folding of the basal membrane. Round spermatids from animals exposed to CCHH presented a significant decrease in energy-dependent cell shape changes. Round spermatid mitochondria of CCHH rats seem to be limited in their ability to handle reducing equivalents. These mitochondria also appear to be uncoupled under basal conditions. Round spermatids from CCHH rats evidence large oxygen consumption (QO2) insensitive to inhibition by cyanide, a process that could be partly related to lipoperoxidation.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.	

Animal Data Study No 5

Animal Data Study No:	5
Study Reference:	Farias JG, Bustos-Obregón E, Tapia PJ, Gutierrez E, Zepeda A, Juantok C, Cruz G, Soto G, Benites J, Reyes JG (2008). Time course of endocrine changes in the hypophysis-gonad axis induced by hypobaric hypoxia in male rats. J. Reprod. Dev. 54 18-21.
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	2008
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- probably no GLP
- some experimental details, such as description of how hypobaric conditions have been achieved, missing
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that exposure of rats under hypobaric conditions (chronic hypobaric hypoxia, CHH) simulating high altitude leads to hormonal changes of the in the pituitary-gonadal axis that may partly explain the effects on spermatogenesis.

Detailed Study Summary and Results:

Animal Data Study No 5

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	N.A. (exposure to air)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Animal Data Study No 5

Test Animals	
Species/strain/sex:	rats / Wistar / male
Number of animals per sex per dose:	Chronic hypobaric exposure (CHH): 32 males Normobbaric control (Nx): 32 males
Age and weight of animals at the study initiation:	age 10 weeks, 251 ± 9 g
Administration/Exposure	
Route of administration	exposure to hypobaric / normobaric conditions in a hypobaric chamber
Doses/concentration levels, rationale for dose level selection:	Hypobaric conditions: 428 torr ; PO2 89.6 mmHg simulating an altitude of 4,600 m
Duration of study and duration	30 days
Frequency of exposure:	chronic exposure
Control group and treatment	normobaric conditions
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Animal Data Study No 5

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- Follicle stimulating hormone (FSH) - Luteinizinng hormone (LH) - Testosterone - Body mass
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	On days 0, 5, 15 and 30 post-exposure, 12 rats were anaesthetized, their body weights were measured and blood samples were collected
Statistical methods used:	The results were analyzed using a 2-way analysis of variance (ANOVA) test followed by Bonferroni analysis. The level of statistical significance was set at $P < 0.05$ for all tests. The data were analyzed using the GraphPad Prism software version 4.0 (GraphPad Software, San Diego, California).

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

The study aims to explain the effects of CHH on the fertility of male rats which are summarized as follows: Exposure of male rats to intermittent (IHH) and chronic hypobaric hypoxia (CHH) produce an increase in haematocrit, decrease in testicular mass, deterioration of interstitial cells, increase of the interstitial space, damage to the germinal epithelium, increase of seminiferous tubule lumen, a strong metabolic stress and loss of spermatogenic cells. The study shows that the effects on CHH on spermatogenesis may be partially related to changes in the hypophysis-gonad hormonal axis, as FSH rises initially due to spermatogenic damage and inhibin B altered feedback but recovers afterwards, being relatively low (as LH is) at longer periods, as found also for testosterone.

Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	Food intake of Nx adjusted to CHH: 15g/rat/day CHH body mass (201 ± 2g) reduced compared to Nx (299 ± 7g) over the entire exposure period
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	At day 5 post exposure, the FSH plasma level of the CHH was increased significantly compared to the Nx group. The FSH of the CHH group decreased thereafter but had a tendency to stay above Nx levels. LH plasma levels decreased over time for CHH. Testosterone plasma level showed a tendency to decrease for CHH.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

This study provides further evidence that hypoxia has significant effects on testis and epididymis.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.

Animal Data Study No:	6
Study Reference:	Farias JG, Puebla M, Acevedo A, Tapia PJ, Gutierrez E, Zepeda A, Calaf G, Juantok C, Reyes JG (2010). Oxidative stress in rat testis and epididymis under intermittent hypobaric hypoxia: protective role of ascorbate supplementation. J. Androl. 31: 314-321.
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	2010
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- probably no GLP
- some experimental details, such as description of how hypobaric conditions have been achieved, missing; but overall for the purpose of
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that intermittent exposure of rats under hypobaric conditions simulating high altitude affects body and testicular weight, reduces epididymal sperm counts, induces lipid peroxidation and reduces glutathione reductase activity in testes and epididymis.

This study therefore provides further evidence that hypoxia has significant effects on testis and epididymis.

Detailed Study Summary and Results:

Animal Data Study No 6

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	N.A. (exposure to air) , Ascorbic acid, NaCl
EC number:	Ascorbic acid : 200-066-2 / NaCl: 231-598-3
CAS number:	Ascorbic acid: 50-81-7 / NaCl: 7647-14-5
Degree of purity:	Ascorbic acid: not disclosed / NaCl: not disclosed
Impurities:	Ascorbic acid: not disclosed/ NaCl: not disclosed
Batch number:	Ascorbic acid: not disclosed/ NaCl: not disclosed

Animal Data Study No 6

Test Animals	
Species/strain/sex:	rats / Wistar / male
Number of animals per sex per dose:	6 males per group / 6 groups in total
Age and weight of animals at the study initiation:	age 10 weeks, 225 ± 14 g
Administration/Exposure	
Route of administration	HH - Hypobaric hypoxia: hypobaric chamber Nx - Normobaric conditions: hypobaric chamber PS - Physiologic NaCl solution: i.p. AA- Ascorbic acid: i.p.
Doses/concentration levels, rationale for dose level selection:	HH: 428 torr; PO2 89.6 mm Hg Rationale: Simulating 4600 m altitude AA: 10 mg / kg bw Rationale: Dose caused significant inhibition of lipid peroxidation induced by $CdCl_2$
Duration of study and duration	32 days
Frequency of exposure:	IHH – intermittent HH: Exposure to HH for 96 h followed by normobaric conditions for 96 h for a total of 32 days. AA : injection every 96 h PS: injection every 96 h Group 3: Nx + AA Group 4: IHH Group 6: IHH + AA
Control group and treatment	Group 1: Nx Group 2: Nx + PS Group 5: IHH + PS
Post exposure observation period:	N.A.
Vehicle:	0.1. ml of physiologic NaCl solution (PS)
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	AA dose administered i.p. caused significant inhibition of lipid peroxidation induced by $CdCl_2$

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	See above
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- Body weight - Testis and epididymis weight - Caudal epididymis sperm count - Haematocrit - Thiobarbituric acid reactive substances (TBARS) - Activity of gluthathione reductase (GR) in testis and epididymis
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	The results were analyzed using a 2-way analysis of variance (ANOVA) test followed by Bonferroni analysis. Two-way ANOVA was performed to determine the presence of a significant interaction between injection (AA) and environment because this would indicate a different effect of AA under IHH in comparison to Nx. The Kruskal-Wallis test (nonparametric) was used to analyze hematocrit results. The level of statistical significance was set at $P < 0.05$ for all tests. The data were analyzed using the GraphPad Prism software version 4.0 (GraphPad Software, San Diego, California).

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

The results of this study revealed that IHH induced lipid peroxidation, a reduction in glutathione reductase activity in testis and epididymis, and a significant decrease in epididymal sperm count. Treatment with AA prevented these changes. In conclusion, AA was capable of decreasing oxidative stress in testis and epididymis under IHH. This protection by AA of the IHH-induced lipid peroxidation can be explained in part by the preservation of glutathione reductase activity in these organs.

Parameters to report (as appropriate, depending on test type)

a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	effects on body weight
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	See above
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

This study provides further evidence that hypoxia has significant effects on testis and epididymis.

Read-across to gallium arsenide: The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.

Animal Data Study No 7

Animal Data Study No:	7
Study Reference:	Hwang GS, Jian CY, Chen TJ, Chen ST, Wang SW (2009). Effects of Hypoxia on Testosterone Release in Rat Leydig cells. Am. J. Physiol. Endocrinol. Metab. 297: E1039-E1049.
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	2009
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GLP
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that exposure of rats under intermittent hypoxic conditions over 4 days leads to an increase in plasma testosterone. In addition in vitro studies with Leydig cells from hypoxic rats led to an increase of testosterone production from testosterone precursors.

Detailed Study Summary and Results:

Animal Data Study No 7

Materials and Methods	
Test type:	Male reproduction toxicity study; no specific guideline followed
Test Substance :	
Name	Artificial atmosphere: Oxygen + Nitrogen
EC number:	Oxygen: 231-956-9 / Nitrogen: 231-783-9
CAS number:	Oxygen: 7782-44-7 / Nitrogen: 7727-37-9
Degree of purity:	Not disclosed
Impurities:	Not disclosed
Batch number:	Not disclosed

Animal Data Study No 7

Test Animals	
Species/strain/sex:	rats / Sprague-Dawley / male
Number of animals per sex per dose:	4 males per group / two groups
Age and weight of animals at the study initiation:	age not specified, 300-350 g
Administration/Exposure	
Route of administration	Hypobaric chamber
Doses/concentration levels, rationale for dose level selection:	12% O_2 + 88% N_2
Duration of study and duration	4 days
Frequency of exposure:	8 h/day (09:00 –17:00)
Control group and treatment	normoxic
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Animal Data Study No 7

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	See above
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- Plasma testosterone concentration - Testosterone secretion/release in response to a) 8-Br-cAMP forskolin and hCG in vitro b) steroidogenic precursors in vitro. c) NAADP in vitro
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	In the in vivo experiment, hypoxic and normoxic rats were euthanized and the blood samples collected. In the in vitro experiment, the enzymatically dispersed rat Leydig cells were prepared and challenged with forskolin (an adenylyl cyclase activator), 8-Br-cAMP (a membrane-permeable analog of cAMP), hCG, the precursors of the biosynthesis testosterone, including 25-OH-C, pregnenolone, progesterone, 17-OH-progesterone, and androstendione, nifedipine (L-type calcium channel blocker), nimodipine (L-type calcium channel blocker), tetrandrine (L-type calcium channel blocker), and NAADP (calcium-signaling messenger causing release of calcium from intracellular stores). The concentrations of testosterone in plasma and medium were measured by radioimmunoassay.
Statistical methods used:	All data were given as means ± SD. In some cases, the means of treatment were tested for homogeneity by analysis of variance, and the difference between specific means was tested for significance by Duncan's multiple range test. In other cases, Student's t-test was employed. A difference between two means was considered statistically significant when $P < 0.05$.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	See above
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
These results suggested that intermittent hypoxia stimulates the secretion of testosterone at least in part via stimulatory actions on the activities of adenylyl cyclase, cAMP, L-type calcium channel, and steroidogenic enzymes.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	The level of plasma testosterone in hypoxic rats was higher than that in normoxic rats. Enhanced testosterone production was observed in rat Leydig cells treated with hCG, 8-Br-cAMP, or forskolin in both normoxic and hypoxic conditions. Intermittent hypoxia resulted in a further increase of testosterone production in response to the testosterone precursors. The activity of 17 - hydroxysteroid dehydrogenase was stimulated by the treatment of intermittent hypoxia in vitro. The intermittent hypoxia-induced higher production of testosterone was accompanied with the influx of calcium via L-type calcium channel and the increase of intracellular calcium via the mechanism of calcium mobilization.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
This study therefore provides further evidence that hypoxia has significant effects on testis and epididymis. The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.	

2.2 Human Data

Human Data Study No 1

Human Data Study No:	1
Study Reference:	Aaseboe U, Gyltnes A, Bremnes RM, Aakvaag A, Sloedal J (1993). Reversal of sexual impotence in male patients with chronic obstructive pulmonary disease and hypoxemia with long term oxygen therapy. J. Steriod Biochem. Molec. Biol. 46: 799-803
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	1993
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- apparently no GCP
- some experimental details missing
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows that erectile impotence in humans caused by respiratory failure and hypoxia in patients with chronic obstructive pulmonary disease (COPD) may be reversed by long term oxygen therapy (LTOT). This was accompanied by a significant increase in serum testosterone and a decrease of sex hormone binding globulin (SHGB). Although no direct measurements on testes or sperm were carried out, this study shows that hypoxemia has an adverse effect on male reproduction and the hormonal homeostasis. In addition, it was found that these adverse effects may be reversible under sufficient oxygen supply.

Detailed Study Summary and Results:

Human Data Study No 1

Materials and Methods	
Test type:	clinical study
Test Substance :	
Name	N.A. (long term oxygen therapy)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Human Data Study No 1

Test Individuals	
Sex:	Male
Number of individuals per sex per dose:	A total number of 19 patients with chronic obstructive pulmonary disease (COPD) were studied.
Age and weight at the study initiation:	Below 75 years to exclude men with an impaired gonadal endocrine function due to age. Weight not disclosed.
Administration/Exposure	
Route of administration	Long term oxygen therapy (LTOT)
Doses/concentration levels, rationale for dose level selection:	7 patients received oxygen therapy for 24 h 12 patients received oxygen therapy for 1 month
Duration of study and duration	1 Month
Frequency of exposure:	See above
Control group and treatment	See above
Post exposure observation period:	1 Month
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Human Data Study No 1

Description of Test Design	
Dosing schedules and pre and post dosing observation periods	See above
Parameters assessed	Sexual function and hormones
Clinical observations performed and frequency, organs examined	N.A.
Statistical methods used:	N.A.

Human Data Study No 1

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions , exposure data	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

In 5 patients under LTOT there was a reversal of functional impotence as evidenced by reappearance of morning erections. Pre- and posttreatment measurements showed a significant increase of pO2, serum testosterone and apparent free testosterone concentration and a significant decrease of sex hormone binding globulin. Serum testosterone increased from low normal or subnormal concentration to normal levels. 7 patients did not respond to LTOT, neither with regard to penile function nor with regard to hormone levels. In addition, the non-responders only showed a slight numerical, but not statistically significant increase of pO2. A 24h oxygen treatment did not lead to any positive effects on sexual function or hormone levels, although there was a clear increase of pO2. This shows that prolonged treatment of hypoxia is necessary to normalise sexual function and hormones.

Parameters to report (as appropriate, depending on test type)

Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	Five of the 12 patients receiving LTOT for one month experienced a reversal of their functional impotence. None of the patients receiving LTOT for 24 h experienced reversal of sexual impotence even though their pO2 levels increased significantly.
Reproductive parameters assessed and details of effects seen	Pre- and posttreatment measurements of pO2, serum testosterone and apparent free testosterone concentration.

Read-across to gallium arsenide:

This study shows that hypoxemia has an adverse effect on male reproduction and hormonal homeostasis, which can be reversed by sufficient oxygen supply.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity.

Human Data Study No 2

Human Data Study No:	2
Study Reference:	Donayre J, Guerra-García R, Moncloa F, Sobrevilla LA (1968). Endocrine studies at high altitude. IV. Changes in the semen of men. J. Reprod. Fert. 16: 55-58
Purpose Flag:	key study
Study Result Type:	experimental result
Study Period:	1967
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GCP
- some experimental details, missing
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study shows adverse effects on sperm in young man under hypoxic conditions at high altitude. Return to normal occurs with return to sea level.

Detailed Study Summary and Results:

Human Data Study No 2

Materials and Methods	
Test type:	Endocrine studies at high altitude
Test Substance :	
Name	N.A. (exposure to hypoxic, hypobaric conditions)
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Human Data Study No 2

Test Individuals	
Sex:	Male
Number of individuals per sex per dose:	A total number of 9 individuals
Age and weight at the study initiation:	18 to 22 years
Administration/Exposure	
Route of administration	Exposure to ambient conditions at Cerro Pasco, Peru (4300 m above sea level)
Doses/concentration levels, rationale for dose level selection:	N.A.
Duration of study and duration	5 (control) + 4 (exposure) weeks
Frequency of exposure:	Continuous exposure
Control group and treatment	total group staying initially 5 weeks at sea level
Post exposure observation period:	8th and 15th days after return to sea level
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Human Data Study No 2

Description of Test Design	
Dosing schedules and pre and post dosing observation periods	Samples were taken on the 7th, 14th and 27th days of the stay at Cerro de Pasco, and two final samples on the 8th and 15th days after return to sea level.
Parameters assessed	Sperm counts were made by haemocytometer and are reported as total number of cells. Motility is expressed as percentage of all cells showing movement. Percentage of live spermatozoa was obtained by staining with eosin-nigrosin ; haematoxylin and eosin were used for counting abnormal forms. Fructose and citric acid were determined in seminal plasma. The pH of fresh semen was also recorded.
Clinical observations performed and frequency, organs examined	Previous physical examinations did not reveal any abnormality in the reproductive and endocrine systems
Statistical methods used:	Not detailed

Human Data Study No 2

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions , exposure data	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

Mean sperm count: a statistically significant and continuous decrease throughout the 42 days of the experiment indicating damage early in the spermatogenic cycle. Sperm motility: a consistent decrease below the level observed at sea altitude. Return to sea level markedly improved motility. Abnormal sperm: a marked and sustained increase at high altitude that continued after return to sea level. Other parameters: seminal pH and fructose were raised, citric acid remained within normal limits.

Overall, most effects occurred rather early, already at the end of the first week.

Parameters to report (as appropriate, depending on test type)

Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen	See above

Read-across to gallium arsenide:

This study shows that hypoxemia has an adverse effect on sperm parameters, which partly reversed by return to sea level.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity.

Human Data Study No 3

Human Data Study No:	3
Study Reference:	Gosney JR (1987). Atrophy of Leydig cells in the testes of men with longstanding chronic bronchitis and emphysema. Thorax 42: 615-619
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	1987
Reliability:	1, reliable
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: This study shows atrophy of Leydig cells due to hypoxaemia in men with long lasting chronic bronchitis and emphysema.	

Detailed Study Summary and Results:

Human Data Study No 3

Materials and Methods	
Test type:	pathological study
Test Substance :	
Name	N.A.
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Human Data Study No 3

Test Individuals	
Sex:	Male
Number of individuals per sex per dose:	Total of 10 subjects + 15 control subjects Ten male subjects with chronic bronchitis and emphysema were selected from those coming to necropsy at the University Department of Pathology at the Royal Liverpool Hospital by application of the following criteria: (1) a clinical history of chronic bronchitis and emphysema lasting at least 15 years, including documentation of the presence of arterial hypoxaemia on successive occasions during this period; (2) morphological changes of chronic bronchitis and emphysema and their sequelae'in the lungs at necropsy and at the subsequent histological examination of bronchopulmonary tissue; (3) a right ventricular fresh weight after trimming of fat at necropsy of over 80 g. Equal care was taken in the selection of 15 control subjects, and the excluding features were strictly applied. No subject with a clinical history or evidence at necropsy of systemic hypertension or appreciable pulmonary disease was included.
Age and weight at the study initiation:	Mean age in both groups: 69

Administration/Exposure	
Route of administration	N.A.
Doses/concentration levels, rationale for dose level selection:	N.A.
Duration of study and duration	N.A.
Frequency of exposure:	N.A.
Control group and treatment	N.A.
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Human Data Study No 3

Description of Test Design	
Dosing schedules and pre and post dosing observation periods	N.A.
Parameters assessed	Atrophy of Leydig cells due to hypoxaemia: Pairs of lungs were fixed in 10% formol-saline, all those from the bronchitic subjects and 10 of the 15 pairs from the control subjects by intratracheal perfusion. Sections from representative blocks of tissue were stained with haematoxylin and eosin and by an elastic and Van Gieson method. Testes were removed at necropsy and weighed, and their dimensions were measured in three diameters with a pair of callipers. The testes were sliced into blocks of tissue 3-4 mm thick, fixed for 24 hours in Bouin's solution, and embedded in paraffin wax. Sections of 4 µm thickness were cut and stained with haematoxylin and eosin, and the proportion of each testis comprising Leydig cells was assessed by point counting. At a magnification of x 100 5000 points were counted. Three components were recognised: Leydig cells, seminiferous tubules, and interstitial tissues with blood vessels and non-Leydig interstitial cells. From the value for the percentage volume of Leydig cells in each testis and by reference to the overall testicular volume, the total volume of Leydig cells in millilitres was calculated for each organ.
Clinical observations performed and frequency, organs examined	N.A.
Statistical methods used:	Student's t test

Human Data Study No 3

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions , exposure data	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance	N.A.

Results:

The testes of 10 men with a history of chronic bronchitis and emphysema of at least 15 years duration were investigated at necropsy. All men had morphological evidence of the cardiopulmonary effects of hypoxia. These cases were compared to a group of 15 matched control subjects. The total volume of Leydig cells in the testes of men with chronic bronchitis and emphysema, was significantly less than the volume in matched controls (0.98 (SD 0.39) and 0.93 (0.42) ml v 1.66 (0.54) and 1.72 (0.52) ml for left and right testes respectively; $p < 0.005$). According to the authors, this atrophy may be a consequence of hypoxic inhibition of pituitary synthesis or release of luteinising hormone.

Parameters to report (as appropriate, depending on test type)

Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen	See above

Read-across to gallium arsenide:

This is another study in humans, which points to the association between hypoxaemic conditions produced by lung diseases and testicular effects.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC. In the applicant's opinion, the effects observed in animal studies are secondary effects of the hypoxaemia/anaemia induced by lung toxicity. This study is further supplementary information supporting this opinion.

2.3 Other Relevant Data

2.3.1 Toxicokinetics

Toxicokinetic Study No 1

Toxicokinetic Study No:	1
Study Reference:	Mast TJ, Greenspan BJ, Dill JA, Stoney KH, Evanoff JJ, Rommereim RL. Inhalation Developmental Toxicology Studies: Gallium Arsenide in Mice and Rats. Final Report. NTIS Technical Report (NTIS/DE91 005300), 1990. Pacific Northwest Laboratory, Richland, WA.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	8/1989
Reliability:	1, reliable without restriction

Rationale for Reliability incl. Deficiencies:

- GLP study conducted within US National Toxicology Program (NTP)

Relevance of the Study:

The report contains data from an experiment in which male mice were exposed to gallium arsenide for 6 hours/day, seven days/week on 12 consecutive days at concentrations of 1, 10 or 37 mg/m³. The lack of detectable gallium and arsenic in the testes suggest that neither gallium nor arsenic are contributing to the “fertility” effects reported in mice after subchronic inhalation exposure. In addition, this study argues against an accumulation potential of gallium in the testes of mice.

Detailed Study Summary and Results:

Toxicokinetic Study No 1

Materials and Methods	
Test type:	Gallium and arsenic concentrations in the testes of mice after repeated inhalation exposure; no specific guideline followed.
Test Substance :	
Name	Gallium arsenide
EC number:	215-114-8
CAS number:	1303-00-0
Degree of purity:	99±1%
Impurities:	Total Max 170 ppm by spark-source mass spectrometry
Batch number:	Lot M051988 , batch 06

Toxicokinetic Study No 1

Test Animals	
Species/strain/sex:	Mice / Swiss CD-1 / male
Number of animals per sex per dose:	3 male
Age and weight of animals at the study initiation:	14 weeks; weight not specified.
Administration/Exposure	
Route of administration	inhalation; whole-body exposure
Doses/concentration levels, rationale for dose level selection:	1, 10 and 37 mg/m^3 rationale not specified
Duration of study and duration	12 days
Frequency of exposure:	6 h/d on 12 consecutive days
Control group and treatment	0 mg/m^3
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	solution, aerosol, 1, 10, 37 mg/m3; stability proven
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	The route was justified with route used in human exposure.

Toxicokinetic Study No 1

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	6 h/d on 12 consecutive days, whole-body exposure
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	testicular concentrations of zinc, gallium and arsenic
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	N.A.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	6 h/d on 12 consecutive days, whole-body exposure
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	MMAD: 0.8 – 1.3 µm GSD: 2.0-2.1
Type or preparation of particles (for studies with aerosols)	preparation of particles was performed with a flexible-brush dust feed mechanism, a Troat Model GEM-T air jet mill, a cyclone separator, an aerosol charge neutralizer, and an aerosol distribution system.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

Remark: Limits of quantitation were 0.29 µg/g for gallium in mouse testes, 0.45 µg/g for arsenic mouse testes. Data reported are not corrected for endogenous amounts present in control animals. Arsenic determinations in testes at all concentrations showed low recoveries. Data are not corrected for low recoveries of these elements.

Parameters to report (as appropriate, depending on test type)

a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	Testicular concentrations of gallium were below the detection limit (0.10 µg/g) in all animals (except one of the 37 mg/m³ group which was probably contaminated). Testicular concentrations of arsenic were below the detection limit (0.15 µg/g) in all animals. Testicular concentrations of zinc were not affected in all groups.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

The bioavailability and assumed accumulation of gallium ions after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.

Toxicokinetic Study No 2

Toxicokin. Study No:	2
Study Reference:	Mast TJ, Greenspan BJ, Dill JA, Stoney KH, Evanoff JJ, Rommereim RL. Inhalation Developmental Toxicology Studies: Gallium Arsenide in Mice and Rats. Final Report. NTIS Technical Report (NTIS/DE91 005300), 1990. Pacific Northwest Laboratory, Richland, WA.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	8/1989
Reliability:	1, reliable without restriction

Rationale for Reliability incl. Deficiencies:

- GLP study conducted within US National Toxicology Program (NTP)

Relevance of the Study:

The report contains data from an experiment in which male rats were exposed to gallium arsenide for 6 hours/day, seven days/week on 12 consecutive days at concentrations of 10, 37 or 75 mg/m³. Gallium and arsenic concentrations in the testes increased concentration-dependently. This suggests that neither gallium nor arsenic are contributing to the “fertility” effects reported in rats and mice after subchronic inhalation exposure because the “fertility” effects in mice were even more severe than in rats although no concentration-dependent increase in either ion was determined in mice (see Toxicokinetic study 1 above).

Detailed Study Summary and Results:

Toxicokinetic Study No 2

Materials and Methods	
Test type:	Gallium and arsenic concentrations in the testes and whole blood of rats after repeated inhalation exposure; no specific guideline followed.
Test Substance :	
Name	Gallium arsenide
EC number:	215-114-8
CAS number:	1303-00-0
Degree of purity:	99±1%
Impurities:	Total Max 170 ppm by spark-source mass spectrometry
Batch number:	Lot M051988 , batch 06

Toxicokinetic Study No 2

Test Animals	
Species/strain/sex:	rat / Sprague Dawley / male
Number of animals per sex per dose:	3 male
Age and weight of animals at the study initiation:	16 weeks; weight not specified.
Administration/Exposure	
Route of administration	inhalation; whole-body exposure
Doses/concentration levels, rationale for dose level selection:	10, 37and 75 mg/m^3 Rationale: same concentrations as for developmental toxicity study
Duration of study and duration	12 days
Frequency of exposure:	6 h/d on 12 consecutive days
Control group and treatment	0 mg/m^3
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	solution, aerosol, 0, 10, 37and 75 mg/m^3 stability proven
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	The route was justified with route used in human exposure.

Toxicokinetic Study No 2

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	6 h/d on 12 consecutive days, whole-body exposure
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	testicular concentrations of zinc, gallium and arsenic, whole blood
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	N.A.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	6 h/d on 12 consecutive days, whole-body exposure
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	MMAD: 0.8 – 1.3 µm GSD: 2.0-2.1
Type or preparation of particles (for studies with aerosols)	preparation of particles was performed with a flexible-brush dust feed mechanism, a Troat Model GEM-T air jet mill, a cyclone separator, an aerosol charge neutralizer, and an aerosol distribution system.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
Remark: Limits of quantitation were 0.29 µg/g for gallium in rat, 0.45 µg/g for arsenic in rat and mouse testes, 0.15 µg/g for gallium in rat whole blood, 1.66 µg/g for arsenic in rat whole blood. Data reported are not corrected for endogenous amounts present in control animals. Gallium and arsenic determinations in whole blood with concentrations less than 1 µg/g, as well as arsenic determinations in testes at all concentrations showed low recoveries. Data are not corrected for low recoveries of these elements.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	Testicular concentrations of gallium were below the detection limit (0.10 µg/g) in control animals; mean levels in 10, 37 and 75 mg/m³-groups were 0.24, 1.02, and 1.70 µg/g, respectively. Testicular concentrations of arsenic were below the detection limit (0.15 µg/g) in control animals; mean levels in 10, 37 and 75 mg/m³-groups were 0.19, 0.56, and 0.82 µg/g, respectively. Testicular concentrations of zinc were not affected in all groups. Whole blood concentrations of gallium were below the detection limit (0.05 µg/g) in controls animals; mean levels in 10, 37 and 75 mg/m³-groups were 0.11, 0.63, and 1.12 µg/g, respectively. Whole blood concentrations of arsenic were 11.7 µg/g in controls animals; mean levels in 10, 37 and 75 mg/m³-groups were 31.2, 87.7, and 126.3 µg/g, respectively.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
The bioavailability and assumed accumulation of gallium ions in the testes after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.	

Toxicokinetic Study No 3

Toxicokin. Study No:	3
Study Reference:	Ando A, Ando I, Hiraki T, Hisada K, Nitta K, Ogawa H. Retention and subcellular distribution of 67Ga in normal organs. Nuklearmedizin 25, 1986, 45-49.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	1986
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GLP
- some details like stability of test solutions, not reported;
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study describes the organ and subcellular distribution, retention/accumulation and elimination of gallium ions after intravenous injection of 67gallium citrate into rats. In contrast to some other tissues there was no remarkable retention/accumulation of gallium in the testes. These results are supplementary evidence that gallium does not have a specific affinity to the testicular/germinal tissue.

Detailed Study Summary and Results:

Toxicokinetic Study No 3

Materials and Methods	
Test type:	- In vivo pharmacokinetic study; no specific guideline followed but adequate for the issue in question. - This study describes the organ and subcellular distribution, retention/accumulation and elimination of gallium ions after intravenous injection of ^{67}Gallium Citrate into rats. - Additional details: animals were non-fasted, 5 animals each were sacrificed 10, 60 min, 3, 24 h, 2, 3, 5, 7 and 10 days after treatment.
Test Substance :	
Name	^{67}Gallium Citrate
EC number:	unknown
CAS number:	27905-02-8
Degree of purity:	As specified by Daiichi Radioisotopes Labs. Ltd. Japan
Impurities:	As specified by Daiichi Radioisotopes Labs. Ltd. Japan
Batch number:	Not reported

Toxicokinetic Study No 3

Test Animals	
Species/strain/sex:	rat / Wistar / male
Number of animals per sex per dose:	number of animals per timepoint: 5
Age and weight of animals at the study initiation:	age: not specified , 153-263 g
Administration/Exposure	
Route of administration	injection into tail vein
Doses/concentration levels, rationale for dose level selection:	0.08M sodium citrate solution; dose(s) used: 0.4 ml, 10 – 100 µCi
Duration of study and duration	10 days
Frequency of exposure:	single injection
Control group and treatment	N.A.
Post exposure observation period:	N.A.
Vehicle:	0.08 M sodium citrate solution
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	Intravenous injection matches the requirements for radiochemical determination of retention kinetics

Toxicokinetic Study No 3

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	single injection
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	- Retention values of ^{67}Ga in organs of normal rats - Adsorption rates into red blood cells of ^{67}Ga - Cumulative urinary excretion rates of ^{67}Ga - Subcellular distribution of ^{67}Ga
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	Blood, skeletal muscle, liver, spleen, kidney, lung, stomach, pancreas, adrenal, heart muscle, brain, small intestine, large intestine, testis, parietal bone, tibia, spinal cord
Statistical methods used:	No specific comments provided

Toxicokinetic Study No 3

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:

There was no remarkable retention/accumulation of gallium in the testes (see following Table).

Table 2 Organ retention values (% dose) of ^{67}Ga in normal rats at various time intervals following intravenous administration of ^{67}Ga-citrate.

	10 min	60 min	3 h	24 h	2 days	3 days	5 days	7 days	10 days
Blood	50.1	29.3	22.6	2.29	0.89	0.59	0.30	0.30	0.22
Muscle	23.2	20.2	16.3	7.31	6.02	6.02	5.59	4.30	4.30
Liver	3.38	3.38	6.72	9.12	9.16	8.75	8.55	8.42	6.27
Spleen	0.46	0.34	0.62	0.94	1.05	1.06	1.01	1.11	1.09
Kidney	1.24	0.72	0.77	0.93	0.94	0.89	0.85	0.81	0.75
Pancreas	0.38	0.14	0.12	0.12	0.12	0.09	0.07	0.07	0.04
Heart muscle	0.64	0.31	0.28	0.14	0.13	0.11	0.10	0.09	0.09
Bone*	9.60	10.9	14.1	27.0	32.0	34.2	36.4	39.0	45.4
Testis	0.25	0.40	0.61	0.37	0.36	0.35	0.32	0.33	0.33

* Bone retention value (% dose) was calculated from retention value (% dose/g) of tibia and bone weight.

Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	See above
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	At 10 min after administration of 67Ga-citrate the retention value of 67Ga in blood was 6.77% dose/g, and this value decreased with time. The values for skeletal muscle, lung, pancreas, adrenal, heart muscle, brain, small intestine, large intestine and spinal cord were the highest at 10 min after administration, and they decreased with time. Conversely, this value in bone increased until 10 days after injection. But in the liver, kidney, and stomach, these values increased with time after administration and were highest 24 h or 48 h after injection. After that, they decreased with time. The value in spleen reached a plateau 48 h after administration, and hardly varied for 10 days. From the results of sub cellular fractionation, it was deduced that lysosome plays quite an important role in the concentration of 67Ga in small intestine, stomach, lung, kidney and pancreas; a lesser role in its concentration in heart muscle, and hardly any role in the 67Ga accumulation in skeletal muscle. In spleen, the contents in nuclear, mitochondrial, microsomal, and supernatant fractions all contributed to the accumulation of 67Ga.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.

Read-across to gallium arsenide:

There was no remarkable retention/accumulation of gallium in the testes.

The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.

Toxicokinetic Study No:	4
Study Reference:	Engelstad B, Luk SS, Hattner RS. Altered 67-Ga citrate distribution in patients with multiple red blood cell transfusions. AJR Am J Roentgenol. 139, 1982, 755-759
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	1982
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GCP
- details on whole-body scintigraphy not reported
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study describes the results of whole-body scintigraphy after 67-gallium citrate injection into two male patients. In contrast to some other tissues there was no remarkable accumulation of gallium in the testes. These results are supplementary evidence that gallium does not have a specific affinity to the testicular/germinal tissue.

Detailed Study Summary and Results:

Toxicokinetic Study No 4

Materials and Methods	
Test type:	In vivo; gallium citrate distribution study in patients. Whole-body scintigraphy after ^{67}Gallium Citrate injection into two male patients.
Test Substance :	
Name	^{67}Gallium Citrate
EC number:	unknown
CAS number:	27905-02-8
Degree of purity:	Not reported
Impurities:	Not reported
Batch number:	Not reported

Toxicokinetic Study No 4

Test Individuals	
Sex:	male
Number of individuals per sex per dose:	total of two males
Age and weight at the study initiation:	87 years / 69 years
Administration/Exposure	
Route of administration	Injection
Doses/concentration levels, rationale for dose level selection:	No further details provided.
Duration of study and duration	N.A.
Frequency of exposure:	Single injection for whole-body scintigraphy
Control group and treatment	N.A.
Post exposure observation period:	N.A.
Vehicle:	No further details provided.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	Single injection for whole-body scintigraphy

Toxicokinetic Study No 4

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	whole-body scintigraphy to detect gallium localizations
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	N.A.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
Increased localizations of gallium were found in kidney, bladder, or bones but not in testes. Thus, this study is supplementary information that gallium does not accumulate in the human testes.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	Whole-body scintigraphy to detect gallium localizations
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	Gallium localization in testes
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
Read-across to gallium arsenide: The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.	

Toxicokinetic Study No 5

Toxicokinetic Study No:	5
Study Reference:	Ishii S, Shishido F, Miyajima M, Sakuma K, Shigihara T, Kikuchi K. Whole-body gallium-67 scintigraphic findings in IgG4-related disease. Clin Nucl Med. 36, 2011, 542-545.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	2011
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GCP
- some details of scintigraphy not provided
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study describes the results of whole-body scintigraphy after ^{67}Gallium Citrate injection into 11 male patients. In contrast to some other tissues there was no remarkable accumulation of gallium in the testes. These results are supplementary evidence that gallium does not have a specific affinity to the testicular/germinal tissue.

Detailed Study Summary and Results:

Toxicokinetic Study No 1

Materials and Methods	
Test type:	In vivo; whole-body scintigraphy study in humans
Test Substance :	
Name	^{67}Gallium Citrate
EC number:	Not known
CAS number:	27905-02-8
Degree of purity:	As specified by Fujifilm RI Pharma, Tokyo, Japan
Impurities:	As specified by Fujifilm RI Pharma, Tokyo, Japan
Batch number:	Not disclosed

Toxicokinetic Study No 1

Test Individuals	
Sex	male
Number of individulas per sex per dose:	11
Age and weight at the study initiation:	47 to 76 Years / weight not reported
Administration/Exposure	
Route of administration	intravenous injection
Doses/concentration levels, rationale for dose level selection:	74 MBq ^{67}Gallium Citrate
Duration of study and duration	N.A.
Frequency of exposure:	N.A.
Control group and treatment	N.A.
Post exposure observation period:	48 and 72 hours after 67-gallium citrate injection
Vehicle:	Details not provided.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	Details not provided.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	Rout of administration: whole-body scintigraphy

Toxicokinetic Study No 1

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	Single injection
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	Whole-body scintigraphy 48 and 72 hours after 67-gallium citrate injection into 11 male patients. Anterior and posterior views of the whole body were obtained on an E.CAM using 2-headed cameras equipped with a middle-energy, parallel-hole collimator, scan speed of 10 cm/min, matrix of 512 x 1024, energy discrimination set to 92 kV ± 20 %, and 185 keV ± 20 % consideration of the 67-gallium photopeaks. Visual analysis of the 67-gallium images.
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	N.A.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
Among the 11 male patients, significant accumulation of Ga-67 was detected in the bilateral pulmonary hila in 10 patients, pancreas in 9, salivary glands in 6, lacrimal glands in 5, periaortic lesions in 2, and lung parenchyma in 1 patient. No significant accumulation of Ga-67 was detected in the testes.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	N.A.
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	N.A.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
Thus, this study is supplementary information that gallium does not accumulate in the human testes. The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.	

Toxicokinetic Study No 6

Toxicokinetic Study No:	6
Study Reference:	Jonkhoff AR, Plaizier MA, Ossenkoppele GJ, Teule GJ, Huijgens PC. High-dose gallium-67 therapy in patients with relapsed acute leukaemia: a feasibility study. Br J Cancer. 72, 1995, 1541-1546.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	1995
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- no GCP
- some details of scintigraphy not provided
- overall deficiencies not relevant to conclusion

Relevance of the Study:

This study describes the results of whole-body scintigraphy after 67-gallium citrate injection into two male patients. In contrast to some other tissues there was no remarkable accumulation of gallium in the testes. These results are supplementary evidence that gallium does not have a specific affinity to the testicular/germinal tissue

Detailed Study Summary and Results:

Toxicokinetic Study No 1

Materials and Methods	
Test type:	Whole-body scintigraphy after intravenous 67-gallium citrate injection (82.8 and 105.0 mCi, 44 and 47 mCi/m² respectively) into two male patients whith relapsed acute leukaemia. 67-gallium citrate volume 10 ml as a rapid intravenous push. No further details provided.
Test Substance :	
Name	^{67}Gallium Citrate
EC number:	Not known
CAS number:	27905-02-8
Degree of purity:	Not disclosed
Impurities:	Not disclosed
Batch number:	Not disclosed

Toxicokinetic Study No 1

Test Species	
Species / Sex	human / male
Number of humans per sex per dose:	1
Age and weight at the study initiation:	22 and 50 Years / weight not reported
Administration/Exposure	
Route of administration	intravenous injection
Doses/concentration levels, rationale for dose level selection:	82.8 mCi and 105.0 mCi ^{67}Gallium Citrate
Duration of study and duration	N.A.
Frequency of exposure:	N.A.
Control group and treatment	N.A.
Post exposure observation period:	Whole-body scintigraphy 24 hours after 67-gallium citrate injection
Vehicle:	Details not provided.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	Details not provided.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	Rout of administration apt for whole-body scintigraphy

Toxicokinetic Study No 1

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	Single injection
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	Whole-body scintigraphy
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	Statistical analysis was performed with Stat-Graphics 2.6 statistical computer program.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
Increased localizations of gallium were found in the skeleton, liver, spleen and bowel but not testes. Thus, this study is supplementary information that gallium does not accumulate in the human testes.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	N.A.
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	N.A.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	N.A.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
Thus, this study is supplementary information that gallium does not accumulate in the human testes. The bioavailability and the assumed accumulation of gallium ions in the testes of rats after inhalation of gallium arsenide was one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.	

2.3.2 Repeated Dose Toxicicity

Repeated Dose Toxicity Study No 1

Rep. Dose Study No:	1
Study Reference:	Venugopal B and Luckey TD. Metal Toxicity in Mammals. Vol. 2 New York, Plenum Press 1978, pp 113-115 citing Dudley HC, Levine MD. Studies of the toxic action of gallium. J Pharmacol Exp Ther 95, 1949, 487-493.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	1949
Reliability:	**4**, not assignable

Rationale for Reliability incl. Deficiencies:

- only very general statements, many details not reported
- but of some use for the purpose of the message

Relevance of the Study:

The study is relevant for the evaluation of gallium arsenide reproductive toxicity because it demonstrates that even very high dietary doses of a soluble gallium compound do not have adverse effects on the testes of rats.

Detailed Study Summary and Results:

Repeated Dose Toxicity Study No 1

Materials and Methods	
Test type:	In vivo; chronic toxicity study
Test Substance :	
Name	Gallium Lactate
EC number:	247-294-9
CAS number:	25850-52-6
Degree of purity:	not specified
Impurities:	not specified.
Batch number:	not specified

Repeated Dose Toxicity Study No 1

Test Animals	
Species/strain/sex:	Rat / unspecified / male
Number of animals per sex per dose:	10
Age and weight of animals at the study initiation:	Qualified “young” / weight 88 – 112g
Administration/Exposure	
Route of administration	feeding
Doses/concentration levels, rationale for dose level selection:	500 or 1000 ppm gallium
Duration of study and duration	26 weeks
Frequency of exposure:	N.A.
Control group and treatment	0 ppm gallium
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Repeated Dose Toxicity Study No 1

Description of Test Design	
Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):	N.A.
Premating exposure period for males and females (P and F1):	N.A.
Dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:	N.A.
Standardization of litters (yes/no and if yes, how and when):	N.A.
Parameters assessed for P and F1 parental animals:	N.A.
Parameters assessed for F1 and F2 pups:	N.A.
Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):	N.A.
Statistical methods used:	N.A.

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions (e.g.: exposure apparatus, method of exposure ("whole body", "oro-nasal", or "head only"), exposure data)	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Results:	
No effects on clinical pathology, body weight and body weight gain, and mortality. Dose-dependently decreased body weights and body weight gain were the only treatment-related effects.	
Parameters to report (as appropriate, depending on test type)	
a) Parental generation (P/F0 and F1, as appropriate):	
Parameters assessed in each generation:	N.A.
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	Body weight gain was 167 g at 1000 ppm, 177 g at 500 ppm and 192 g in controls (male and female rats together). One 500 ppm female rat and one control male rat died. General condition was not affected.
Reproductive parameters assessed and details of effects seen (e.g. estrous cycle length and pattern, sperm examination, mating index, gestation index, gestation length, litter size, litter weight):	N.A.
b) Pups (F1 and F2, as appropriate):	
Body weight and body weight gain, at various ages, as available	N.A.
Standardization of litters (yes/no and if yes, how and when)	N.A.
Parameters assessed in each generation	N.A.
Clinical observations performed including frequency, organs examined at necropsy, other endpoints examined (e.g. anogenital distance)	N.A.
Read-across to gallium arsenide:	
The lack of toxicity to the testes at these high doses of a soluble gallium salt suggests that the gallium ion has no specific effects on the testes in rats. Testicular effects in studies with gallium arsenide have been one of the reasons why gallium arsenide has been classified into Category 1B by the RAC.	

2.3.3 Additional Relevant Information

Additional Relevant Information Study No 1

Add. Inf. Study No:	1
Study Reference:	Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect. 116, 2008, 1473-1479.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	2008
Reliability:	2, reliable with restriction

Rationale for Reliability incl. Deficiencies:

- Some experimental detail not provided
- overall deficiencies not relevant to conclusion

Relevance of the Study:

The authors measured semen quality and metals in blood (arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, selenium, zinc) among 219 men recruited through two infertility clinics. There was no association between arsenic concentrations in blood and semen quality.

Detailed Study Summary and Results:

Additional Relevant Information Study No 1

Materials and Methods	
Test type:	Epidemiologic study of environmental contaminants and male reproduction. The protocols of the study were approved by the committees on research ethics at all participating institutions, and informed consent was obtained from all participants.
Test Substance :	
Name	N.A.
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Additional Relevant Information Study No 1

Test Individuals	
Sex:	Male
Number of individuals per sex per dose:	Total of 219 individuals
Age and weight at the study initiation:	34.2 ± 5.6 Years
Administration/Exposure	
Route of administration	N.A. (exposure to environmental conditions)
Doses/concentration levels, rationale for dose level selection:	N.A.
Duration of study and duration	N.A.
Frequency of exposure:	N.A.
Control group and treatment	N.A.
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Additional Relevant Information Study No 1

Description of Test Design	
Dosing schedules and pre and post dosing observation periods	N.A.
Parameters assessed	blood metal levels of: As, Cd, Cr, Pb, Mn, Hg, Mo, Se, Tl, Zn The limits of detection (LOD) for the blood metal levels were as follows: As, 4.0 µg/L; Cd, 0.2 µg/L; Cr, 0.5 µg/L; Pb, 0.3 µg/dL; Mn, 1.0 µg/L; total Hg, 0.2 µg/L; Mo, 1.0 µg/L; Se, 5 µg/L; Tl, 0.1 µg/L; and Zn, 50 µg/L.
Clinical observations performed and frequency, organs examined	The study population included fertile men and men with a range of fertility problems. Men between 18 and 55 years of age currently attempting to conceive a pregnancy with their partner were enrolled. Men with diabetes, thyroid or adrenal disorders, genetic disorders related to fertility, testicular cancer, bilateral orchiectomy, or taking hormone therapy were excluded.
Statistical methods used:	Data analysis based on SAS, version 9.1 (SAS Institute Inc., Cary, NC). Calculation of descriptive statistics on subject demographics, along with the distributions of blood metal concentrations and semen-quality parameters. Bivariate analysis was conducted between all semen quality, metal concentration, and variables to investigate differences between distributions or categories and the potential for confounding. Correlations or differences between groups were tested statistically using parametric or non parametric methods where appropriate.

Additional Relevant Information Study No 1

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions , exposure data	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Additional Relevant Information Study No 1

Results:	
As, Cu, Pb, total Hg, and Zn were > LOD for most samples. Fifty percent of samples had Cd and thallium levels > LOD, whereas 30% of samples had levels > LOD for Cr and Mo.	
Parameters to report (as appropriate, depending on test type)	
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen	The Final logistic regression models for below-reference sperm concentration and morphology when considering multiple metals and other covariates did not show an association of these parameters with the As blood concentration. As was positively associated with semen volume.
Read-across to gallium arsenide:	
Read-across to gallium arsenide: This is supplementary information for the hypothesis that arsenic is most likely not a contributing factor to the testicular effects observed in animal studies after inhalation or intratracheal instillation of gallium arsenide.	

Additional Relevant Information Study No 2

Add. Inf. Study No:	2
Study Reference:	Meeker JD, Rossano MG, Protas B, Padmanahban V, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ. Environmental exposure to metals and male reproductive hormones: circulating testosterone is inversely associated with blood molybdenum. Fertil Steril. 93, 2010, 130-140.
Purpose Flag:	key study; robust study summary
Study Result Type:	experimental result
Study Period:	2010
Reliability:	1, reliable

Rationale for Reliability incl. Deficiencies:

- very recent carefully conducted, reported study and analysis of the results.

Relevance of the Study:

The authors measured male reproductive hormone levels and metals in blood (arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, selenium, zinc) among 219 men recruited through two infertility clinics. The study population included fertile men and men with a range of fertility problems. Men between 18 and 55 years of age currently attempting to conceive a pregnancy with their partner were enrolled. Men with diabetes, thyroid or adrenal disorders, genetic disorders related to fertility, testicular cancer, bilateral orchiectomy, or taking hormone therapy were excluded. The protocols of the study were approved by the committees on research ethics at all participating institutions, and informed consent was obtained from all participants.

There was no association between arsenic concentrations in blood and male reproductive hormone levels.

Detailed Study Summary and Results:

Additional Relevant Information Study No 2

Materials and Methods	
Test type:	Epidemiologic study of environmental contaminants and male reproductive hormones. The protocols of the study were approved by the committees on research ethics at all participating institutions.
Test Substance :	
Name	N.A.
EC number:	N.A.
CAS number:	N.A.
Degree of purity:	N.A.
Impurities:	N.A.
Batch number:	N.A.

Additional Relevant Information Study No 2

Test Individuals	
Sex:	Male
Number of individuals per sex per dose:	Total of 219 individuals
Age and weight at the study initiation:	median (25th, 75th percentile) age of 34 (30,38) years / median BMI of 28 (25,33).
Administration/Exposure	
Route of administration	N.A. (exposure to environmental conditions)
Doses/concentration levels, rationale for dose level selection:	N.A.
Duration of study and duration	N.A.
Frequency of exposure:	N.A.
Control group and treatment	N.A.
Post exposure observation period:	N.A.
Vehicle:	N.A.
Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:	N.A.
Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:	N.A.
If other route of administration than the oral route is chosen provide a justification:	N.A.

Description of Test Design	
Dosing schedules and pre and post dosing observation periods	Blood samples were collected from participants during a morning clinic visit.
Parameters assessed	Blood samples were collected from participants during a morning clinic visit. Most of the hormone assays were performed by the Immulite Assay System. FSH, LH, and sex hormone binding globulin (SHBG) were measured by solid phase, two site, chemiluminescent enzyme immunometric assay. For testosterone (T) measurement a solid phase, competitive chemiluminescent enzyme immunoassay was employed. Blood samples were assayed for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), total mercury (Hg), molybdenum (Mo), selenium (Se), thallium (Tl) and zinc (Zn).
Clinical observations performed and frequency, organs examined	Participants were recruited at two Michigan infertility clinics without knowledge of male factor infertility. Since couples may present at the clinics for problems relating to either male or female fertility problems (or both), the study population includes fertile men and men with a range of fertility problems. After obtaining informed consent, men between 18 and 55 years of age currently attempting to conceive a pregnancy with their partner were enrolled. Men with diabetes, thyroid or adrenal disorders, genetic disorders related to fertility, testicular cancer, unilateral orchiectomy, or taking hormone therapy were excluded.
Statistical methods used:	Data analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics on subject demographics were calculated, along with the distributions of blood metal concentrations and hormone levels. Bivariate analysis was conducted between all hormone, metal concentration, and demographic variables to investigate differences between distributions or categories and the potential for confounding. Correlations or differences between groups were tested statistically using parametric or non-parametric methods where appropriate.

Additional Relevant Information Study No 2

Information Requirements for Inhalation Studies:	
Type of inhalation exposure and test conditions , exposure data	N.A.
Analytical verification of test atmosphere concentrations	N.A.
Particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)	N.A.
Type or preparation of particles (for studies with aerosols)	N.A.
Information requirements for dermal studies:	
Area covered (e.g. 10% of body surface)	N.A.
Occlusion (e.g. semi-occlusive)	N.A.
Total volume applied	N.A.
Removal of test substance (e.g. water or solvent)	N.A.

Additional Relevant Information Study No 2

Results:

The limits of detection (LOD) for the blood metal levels were as follows: As, 4.0 µg/L; Cd, 0.2 µg/L; Cr, 0.5 µg/L; Pb, 0.3 µg/dL; Mn, 1.0 µg/L; Hg, 0.2 µg/L; Mo, 1.0 µg/L; Se, 5 µg/L; Tl, 0.1 µg/L; and Zn, µg/L.

Parameters to report (as appropriate, depending on test type)	
Clinical observations (e.g. body weight, body weight gain, clinical signs, food consumption):	See above
Reproductive parameters assessed and details of effects seen	As concentrations found in the blood were well above LOD (50% Percentile: 8.10 µg/L, Max: 25.5 µg/L). In the final linear regression models for hormone levels when considering multiple metals and other potentially important covariates there was no statistically significant association with As blood levels detected.

Read-across to gallium arsenide:

This is supplementary information for the hypothesis that arsenic is most likely not a contributing factor to the testicular effects observed in animal studies after inhalation or intratracheal instillation of gallium arsenide.

Additional Relevant Information Study No 3

Add. Inf. Study No:	3
Study Reference:	ATSDR. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services. August 2007.
Purpose Flag:	key study; robust study summary
Study Result Type:	Review
Study Period:	N.A.
Reliability:	N.A.
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: This large review has among others evaluated a number of experimental studies regarding possible effects of inorganic arsenic compounds on male reproductive organs and fertility. No studies were found reporting specific male reproductive toxicity. No studies were located regarding male reproductive effects in humans after exposure to inorganic arsenicals.	

Summary and results:

This review cites results from the following relevant studies: Intraperitoneal injection of sodium arsenite into mice did not affect spermatogonia and sperm morphology, and did not induce dominant lethal mutations.

Read-across to gallium arsenide:

This is supplementary information for the hypothesis that arsenic is most likely not a contributing factor to the testicular effects observed in animal studies after inhalation or intratracheal instillation of gallium arsenide.

Additional Relevant Information Study No 4

Add. Inf. Study No:	4
Study Reference:	Bomhard EM. Zur Frage einer primären Hoden-/Neben-hoden-/Spermientoxizität von Arsen oder Gallium. Unpublished communication to the Working Group "Reprotoxicity" of the German Subcommittee "UAIII". July 19, 2011. [In German]
Purpose Flag:	key study; robust study summary
Study Result Type:	Review
Study Period:	2011
Reliability:	N.A.
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: This review evaluated a number of more recent experimental studies regarding possible effects of inorganic arsenic compounds on male reproductive organs and fertility. In addition, human and experimental data on gallium are reviewed. Relatively high-dose oral or parenteral exposure to highly soluble inorganic arsenicals seem to have adverse effects on rat and mouse testes and sperms. The small amounts of bioavailable arsenic ions after inhalation of gallium arsenide, however, are not considered to contribute to the observed male "fertility" effects.	

Summary and results:

The available literature on adverse effects of gallium and arsenic compounds was evaluated with regard to the question of a primary effect on male fertility, as postulated by RAC.

Valid indications of a potential for primary adverse effects on male fertility could not be found.

Arsenic in its highly soluble compounds sodium arsenite and diarsenic trioxide with high bioavailability caused testicular damage and adverse sperm effects at relatively high oral or parenteral doses in rats and mice, but not after intratracheal instillation of diarsenic trioxide into rats and hamsters. A number of data point to an accumulation potential of soluble inorganic arsenic compounds in male genital organs of laboratory animals. However, in the 2-year inhalation study with GaAs there was no accumulation of arsenic in the testes. A contribution of the bioavailable arsenic concentrations from GaAs in the NTP inhalation studies and the studies with intratracheal instillation to the testicular/sperm effects is thus not plausible.

Read-across to gallium arsenide:

The evaluation of all findings of the studies on GaAs together with the literature data on the effects of gallium and arsenic compounds lends support to the conclusion that hypoxaemia/anaemia following lung damage played the decisive role in the testicular effects on male fertility.

Additional Relevant Information Study No 5

Add. Inf. Study No:	5
Study Reference:	Bomhard EM and Gelbke H-P. Are the haematological and fertility effects observed in the inhalation/intratracheal instillation studies primary or secondary effects of gallium arsenide exposure? Unpublished Document prepared for FCM; Sept. 16, 2011
Purpose Flag:	key study; robust study summary
Study Result Type:	Review
Study Period:	2011
Reliability:	N.A.
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: This in-depth comparative evaluation of the NTP and other studies with regard to lung, haematological and male reproductive effects, their concentration- and time-related occurrence in both rats and mice in combination with the data on bioavailability presents new data on the most likely mode of action of the „fertility“ effects. It demonstrates that neither gallium nor arsenic ions are directly involved in the testicular/sperm effects and explains why other particular substances did not have the same profile of effects despite of significant lung toxicity.	

Summary and results:

During the break out session on gallium arsenide on 13.09.2011 the question was raised by RAC as to whether the effects observed in the NTP inhalation studies i.e. the haematological and sperm/epididymides/testicular changes in rats and mice had to be seen as primary effects due to systemically available substance or metabolites or secondary effects of the lung toxicity. One hypothesis brought into the discussion was that, if it is a secondary effect, then this constellation of findings should also be found whenever particulate matter causes chronic lung inflammation.
The reworking of the subchronic NTP studies on gallium arsenide clearly revealed that, at concentrations that affected epididymides/testes and/or sperm parameters, massive damage to the lungs with consecutive blood effects was present, which definitely infers an existing undersupply of the organism with oxygen. The germinal epithelium and the spermatozoa are especially sensitive to oxygen depletion.

A comparative examination with other particulate substances (including metal compounds) administered by inhalation revealed that gallium arsenide differs in its profile and sequence of toxicity to the lungs from almost all other considered lung-damaging substances. This explains the rather unique haematological effects and the testicular toxicity in subchronic inhalation studies/experiments with intratracheal instillation.

Read-across to gallium arsenide:

Together with other data from human and experimental animal studies at hypoxaemic conditions, as well as comparative evaluations of other compounds, this led to the conclusion that systemically available arsenic ions and/or gallium ions do not play a role in the observed effects after GaAs treatment. Thus, the classification and labelling of gallium arsenide in Category 1B-H360F is not supported by weight of evidence.

Additional Relevant Information Study No 6

Add. Inf. Study No:	6
Study Reference:	IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Drinking-Water Disinfectants and Contaminants, including Arsenic. WHO, Lyon, France, Vol. 84, 2004.
Purpose Flag:	
Study Result Type:	Review
Study Period:	2004
Reliability:	N.A.
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: This large review has among others evaluated the available data on possible effects of inorganic and organic arsenicals via drinking water exposure on male reproductive organs and fertility.	

Summary and results:

No studies were found reporting specific male reproductive toxicity.

Read-across to gallium arsenide:

This is supplementary information for the hypothesis that arsenic is most likely not a contributing factor to the testicular effects observed in animal studies after inhalation or intratracheal instillation of gallium arsenide.

Additional Relevant Information Study No 7

Add. Inf. Study No:	7
Study Reference:	• NTP 1993. Toxicology and Carcinogenesis Studies of Talc (CAS No. 14807-96-6) (Non-Asbestiform) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Nr. 421. • NTP 1996. Toxicology and Carcinogenesis Studies of Nickel Subsulfide (CAS No. 12035-72-2) in F344 Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Nr. 453. • NTP 1997. Toxicology and Carcinogenesis Studies of Molybdenum Trioxide (CAS No. 1313-27-5) in F344 Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Nr. 462. • NTP 2001. Toxicology and Carcinogenesis Studies of Indium Phosphide (CAS No. 22398-80-7) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Nr. 499.
Purpose Flag:	
Study Result Type:	Review
Study Period:	2004
Reliability:	N.A.
Rationale for Reliability incl. Deficiencies:	
Relevance of the Study: These (and other similar) studies have been used to argue against the hypothesis of an association between lung toxicity/hypoxaemia and testicular effects.	

Summary and results:

All these compounds induced chronic pulmonary lesions (basically fibrosis and inflammation) but no testicular/sperm toxicity in the subchronic toxicity studies. However, none of the subchronic inhalation studies performed with these compounds from the NTP data bank showed a spectrum of histopathological, haematological (partly not examined) or spermatological effects (partly not examined) similar to that of GaAs. In addition, the lung effects were clearly different quantitatively and/or qualitatively. One of the characteristics of GaAs is the initially much more pronounced alveolar proteinoses compared with the other substances, with an initially less pronounced inflammation in the lungs, combined with haemolytic effects.

Read-across to gallium arsenide:

This highlights the special status of GaAs in comparison with the other evaluated compounds, and also offers a plausible explanation for the hypothesis of hypoxaemia-induced testicular and sperm damage.

3. Overall Relevance of the Provided Information

The data provided and summarized below argue against a potential of gallium and arsenic ions for a specific toxicity to the testes/sperms:

Gallium concentrations in serum and whole blood of male F344 rats again were extremely low after daily whole-body exposure (6 h/day, 5 days/week) to GaAs concentrations of 1.0 mg/m³ for up to 18 months (? 0.05 µg/g in whole blood, thus more than 1000-fold less than arsenic levels; ? 0.11 µg/g in serum), at which concentrations marked clinical signs and gross pathology indicated significant lung toxicity. At the lower but still pulmonary toxic concentrations of 0.1 and 0.01 mg/m³, gallium was either below the detection limit of 0.042 µg/g in serum or basically at the same whole blood level as in controls (NTP 2000).

Administration of very high levels of water soluble gallium compounds (e.g. dietary levels of up to 1000 ppm gallium as gallium trichloride) to rats for 26 weeks did not reveal significant toxic effects (Dudley and Levine 1949 cited in Venugopal and Luckey 1978).

Swiss CD-1 mice were dosed with GaAs by inhalation (same sample as used in 16-day and 14-week NTP inhalation studies) at concentrations of 0, 1, 10 or 37 mg/m³ on 6 hours/day, 7 days/week for consecutive 12 days. Testicular concentrations of gallium were below the detection limit (0.10 µg/g) in all animals (except one of the 37 mg/m³ group which was probably contaminated) (Mast et al. 1990).

Male Sprague-Dawley rats were dosed with GaAs by inhalation (same sample as used in 16-day and 14-week NTP inhalation studies) at concentrations of 0, 10, 37, or 75 mg/m³ on 6 hours/day, 7 days/week for consecutive 12 days. Gallium and arsenic increased concentration-dependently. This suggests that neither gallium nor arsenic are contributing to the “fertility” effects reported in rats and mice after subchronic inhalation exposure because the “fertility” effects in mice were even more severe than in rats although no concentration-dependent increase in either ion was determined in the testes of mice (Mast et al. 1990).

High subcutaneous doses of gallium nitrate to male mice (up to 96 mg/kg bodyweight per day for

14 days) produced no indications of an impairment of fertility (Colomina et al. 1993).

Intravenous injection of 0.4 ml gallium-67 citrate per animal (10 – 100 µCi; weight of animals not specified) into Wistar rats did not result in an accumulation of gallium in the testes up to 10 days after treatment (Ando et al. 1986).

Whole-body scintigraphy in patients did not reveal an accumulation of gallium-67 in testes and epididymides (Engelstad et al. 1982; Ishii et al. 2011; Jonkhoff et al. 1995).

Based on this analysis, the effects on blood and testes are secondary to the lung toxicity, not a direct effect of gallium arsenide, and therefore a more appropriate classification of gallium arsenide regarding reproductive effects should be Category 2 or no classification.

4. Comparison with the CLP and DSD Criteria

The current RAC opinion for classification of GaAs as Repr. 1B-H360F rely on the assumption that the testicular/sperm effects observed in repeated-dose intratratracheal instillation/subchronic inhalation studies with mice, rats and/or hamsters were primary effects and not a secondary non-specific consequence of lung toxicity. In the opinion of the RAC, this classification was „also supported by the potential of gallium to accumulate in rat testis following inhalation exposure“.

For a classification as a Category 1B (Presumed human reproductive toxicant) substance the CLP regulation has defined the following criteria:

“The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development **in the absence of other toxic effects** (emphasis by the current athors), or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.”

For a classification as a Category 1 (Substances known to impair fertility in humans) substance the DSD regulation has defined the following criteria:

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

For a classification as a Category 2 (Substances which should be regarded as if they impair fertility in humans) the DSD regulation has defined the following criteria:
There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of: clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects, other relevant information.
As stated above, the presented arguments and literature data in our opinion do not provide clear evidence of an adverse effect on fertility **in the absence of other toxic effects.** Instead clear evidence is provided that the primary events are lung effects leading to haematological changes and hypoxaemia, which than trigger the testicular effects. Thus,
classification (if at all) into Category 2 of the CLP regulation (Suspected human reproductive toxicant) is more appropriate.

[CLP Criteria for Category 2: “Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects” (ECHA 2011).]

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