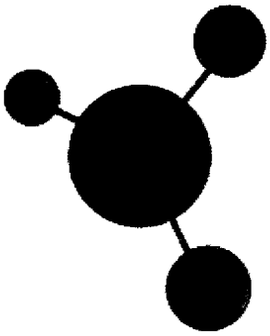

IUCLID 5

Printing Date 2007-12-05 13:42:56 CET

Restriction of specific regulatory purposes

Confidentiality

Owner	N-Butylbenzenesulphonamide / N-butylbenzenesulphonamide / N-butylbenzenesulfonamide / N-butylbenzenesulfonamide / 3622-84-2 / Proviron Fine Chemicals nv / Oostende / Belgium
Legal entity owner	Proviron Fine Chemicals nv / Oostende / Belgium

Endpoint study record: Toxicity to reproduction.001

UUID	IUC5-e94bf54a-0296-41c1-a14a-0ed417bfe04e
Dossier UUID	0
Author	bco / Proviron Industries NV / Hemiksem / other:
Date	2007-12-03 15:35:53 CET
Remarks	

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	2007
Reliablility	1 (reliable without restriction)		

Data source

Reference

Reference type	study report		
Author	International Institute of Biotechnology and Toxicology (IIBAT)	**Year**	2007
Title	Reproduction and development toxicity study with N-n-Butylbenzene sulphonamide in Wistar Rats		
Bibliographic source	Study report		
Testing laboratory	IIBAT	**Report no.**	06334
Owner company	Proviron Fine Chemicals N.V.		
Company study no.	06334	**Report date**	2007-10-31

Data access

data submitter is data owner

Data protection claimed

yes

Materials and methods

Test type

one-generation study

Test guideline

Qualifier	according to
Guideline	OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)
Deviations	

GLP compliance

yes (incl. certificate)

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number

Identity 3622-84-2

Identifier EC number

Identity 222-823-6

Identifier IUPAC name

Identity N-butylbenzenesulfonamide

Details on test material

- Name of test material (as cited in study report): N-n-butyl benzenesulphonamide (BBSA)
- Substance type: mono constituent substance-organic
- Physical state: clear liquid
- Analytical purity: 99.88%
- Impurities (identity and concentrations): 0.1% Diphenylsulphon/0.1% water/0.1% Butylbenzeensulphate/0.02% Butylamine
- Composition of test material, percentage of components: 99.88% BBSA
- Isomers composition:
- Purity test date: 13/10/2006
- Lot/batch No.: 200610130015
- Expiration date of the lot/batch: 13/10/2008
- Stability under test conditions: stable
- Storage condition of test material: Dry, cool and well-ventilated place
- Other:

Confidential details on test material

- Analytical purity: 99.88%
- Impurities (identity and concentrations): 0.1% Diphenylsulphon/0.1% water/0.1% Butylbenzeensulphate/0.02% Butylamine
- Composition of test material, percentage of components: 99.88% BBSA
- Purity test date: 13/10/2006
- Lot/batch No.: 200610130015
- Expiration date of the lot/batch:13/10/2008
- Isomers composition:
- Other:

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: The Animal house facilities of Department if Toxicology, IIBAT
- Age at study initiation: (P) 12-15 wks; (F1) 0 wks:
- Weight at study initiation: (P) Males: 300-330 g; Females: 200-220
- Housing: Standard polypropylene rat cages with stainless steel top grill. Cleaned, sieved and autoclaved paddy husk was used as bedding material
During mating either sex in ratio of 1:1 were accommodated with special cages meant for mating of later pregnant rats were housed
individually.
- Use of restrainers for preventing ingestion (if dermal): no
- Diet (e.g. ad libitum): Standard rodent pellet feed. routinely analyzed; ad libitum
- Water (e.g. ad libitum): Reverse osmosis water. Routinely analyzed; ad libitum
- Acclimation period: 5 days prior to test in the test room
- Fasting: Feed alone was withdrawn over-night prior to sacrifice.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.6 - 21.2
- Humidity (%): 59 - 64
- Photoperiod (12 hrs dark /12 hrs light):

IN-LIFE DATES: From: 02/04/2007 To: 30/05/2007

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS:

DIET PREPARATION
- Rate of preparation of diet (frequency):daily
- Mixing appropriate amounts with (Type of food): standard rodent pellet feed

- Storage temperature of food:no data

VEHICLE
- Justification for use and choice of vehicle (if other than water): no data
- Concentration in vehicle: no data
- Amount of vehicle (if gavage): no data
- Lot/batch no. (if required): no data
- Purity:no data

Details on mating procedure

- M/F ratio per cage:1:1
- Length of cohabitation: no data
- Proof of pregnancy: vaginal plug / sperm in vaginal smear, referred to as day 0 of pregnancy
- After unsuccessful pairing replacement of first male by another male with proven fertility.
- Further matings after two unsuccessful attempts: [yes (explain)]: Until two weeks have elapsed.
- After successful mating each pregnant female was caged (how): no data
- Any other deviations from standard protocol:

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

analyzed by Analytical Chemistry, IIBAT

Duration of treatment / exposure

2 weeks prior to mating, after acclimization.
Dosing was continued in both sexes during the mating period.
Males were further dosed after the mating period untill the min. dosing period of 28d has been completed.
Dosing of confirmed females was continued throughout the pregnancy and including, day 3 post partum.

Frequency of treatment

Daily

Details on study schedule

- F1 parental animals not mated until [...] weeks after selected from the F1 litters.

- Selection of parents from F1 generation when pups were [...] days of age.
- Age at mating of the mated animals in the study: 12-15 weeks

Doses / concentrations

0-100-200 and 400 mg/kg b.w.

Basis nominal conc.

No. of animals per sex per dose

12 animals/group/sex

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: a range finding study
- Rationale for animal assignment (if not random): standard species used in reproduction/developmental toxicity studie
- Other:

Positive control

Control group were treated similary but with corn oil alone. All dams were allowed to litter through the natural birth and the size, weight and sex of litters were recorded at parturition (day 0) and the day 4 of post partum.

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: males: observed for 28 days (pre and post mating)
females: observed for 54 days (premating, mating, gestation, parturition and post partum)
- Cage side observations checked in table; yes.

DETAILED CLINICAL OBSERVATIONS: yes

BODY WEIGHT: Yes
- Time schedule for examinations: prior to the administration of the test substance (day 0) and weekly during the entire observation period and at
termination

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as mg food/kg body weight/day: yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: yes

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): No
- Time schedule for examinations:/

TOXICITY SIGNS: All groups of animals twice daily preferably after dosing in morning and later in the evening session.

GESTATION AND ONSET OF DELIVERY: The onset of labour, thelitter size, the sex-ratio and weight of pups at birth (day 0) and day 4 post partem
was recorded. Sex ratio (m/f) and live pups/dam were calculated.

MORTALITY/MORBIDITY: All animals were observed twice daily for mortality/morbidity, those found moribund/severly exhibiting toxic signs were
necropsied and recorded.
CAGE SIDE OBSERVATIONS: Yes / No / No data
- Time schedule:
- Cage side observations checked in table.yes

DETAILED CLINICAL OBSERVATIONS: Yes / No / No data
- Time schedule:

BODY WEIGHT: Yes / No / No data
- Time schedule for examinations:

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

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): Yes / No / No data
- Time schedule for examinations:

OTHER:

Estrous cyclicity (Parental animals)

no data

Sperm parameters (Parental animals)

Parameters examined in P male parental generations:
[testis weight, epididymis weight, daily sperm production, sperm count in testes, sperm count in epididymides, enumeration of cauda epididymal sperm reserve, sperm motility, sperm morphology, other:]
Test substances related adverse histopahological findings were observed in testis and epididymides of male rats in high dose groups. In testis
these findings consisted of sertolicell vacuolation (8/12), spermatid retention (6/12), multinucleated giant cells (5/12), desquamation of germ cells
(4/12), absence of spermatids (2/12) and atrophy of testis (1/12).
Test substance related adverse findings in the epididymides were desquamated germ cells (7/12) and oligospermia (4/12). These effects were in correspondence to the effects observed in the testis.

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: [yes/no]
- If yes, maximum of [...] pups/litter ([...]/sex/litter as nearly as possible); excess pups were killed and discarded.

PARAMETERS EXAMINED
The following parameters were examined in F1 offspring:
number and sex of pups: Test substance related effect was observed on mean litter size in high dose group at day 0 and day 4 post partum. Mean litter size in high dose group was 2.50 followed by 8.33, 10.08 and 9.75 in intermediate, low an control groups at day 0, while it was 1.08, 7.92, 9.33 and 9.17 in high, intermediate, low and control group respectively at day 4 post partum. No test substance related effect was observed on sex ratio of the pups.
stillbirths: no data
live births: Test substance releated effect was observed on the number of dams delivered

with live pups in high and intermediate dose group.
Only 50% females delivered with live pups in high dose group. In intermediate group 82.33% females delivered with live pups. 100% dams delivered with live pups in both control and low dose groups.
postnatal mortality:test substance related effect was observed on loss of offspring in high dose group. There was increased rate of loss of offspring in high dose group.
presence of gross anomalies: in high dose group 1/3 dams delivered abnormal pups. Anasarca in whole litter of dam.
weight gain: Test substance related effect was observed in mean litter weight on day 0 and day 4 post partum in high dose group. Mean litter weight in high dose group at day 0 was 11.6 followed by 46.7, 54.2 and 54.1 in intermediate, low and control groups respectively. Mean litter weight in high dose group day 4 post partum was 55.7 followed by 59.7, 72.0 and 70.6 in intermediate, low and control group respectively.
physical or behavioural abnormalities, no data
other:]

GROSS EXAMINATION OF DEAD PUPS:
yes , for external and internal abnormalities; possible cause of death was not determined for pups born or found dead.

Postmortem examinations (Parental animals)

SACRIFICE
- Male animals: All surviving animals [describe when, e.g. as soon as possible after the last litters in each generation were produced.]: males were sacrified after the mating period when until the minimum dosing period of 28 days has been completed.
- Maternal animals: All surviving animals [describe when, e.g. after the last litter of each generation was weaned.]: Dams with offspring were sacrified on day 4 post partum.

GROSS NECROPSY
- Gross necropsy consisted of [external and internal examinations including the cervical, thoracic, and abdominal viscera.]: At the time of sacrifice or death during the study, the adult animals were examined macroscopically for any abnormalities or pathological changes. Special attention were paid to the organs of the reproductive system. All gross pathological changes are recorded individually for each animal. The number of implantation sites were recorded. The counting of corpora lutea were done. Dead pups and pups sacrified at day 4 post partum, or shortly thereafter, were carefully examined externally for gross abnormalities. The ovaries, epididymides, accessory sex organs and all organs showing macroscopic lesions of all adult animals were preserved in 10% neutral buffered formalin. Testes were preserved in Bouin's fixative.

HISTOPATHOLOGY / ORGAN WEIGHTS
The tissues indicated in Table [#] were prepared for microscopic examination and weighed, respectively.:
Weights of following organs of all male adult animals were recorded: testes and the epididymides
Testes were evaluated with special emphasis on stages of spermatogenesis in all dose groups. Seminal vesicle, prostate and ovaries were evaluated from control and high dose groups.
The following grading system was used for histopathological evaluation in the study,
1. minimal
2. mild
3. moderate
4. marked
5. severe

Postmortem examinations (Offspring)

SACRIFICE
- The F1 offspring were sacrificed at 4 days of age.
- These animals were subjected to postmortem examinations (macroscopic and/or microscopic examination) as follows: /

GROSS NECROPSY
- Gross necropsy consisted of external examinations carefully examined externally for gross abnormalities

Statistics

The body weight, feed consumption and organ weight data were statistically analysed using one way ANOVA.

Reproductive indices

no data

Offspring viability indices

Dam with live pups.
Loss of offspring
external abnormalities in pups

Results and discussions

Effect levels

Endpoint	NOAEL
Generation	P
Sex	male/female
Effect level	200 mg/kg b.w
Basis for effect level / Remarks	overall effects clinical signs; mortality; In the 400 mg/kg b.w treated group, few animals exhibited abnormal gait, respiratory distress, tremor, hunched posture, dullness, salivation, piloerection and lethargy throughout the experimental period. body weight; Statistical significant decreases in body weight were observed in males of high dose group from second week till termination. There was statistical significant decrease in body weight of female in high dose during late pregnancy (3rd and 4th week) and at terminal sacrifice (day 4 post partum) were also observed. Significant increases in body weight were also observed in low dose females during premating. food consumption; was decreased significantly during the first week of premating in both the sexes in dose related pattern in intermediate and high dose related pattern in intermediate and high dose which recovered in later period. Feed consumption during gestation in female was also decreased when compared to control. gross pathology; no test substance related gross pathological observations were observed in any of the treated groups of male and female rats. organ weights; histopathology; mating index; fertility index; % females mating > 1st estrous; number of implantation sites; duration of pregnancy; birth index; live birth index; pregnancy index; litter size; litter weight; pup weight; sex ratio; survival index; viability index; lactation index; sperm characterization; other:

Observations: parental animals

Clinical signs (parental animals)

yes

Body weight and food consumption (parental animals)

yes

Test substance intake (parental animals)

yes

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

yes

Reproductive performance (parental animals)

yes

Organ weights (parental animals)

yes

Gross pathology (parental animals)

yes

Histopathology (parental animals)

yes

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
In the high dose treated group, few animals exhibited abnormal gait, respiratory distress, tremor, hunched posture, dullness, salivation, piloerection and lethargy.
2 females were found death dead during mating period in high dose group. Based on gross pathologie, cause of death was uncertain. No mortality was observed in the doses of control, 100, 200 mg/kg b.w. No toxicity signs were observed in dams and pups in the doses of 100 and 200 mg/kg b.w throughout the observation period.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
Statistical significant decreases in body weight were observed in males of high dose group from second week till termination. There was statistical significant decrease in body weight of female in high dose during late pregnancy (3rd and 4th week) and at terminal sacrifice (day 4 post partem) were also observed. Significant increases in body weight were also observed in low dose females during premating.
Feed consumption was decreased significantly during the first week of premating in both the sexes in dose related pattern in intermediate and high dose which recovered in later period. Feed consumption during gestation in female was also decreased when compared to control.

TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)

REPRODUCTIVE FUNCTION: ESTROUS CYCLE (PARENTAL ANIMALS)

REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)
Test substances related adverse histopahological findings were observed in testis and epididymides of male rats in high dose groups. In testis
these findings consisted of sertolicell vacuolation (8/12), spermatid retention (6/12), multinucleated giant cells (5/12), desquamation of germ cells
(4/12), absence of spermatids (2/12) and atrophy of testis (1/12).
Test substance related adverse findings in the epididymides were desquamated germ cells (7/12) and oligospermia (4/12). These effects were in correspondence to the effects observed in the testis.

REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)
9/12 females in the high dose group which mated with high dose males were infertile. Above mentioned microscopic finding in the testis and epididymides of high dose males which mated with high dose females could be attributed to the infertility. However, two mating pairs in the high dose group did not exhibit any histopathological changes in testis,

epididymide, prostate, seminal vesicle and ovary which could explain their infertility.
All other changes observed in the testis and epididymides were either does not have dose response relationship or does not effect the fertility or does not have relationship between the lesions observed in the epididymides and testis. Hence, all other findings in the testis and epididymides were spontaneous, incidental and their relation with test substance administration is uncertain.
No test substance related findings were observed in seminal vesicles, prostate and ovaries. All findings observed in these organs were spontaneous or incidental.

ORGAN WEIGHTS (PARENTAL ANIMALS)
Statistically significant decrease in absolute testis and epididymides weight of high dose male rats was observed. This effect was correlated histologically with atrophy of testis, absence and depletion of spermatids in testis and oligospermia in epididymides and considered test substance related adverse effects.

GROSS PATHOLOGY (PARENTAL ANIMALS)
No test substance related gross pathological observations were present in the male and female rats.

HISTOPATHOLOGY (PARENTAL ANIMALS)
All other changes observed in the testis and epididymides were either does not have dose response relationship or does not effect the fertility or does not have relationship between the lesions observed in the epididymides and testis. Hence, all other findings in the testis and epididymides were spontaneous, incidental and their relation with test substance administration is uncertain.
No test substance related findings were observed in seminal vesicles, prostate and ovaries. All findings observed in these organs were spontaneous or incidental.

OTHER FINDINGS (PARENTAL ANIMALS)

Observations: offspring

Viability (offspring)

yes

Clinical signs (offspring)

yes

Body weight (offspring)

yes

Sexual maturation (offspring)

not examined

Organ weights (offspring)

not examined

Gross pathology (offspring)

yes

Histopathology (offspring)

not examined

Details on results (offspring)

VIABILITY (OFFSPRING)
Test substance relted effect was observed on loss of offspring in high dose group. There was increased rate of loss of ofspring in high dose group (pre implantation, post implantation and post partum).

CLINICAL SIGNS (OFFSPRING)
In high dose groups, 1/3 dams delivered abnormal pups. (anasarca in whole litter of dam)

BODY WEIGHT (OFFSPRING)
Test substances related effect was observed in mean pup weight on day 0 and day 4 post partum. Mean pup weight in high group day 0 was 1.6 followed by 5.1, 5.4 and 5.6 in intermediate, low and control group respectively. Mean pup weight in high dose at day 4 post partumm were 0.5 followed by 6.4, 7.2 and 7.8 in low and control group respectively.

SEXUAL MATURATION (OFFSPRING)
no data

ORGAN WEIGHTS (OFFSPRING)
no data

GROSS PATHOLOGY (OFFSPRING)
External abnormalities in pups: Anasarca was observed.

HISTOPATHOLOGY (OFFSPRING)
no data

OTHER FINDINGS (OFFSPRING)

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Based on the above findings, toxicity signs in few animals treated with high dose (400 mg/kg b.w), hunched posture, abnormal gait, tremor, respiratory distress, dullness, salivation, pilo-erection. Test substance related adverse effect on body weight changes and on most of the reproduction parameters, statistically decrease in absolute weight of testis and epididymis in high doses group (400 mg/kg b.w). It is conclused that the dose 100 and 200 mg/kg b.w of N-n-butylbenzene sulphonamide was considered to be safe and non-toxic, while 400 mg/kg b.w of N-n-butylbenzenesulphonamide was considered toxic to Wistar rats in terms of reproduction, therefore the NOAEL of the test substance was regarded as 200 mg/kg b.w.

Executive summary

IUCLID 5

Printing Date 2007-12-05 14:02:03 CET

Restriction of specific regulatory purposes

Confidentiality

Owner	N-Butylbenzenesulphonamide / N-butylbenzenesulphonamide / N-butylbenzenesulfonamide / N-butylbenzenesulfonamide / 3622-84-2 / Proviron Fine Chemicals nv / Oostende / Belgium
Legal entity owner	Proviron Fine Chemicals nv / Oostende / Belgium

Endpoint study record: Short-term toxicity to fish.001

UUID	IUC5-d8d84d80-ca65-4cb0-b978-9d390bc2ebeb
Dossier UUID	0
Author	pvd / Proviron Fine Chemicals nv / Oostende / Belgium
Date	2007-12-03 16:40:15 CET
Remarks	

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	2006
Reliablility	1 (reliable without restriction)		

Data source

Reference

Reference type	study report		
Author	Rajini A. Chittibabu	**Year**	2006
Title	Acute toxicity of BBSA to freshwater fish		
Bibliographic source			
Testing laboratory	IIBAT	**Report no.**	06011
Owner company	Proviron Fine Chemicals		
Company study no.	06011	**Report date**	2006-05-03

Data access

data submitter is data owner

Data protection claimed

yes

Materials and methods

Test guideline

Qualifier	according to
Guideline	OECD Guideline 203 (Fish, Acute Toxicity Test)
Deviations	

GLP compliance

yes (incl. certificate)

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	3622-84-2
Identifier	EC number
Identity	222-823-6

Identifier IUPAC name

Identity N-butylbenzenesulfonamide

Details on test material

- Name of test material (as cited in study report): N-n-butyl benzenesulphonamide (BBSA)
- Substance type: mono constituent substance-organic
- Physical state: Clear viscous liquid
- Analytical purity: 99.89
- Impurities (identity and concentrations): 0.1% Diphenylsulphon/0.1% water/0.1% Butylbenzeensulphate/0.02% Butylamine
- Composition of test material, percentage of components: 99.89% BBSA
- Isomers composition:
- Purity test date: 08/01/2006
- Lot/batch No.: 200601080022
- Expiration date of the lot/batch: 08/01/2008
- Stability under test conditions: stabil
- Storage condition of test material: room temperature
- Other:

Confidential details on test material

- Analytical purity: 99.89%
- Impurities (identity and concentrations): 0.1% Diphenylsulphon/0.1% water/0.1% Butylbenzeensulphate/0.02% Butylamine
- Composition of test material, percentage of components: 99.89% BBSA
- Purity test date: 08/01/2006
- Lot/batch No.: 200601080022
- Expiration date of the lot/batch:08/01/2008
- Isomers composition:
- Other:

Details on properties of test surrogate or analogue material

PHYSICO-CHEMICAL PROPERTIES
- Melting point: -30°c
- Boiling point: 314°c
- Vapour pressure: < 0.001hPa
- Water solubility (under test conditions): 36.66 mg/l
- Henry's law constant: 2.17 E-006 atm-m³/mole
- log Pow: 2.1
- pKa:
- Stability in water:
- Stability in light:
- pH dependance on stability:

OTHER PROPERTIES (if relevant for this endpoint)
- Results of test for ready biodegradability: not readily biodegradable
- Other:

Details on sampling

- Concentrations: 38 mg/l
- Sampling method:Representative water samples of exposure medium, control and solvent control was collected during the conduct of the experiment at
0 and 24h and sent to the department of analytical chemistry for the verification of concentration and stability.

- Sample storage conditions before analysis: no data

Details on analytical methods

DETAILS ON PRETREATMENT
- Centrifugation: /
- Filtration: /
- Digestion (acid used, method: e.g. micro-oven): /
- Extraction (solvent used, method: e.g. liquid-liquid, SPE): /

- Clean up method:e.g. chemical used for chemistry method (Cu, Hg, ...) or phase and solvent used for SPE method: /
- Derivatisation method if used:/
- Concentration (method): /

IDENTIFICATION AND QUANTIFICATION OF TEST SUBSTANCE/PRODUCT
- Separation method (e.g. HPLC, GC): HPLC
- Conditions (column, mobile phase, etc.): no data
- Detection method (e.g. ECD, UV, MS, ICP-AES, ICP-MS): /
- Detection limits (LOD, LOQ) (indicate method of determination/calculation): /
- Reproducibility in % (indicate method of evaluation; should be given for stated concentration levels): /
- Linearity range: /
- Internal or external calibration: /
- Extraction recovery (indicate if results are corrected or not for recoveries): /
- Method of confirmation of identity of measured compound:/

Vehicle

yes

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)
- Method: the final solution was warmed btween 50-60℃ and kept on a magnetic stirrer for 30 minutes to obtain clear solution. Then it was allowed to cool at room temperature and the same was transferred to the exposure medium.
- Eluate:
- Differential loading:
- Controls: control group (without test substance) and solvent control
- Chemical name of vehicle (organic solvent, emulsifier or dispersant): acetone as organic solvent
- Concentration of vehicle in test medium (stock solution and final test solution(s) including control(s)): the solvent used in the exposure medium did not exceed 0.1 ml/l
- Evidence of undissolved material (e.g. precipitate, surface film, etc): no

Test organisms

Test organisms (species)

Brachydanio rerio (new name: Danio rerio)

Details on test organisms

TEST ORGANISM
- Common name: Brachydanio Rerio
- Strain:
- Source: supplied from a commercial fish farm.
- Age at study initiation (mean and range, SD):
- Length at study initiation (length definition, mean, range and SD): 3 +- 0.5 cm
- Weight at study initiation (mean and range, SD):
- Method of breeding: /
- Feeding during test
- Food type: commercially available aquarium fish feed
- Amount:
- Frequency: daily

ACCLIMATION
- Acclimation period:7 days
- Acclimation conditions (same as test or not): same as test
- Type and amount of food: commercially available aquarium fish feed
- Feeding frequency: feeding was stopped 24h prior to the commencement of the experiment
- Health during acclimation (any mortality observed): healthy

QUARANTINE (wild caught)
no wild caught
- Duration:
- Health/mortality:

Study design

Test type

semi-static

Water media type

freshwater

Total exposure duration

96 h **Remarks**

Post exposure observation period

The fish was quarantined for a min. of at least 12d in the laboratory before they are used for testing and the fish was fed with
commercially available aquarium fish feed, daily.
Body weight was recorded and length measured in randomly selected ten fish prior to the commencement of the acclimatization.
The fish was acclimatized 7 days prior to the experiment in the test room and the feeding was stopped 24h prior to the commencement of the
experiment.

Test conditions

Hardness

control: 200 CaCO3 mg/l
Solvent control (acetone not exceeding 0.1 ml/l): 200 CaCO3 mg/l
38 mg/l: 20/04/2006: 184 mg/l CaCO3 -- 24/04/2006: 194 mg/l CaCO3.

Test temperature

control: ca. 22.5°c
Solvent control (acetone not exceeding 0.1 ml/l): ca. 22.5°c
38 mg/l: ca. 22.5 - 25.4 °c

pH

control: 8.21-8.26
Solvent control (acetone not exceeding 0.1 ml/l): 8.20 - 8.28
38 mg/l: 8.20 - 8.38

Dissolved oxygen

control: 7.07 - 7.22 mg/l
Solvent control (acetone not exceeding 0.1 ml/l): 7.17 - 7.31 mg/l
38 mg/l: 7.19 - 7.28 mg/l

Salinity

NO DATA

Nominal and measured concentrations

NO DATA

Details on test conditions

TEST SYSTEM
- Test vessel:glass aquaria (10l)
- Type (delete if not applicable): open / closed
- Material, size, headspace, fill volume: 10l
- Aeration:
- Type of flow-through (e.g. peristaltic or proportional diluter):
- Renewal rate of test solution (frequency/flow rate):renewed daily

- No. of organisms per vessel:10
- No. of vessels per concentration (replicates):5
- No. of vessels per control (replicates):1
- No. of vessels per vehicle control (replicates):1
- Biomass loading rate:

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water:
- Total organic carbon:
- Particulate matter:
- Metals:
- Pesticides:
- Chlorine:
- Alkalinity:
- Ca/mg ratio:
- Conductivity:1120-1158µS
- Culture medium different from test medium:
- Intervals of water quality measurement:

OTHER TEST CONDITIONS
- Adjustment of pH:8.20-8.38
- Photoperiod:
- Light intensity:12h light, 12h darkness

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :

TEST CONCENTRATIONS
- Spacing factor for test concentrations:
- Justification for using less concentrations than requested by guideline:
- Range finding study
- Test concentrations:
- Results used to determine the conditions for the definitive study:

Reference substance (positive control)

yes

Any other information on materials and methods incl. tables

Results and discussions

Effect concentrations

Duration	96 h
Endpoint	LC50
Effect conc.	> 38 mg/L
Nominal/Measured	meas. (initial)
Conc. based on	test mat.
Basis for effect	mortality
Remarks (e.g. 95% CL)	

Details on results

- Behavioural abnormalities: no signs of toxicity or behavioural abnormalities were observed in fish exposed to control and 38 mg/l BBSA.
- Observations on body length and weight:
- Other biological observations:
- Mortality of control: no mortality was observed in control group, solvent control group and fish exposed to 38 mg/l
- Other adverse effects control:
- Abnormal responses:
- Any observations (e.g. precipitation) that might cause a difference between measured and nominal values:/
- Effect concentrations exceeding solubility of substance in test medium:/

Results with reference substance (positive control)

- Results with reference substance valid? yes
- Mortality: no
- LC50: > 38 mg/l
- Other:

Reported statistics and error estimates

NO DATA

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

From the experiment, the LC50 of BBSA supplied by M/s. Proviron Fine Chemicals N.V at 24, 48, 72 and 96h were considered as > 38 mg/l, respectively.

IUCLID 5

Printing Date 2007-12-05 14:07:53 CET

Restriction of specific regulatory purposes

Confidentiality

Owner	N-Butylbenzenesulphonamide / N-butylbenzenesulphonamide / N-butylbenzenesulfonamide / N-butylbenzenesulfonamide / 3622-84-2 / Proviron Fine Chemicals nv / Oostende / Belgium
Legal entity owner	Proviron Fine Chemicals nv / Oostende / Belgium

Endpoint study record: Genetic toxicity in vitro.chromosomal aberration test

UUID	IUC5-ad61cf43-1d66-4b30-98db-1dfdda0902d1
Dossier UUID	0
Author	pvd / Proviron Fine Chemicals nv / Oostende / Belgium
Date	2007-11-26 16:23:54 CET
Remarks	

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	29/06/2006 - 29/07/2006
Reliablility	1 (reliable without restriction)		

Data source

Reference

Reference type	study report		
Author	Dr.V.Thanikaivel (IIBAT)	**Year**	2006
Title	In Vitro Cytogenetic Assay measuring chromosomal Aberration frequencies induced by N-n-Benzenesulphonamide (BBSA) in human lymphocytes		
Bibliographic source			
Testing laboratory	International Institute of Biotechnology and Toxicologie (IIBAT)	**Report no.**	06097
Owner company	Proviron Fine Chemicals N.V		
Company study no.	06097	**Report date**	2006-08-29

Data access

data submitter is data owner

Data protection claimed

yes

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	
Guideline	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)
Deviations	

GLP compliance

yes (incl. certificate)

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	3622-84-2
Identifier	EC number
Identity	222-823-6
Identifier	IUPAC name
Identity	N-butylbenzenesulfonamide

Details on test material

- Name of test material (as cited in study report): N-n-butyl benzenesulphonamides (BBSA)
- Substance type: mono constituent substance-organic
- Physical state: liquid
- Analytical purity: 99.89 %
- Impurities (identity and concentrations): $\leqslant$ 0,1%water, $\leqslant$ 0.01% butylamine, $\leqslant$ 0.1% diphenylsulfon, $\leqslant$ 0.2% benzeendibutylsulphonamine, $\leqslant$ 0.1% dibutylbenzeensulfonamine, $\leqslant$ 0.1% butylparachlorobenzeensulfonamine
- Composition of test material, percentage of components: 99.89 % N-n-butyl benzenesulphonamides
- Isomers composition:
- Purity test date: 08/01/2006
- Lot/batch No.: 200601080022
- Expiration date of the lot/batch: 08/01/2008
- Stability under test conditions: stable
- Storage condition of test material: stored at room temperature
- Other:

Confidential details on test material

- Analytical purity: 99.89%
- Impurities (identity and concentrations):): $\leqslant$ 0,1%water, $\leqslant$ 0.01% butylamine, $\leqslant$ 0.1% diphenylsulfon, $\leqslant$ 0.2% benzeendibutylsulphonamine, $\leqslant$ 0.1% dibutylbenzeensulfonamine, $\leqslant$ 0.1% butylparachlorobenzeensulfonamine
- Composition of test material, percentage of components: 99,89 % N-n-butyl benzenesulphonamides
- Purity test date: 08/01/2006
- Lot/batch No.: 200601080022
- Expiration date of the lot/batch: 08/01/2008
- Isomers composition:
- Other:

Method

Target gene

cultured human lymphocytes.
Lymphocytes in peripheral blood obtained from a healthy adult male non-smoking donor without any recent history of illness and under no medication was used for this study.

Species/strain

Species/strain	lymphocytes:
Details on mammalian cell lines (if applicable)	- Type and identity of media: Lymphocytes in peripheral blood obtained from a healthy adult male non-smoking donor without any recent history of illness and under no medication was used for this study. - Properly maintained: yes - Periodically checked for Mycoplasma contamination: no - Periodically checked for karyotype stability: no - Periodically "cleansed" against high spontaneous background:no
Additional strain characteristics	not applicable

Metabolic activation	with and without
Metabolic activation system	S9

Test concentrations

pre test: 2000, 1000, 500 µg/ml BBSA.
Main study: 550, 275, 138, 69 and 35µg/ml BBSA.

Vehicle

- Vehicle(s)/solvent(s) used: DMSO
- Justification for choice of solvent/vehicle:

Controls

Negative controls	yes cultures without the test substance
Solvent / vehicle controls	yes cultures with 0.1ml of DMSO
True negative controls	yes 1 µg/ml Mitomycin C
Positive controls	yes 20µg/ml 20 µg/ml
Positive control substance	cyclophosphamide
Remarks	

Details on test system and conditions

METHOD OF APPLICATION: in medium; in agar (plate incorporation); preincubation; in suspension; as impregnation on paper disk
Cultures were incubated with PHA-M for 48h @ 37°c +-0.5°c. Following this, testsubstance BBSA was added to the culture at 550, 275, 138, 69 and 35µg/ml with and without S9.

DURATION
- Preincubation period: 4h
- Exposure duration: subsequently, cultures were washed free of test substance and incubated in freshly prepared medium with all aditions (except PHA-M and S9). Cultures were incubated at 37+-0.5°c for 32h.
- Expression time (cells in growth medium): Cultures were incubated at 37+-0.5°c for 32h.
- Selection time (if incubation with a selection agent): /
- Fixation time (start of exposure up to fixation or harvest of cells): one hour prior to harvest

SELECTION AGENT (mutation assays): /
SPINDLE INHIBITOR (cytogenetic assays): Colchicine
STAIN (for cytogenetic assays): Giemsa

NUMBER OF REPLICATIONS: thousand consecutive cells were examined.

NUMBER OF CELLS EVALUATED:thousand consecutive cells were examined and cells in metaphase were enumerated. Two hundred well spread, intact metaphases were analysed.

DETERMINATION OF CYTOTOXICITY
- Method: mitotic index; other: chromosomal aberrations

OTHER EXAMINATIONS:
- Determination of polyploidy: yes

- Determination of endoreplication: /
- Other: /

OTHER:

Evaluation criteria

- the overall aberration frequencies
- the percentage of cells with aberrations
- the percentage of cells with more than one aberration
- any evidence for increased amounts of damage with increasing dose, (i.e.) a positive dose response
- the estimated number of breaks involved in production of the different types of aberrations observed (i.e.,) complex aberrations, may have more significance than simple breaks.

Statistics

The percent frequencies of numerical aberrations, mean frequency of total structural aberrations per metaphases and mitotic indices were statistically compared between the various treatment groups, using Student 't',test for significance.
The type of aberation, its frequency, the statistical significance of any increase and its correlation to concentration in a given time period were all considered in the evaluation of the mutagenic potential of the test substance.
The criteria for a positive response are either a statistically significance increase in the number of metaphases with structural aberrations at one concentration, or a statistically significant, concentration-related increase in the number of metaphases with structural aberrations. The final decision was based upon scientific judgement.

Any other information on materials and methods incl. tables

Results and discussions

Test results

Species/strain	lymphocytes:
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	not determined
Cytotoxicity	yes 1000 - 2000 µg
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Effects of pH: not determined
- Effects of osmolality: not determined
- Evaporation from medium: no
- Water solubility: moderately soluble
- Precipitation:no
- Other confounding effects: no

RANGE-FINDING/SCREENING STUDIES:
Pre test, 3 concentrations of BBSA: 2000, 1000 and 500 µg/ml of culture was tested on human lymphocytes with and without metabolic activation system S9 to test cytotoxicity. Cytotoxicity was observed at 2000 and 1000 µg of test substance with and without

metabolic activation system. The percent mean mitotic index of 500µg/ml of culture without S9 was 6,8 and with S9 6,5.

COMPARISON WITH HISTORICAL CONTROL DATA:
no data

ADDITIONAL INFORMATION ON CYTOTOXICITY:

Remarks on results including tables and figures

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Under the conditions of the above experiment, no evidence of induction of numerical and structural aberrations was observed at the test dose levels, however, at 550 µg/ml concentration of the test substance, numerical aberration (polyploidy) was recorded. The percentage of polyploidy recorded at this concentration falls within the limit of the historical data.
Hence, N-n-benzenesulphonamide was considered as non-mutagenic in in vitro chromosomal aberration assay using human lymphocytes.

Executive summary