Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy) but-2-ylamine

EC Number: 447-920-2
CAS Number: -

CLH-O-0000001412-86-132/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: XTJ 568

Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine

EC Number: **447-920-2**

CAS Number: **-**

Index Number: **-**

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DG 5 / Department of Product Policy and chemical Substances / Management of Chemical Substances

BELGIUM

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
EC number:	447-920-2
CAS number:	-
Annex VI Index number:	-
Degree of purity:	>80.0-<100.0% (w/w)
Impurities:	See Confidential annex

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No current entry in annex VI, table 3.1
Current proposal for consideration by RAC	Acute Tox. 4, H302 Skin Corr. 1B, H314 Eye Dam. 1, H318 Repr. 2, H361fd
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4, H302 Skin Corr. 1B, H314 Eye Dam. 1, H318 Repr. 2, H361fd

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	None	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	None	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	None	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	None	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	None	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	None	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	None	Not classified	Data lacking

3.1.	Acute toxicity - oral	**Acute Tox.4, H302**			
	Acute toxicity - dermal	Not classified	None	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	None	Not classified	Data lacking
3.2.	Skin corrosion / irritation	**Skin Cor.1B, H314**			
3.3.	Serious eye damage / eye irritation	**Eye Dam. 1 H318**			
3.4.	Respiratory sensitisation	Not classified	None	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	None	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	None	Not classified	Data Lacking
3.7.	Reproductive toxicity	**Repr. Cat.2, H361fd**			Data lacking for lactation
3.8.	Specific target organ toxicity –single exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	None	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Not classified	None	Not classified	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	Not classified	None	Not classified	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Danger
Pictograms : GHS05,GHS07, GHS08
Hazard statements: H302, H314, H361fd
Precautionary: NA, P-statements are not included in Annex VI

Proposed notes assigned to an entry:

/

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There exist no current classification in annex VI of the CLP regulation

The substance XTJ 568 was notified to the Belgian Competent authority in the frame of dir. 67/548/EEC (NONS) in 2004. Based on the available physiological, toxicological and ecotoxicological data at the time of notification the Belgian Competent Authority proposed to label the substance as C, R22, R34, R52/53.

A risk assessment was performed in the frame of Art. 16(1) of Dir. 67/548/EEC and the notifier was asked to submit further information. Two studies were requested : Prenatal development toxicity test (OECD 414) in combination with 1 or 2 generation reproduction toxicity study (OECD 415/416) and submitted to ECHA in 2011. Following art. 135(2) of regulation 1907/2006 a new risk assessment of the updated dossier had to be conducted by the Belgian Competent Authority in accordance with art. 52 thereof. The updated risk assessment was finalized in 2012 and resulted in a proposal of classification inter alia for the hazard class reproductive toxicity. Proposed classification : C, Xn, R22, R34, R62, R63, R52/53.

Besides the two requested tests the notifier performed 14 new studies for regulatory purposes outside of Europe, which were submitted to ECHA in 2012. Those new studies are listed in section 7 of this document.

2.2 Short summary of the scientific justification for the CLH proposal

Human toxicology

Based on the available results of the human and animal toxicity studies, classification is required for different endpoints :

- two acute toxicity studies via oral route revealed LD_{50} between 300 and 2000 mg/kg bw/d (1000 mg/kg bw/d for the first study and between 200 and 2000 mg/kg bw/d for the second study). A classification as acute toxicity via oral route category 4 is warranted.

- For the skin corrosivity, one study showed a full thickness destruction of skin as wounds with serious exudate, grey discoloration and signs of necrosis immediately after removal of dressing which is stayed 1 hour. Therefore, a classification as skin corrosivity in category 1B is needed.

- A two generation study performed in rats revealed some variations in the fertility parameters : statistically significant minimal or slight multifocal seminiferous degeneration in testis and slight seminiferous cell debris in epididymis. These results are confirmed by the sperm evaluation which showed a statistically significant decrease of the sperm concentration and the quality of sperm was affected : statistically significant decrease the percentage of the sperm cells with normal morphology and the number of sperm with a separated head was increased. In females, an irregular cycle is also observed together with modifications in vagina and uterus, however the female reproduction parameter are unaffected. A classification for the reprotoxicity is required for the fertility.

Environment

The latest proposal for Classification and labelling was communicated to the registrant via the Risk Assessment report. Based on the newly available results of the aquatic toxicity tests and the degradability study, a review is necessary.

When applying the **CLP** criteria (2nd ATP), those are not met and the substance should **not** be classified as **hazardous for the environment.**

- Not rapidly degradable
- No potential to bioaccumulate
- Toxicity :
  - Acute toxicity :
    Most sensitive species : *Daphnia magna* : 48hEC50=88mg/l (nominal), (Bauwman, L.M., 2004b)
    EC50>1mg/l => **No classification**
  - Chronic toxicity :
    Most sensitive species : *pseudokirchnerella subcapitata (former Selenastrum capricornutum)* : 72hNOErC = 4,6 mg/l (nominal), (Bauwman, L.M., 2004c)
    Chronic toxicity studies available for all trophic levels, substance not rapidly degradable and NOEC >1mg/l => **No classification**

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No current harmonised classification and labelling in Annex VI, Table 3.1 of CLP.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No current harmonised classification and labelling in Annex VI, Table 3.2 of CLP.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Self-classification and labelling from ECHA dissemination website (registration dossier v.2013) :

Classification	Labelling
Acute tox. 4 , H302 Skin corr. 1B, H314 Eye Damage 1, H318	GHS05, GHS07 H302, H314 P280, P301+330+331, P303+361+353, P310, P305+351+338

Three different notifications exist in the C&L Inventory:

Classification	
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**
Acute tox 4 Skin Corr. 1B Eye Dam. 1	H302 H314 H318
Acute tox 4 Skin Corr. 1B Aquatic Chronic 3	H302 H314 H412
Acute tox. 3 Skin Corr. 1B Eye Dam. 1 Aquatic Chronic 3	H301 H314 H318 H412

RAC general comment
The substance to be classified is a multiconstituent substance, defined as the reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine (trade name XTJ-568), roughly containing the two components in a 3:1 ratio. This polyetherdiamine is highly soluble in aqueous solutions, resulting in a basic pH. However, for the reproductive toxicity studies, a dihydrochloride salt of the substance was used, which dissociates in water to form the free diamine. The substance to be classified will be referenced throughout the document by its trade name, XTJ-568. The CLH proposal addressed six endpoints; although other endpoints were also commented on during the public consultation, this opinion only covers those proposed by the dossier submitter.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There exist no entry in annex VI of CLP and the substance is notified in the C&L inventory, resulting in three different self-classifications.

A substance that fulfills the criteria for reproduction category 2 shall normally be subject harmonized classification and labeling (Art. 36, 1. of regulation 1272/2008).

The currently available data (the notifier performed 14 new studies for regulatory purposes outside of Europe, which were submitted to ECHA in 2012) justify classification of the substance as Acute Tox. 4, H302; Skin Corr. 1B, H314; Eye Dam. 1, H318 and Repro. 2, H361fd.

Furthermore, the e-MS disagrees with the current self-classification by the notifiers and registrant.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

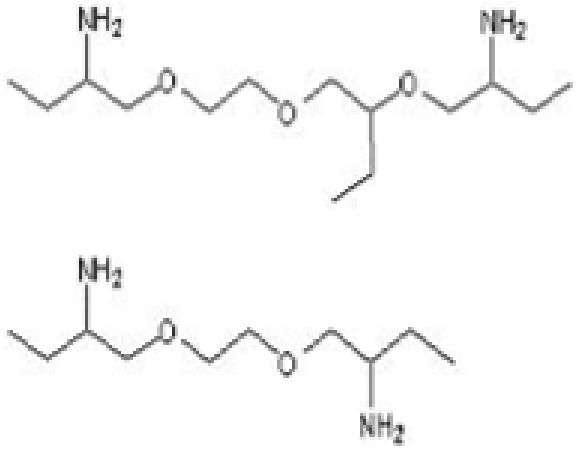
1.1 Name and other identifiers of the substance

The substance **Reaction mass of 1-[2-(2-aminobutoxy) ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy) ethoxy]methyl}propoxy) but-2-ylamine** is an organic multi-constituent substance.

Table 4: Substance identity

EC number:	447-920-2
EC name:	NA
CAS number (EC inventory):	NA
CAS number:	NA
CAS name:	NA
IUPAC name:	Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
CLP Annex VI Index number:	NA
Molecular formula:	C10H24N2O2 C14H32N2O3
Molecular weight range:	1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine : 276 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine : 204

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	19.0 % (w/w)	>= 15.0 — <= 25.0 % (w/w)	
1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine	65.0 % (w/w)	>= 58.0 — <= 72.0 % (w/w)	

Current Annex VI entry: not applicable

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
See confidential annex			

Current Annex VI entry: not applicable

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry: not applicable

1.2.1 Composition of test material

Study	Batch n°	Purity %
Physicochemical properties	8191-34	97.8% (expressed as primary amine, % of total)
Toxicological studies	8191-34	97.8%
	OG704	97% (primary amine)
	AD31GF	97%
	8666-080C	~ 100%
Ecotoxicological studies	8191-34	97.8%
	OG704	~ 100%
	DR32630507	90%

1.3 Physico-chemical properties

For physicochemical tests the material used was :

Study	Batch n°	Purity %
Physicochemical properties	8191-34 (if otherwise, it is mentioned in the summary table)	97.8% (expressed as primary amine, % of total)

Table 8: Summary of physico - chemical properties

Property	**Method**	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa		Clear colourless liquid	Several GLP studies	
Melting/freezing point	EC A.1 and OECD102 GLP	Below -81°C at 1013 hPa	Anonymous 3, 2003	A preliminary test indicated a freezing point below the lower limit of measurement (-20 °C); a main study was therefore not conducted.
Boiling point	EC A.2 and OECD103 GLP	Decompose at and above 148°C	Anonymous 4, 2003	The substance was concluded to decompose before its boiling temperature.
Relative density	EC A.3 and OECD109 GLP	0.94 at 20°C	Anonymous 5, 2003	
Vapour pressure	EC A.4 and OECD104 GLP	3.4 Pa at 20°C	Anonymous 6, 2003	Recommended measurement range for the static technique is 10 to 10E5 Pa. In the current study a capacitance manometer with a measuring range of 6x10E-3 to 2x10E5 Pa was applied extending the recommended measurement range to a lower limit of at least 10E-1 Pa as indicated in the test guideline.
Surface tension	EC A.5 and OECD115 GLP	62.4 mN/m at test conc. of 1.045g/L and 20°C	Anonymous 7, 2003	The substance is concluded not to have surface active properties.
Water solubility	EC A.6 and OECD105 GLP	>500g/L at 20°C	Anonymous 8, 2003	The test substance is concluded to be miscible with water in at least a 1:1 (w:v) ratio (i.e. water solubility of the test substance is > 1000 g substance/L water). As the water solubility is defined as units of mass per volume of solution, the water solubility is > 500 g/L, at 20 °C. A pH of 12.5 was measured at this concentration.

Partition coefficient n-octanol/water	EC A.8 and OECD117 GLP	2.0 at 24°C	Anonymous 9, 2004	The pH of the mobile phase was 11 at which >90% of the substance is in its non-ionised form. In addition to the log Pow of the main component, additional log Pow values could be calculated for two impurities (log Pow = 1.9 and 3.2).
Flash point	EC A.9 and DIN EN 22719 GLP	126°C at 101 325 Pa	Anonymous 10, 2003	
Flammability	EC A.12 and A.13 GLP	Non flammable	Anonymous 11, 2003a Anonymous 12, 2003b	non flammable Based on the theoretical assessment and experience in handling it is concluded that the submission substance is incapable of developing dangerous amounts of (flammable) gas when coming into contact with water. The submission substance is concluded not to be flammable in contact with air as based on the chemical structure no pyrophoric properties are expected. Experience in handling confirms that the substance does not ignite coming into contact with air.
Explosive properties	EC A.14 GLP	Non explosive	Anonymous 13, 2003	XTJ 568 is concluded not to be explosive as based on the chemical structure the substance does not contain any chemically instable or highly energetic groups that might lead to an explosion.
Self-ignition temperature	EC A.15, DIN 51794 and IEC standard 79-4 GLP	255°C at 1013 hPa	Anonymous 14, 2003	

Oxidising properties	Chemical structure	Non oxidising		Considering the chemical structure of the substance, XTJ 568 is considered not to contain any chemical groups indicating oxidising properties as the oxygen present in the molecule is chemically only bounded to carbon. Further testing was omitted and the submission substance is concluded to be incapable of reacting exothermically with combustible materials.
Granulometry				
Stability in organic solvents and identity of relevant degradation products				
Dissociation constant	Calculated from structure of main component	pKa of 9.9 and 9.3 for both of the R-NH3+ groups	Anonymous 16, 2004	For complex mixtures (e.g. UVCBs) containing ionisable components the assessment of pKa is clearly complicated. Estimation of the pKa values of the main component is therefore considered as an alternative. In addition to the pKa values for the R- NH3+ groups, pKa values were calculated for the R-O-R groups to be -4.7 and -5.3
Viscosity Batch n° DR32630507 Purity : 97.2%	OECD 114 GLP	9.16 mm^2/s and 4.69 mm^2/s at 20°C and 40°C	Anonymous 15, 2011	

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Not relevant for this dossier.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EU Method A.12 (Flammability (Contact with Water)) (Statement)	Non flammable	1 (reliable without restriction) key study Expert statement Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine Based on the theoretical assessment, the submission substance is expected not to be sensitive to flame, shock or friction and thus not to present any risk of explosion	Anonymous 11 (2003a)
EU Method A.13 (Pyrophoric Properties of Solids and Liquids) (Statement)	non flammable Ignition on contact with air: (No experimental results.)	1 (reliable without restriction) key study Expert statement Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	Anonymous (2003b)

closed cup ISO No., other: DIN EN 22719 (1993); Pensky-Martens EU Method A.9 (Flash-Point)	Flash point: 126 °C at 102.02 kPa	1 (reliable without restriction) key study experimental result Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy The preliminary test revealed a flash-point of 126 °C. In the main study in both replicates the flash-point was also determined to be 126 °C. The flash-point was corrected for the atmospheric pressure, which was recorded to be 102.02 kPa, to be 126 °C.	Anonymous 10 (2003)
EC A.14	Non explosive	1 (reliable without restriction) key study Expert statement Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine Based on the theoretical assessment, the submission substance is expected not to be sensitive to flame, shock or friction and thus not to present any risk of explosion.	Anonymous 13, 2003
EC A.15, DIN 51794 and IEC standard 79-4	Not self-igniting 255°C at 1013 hPa	-	Anonymous 14, 2003

Chemical structure	Non oxidising	XTJ 568 does not contain any chemical groups indicating oxidising properties as the oxygen present in the molecule is chemically only bounded to carbon	

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of physicochemical properties

None of the reported physico-chemical properties result in a classification when comparing the results with the criteria of the CLP legislation.

3.1.2 Comparison with criteria

The substance XTJ 568 is considered non-flammable. Based on the theoretical assessment in a GLP study according to test method EC A.12 (statement) and A.13 (statement) (Anonymous 11 and 12, 2003) and experience in handling it is concluded that the substance is incapable of developing dangerous amounts of (flammable) gas when coming into contact with water. The substance is concluded not to be flammable in contact with air as based on the chemical structure no pyrophoric properties are expected. Experience in handling confirms that the substance does not ignite coming into contact with air.

The flash-point of XTJ 568 was determined to be 126 °C at 101 325 Pa and is therefore outside the range relevant for classification and labelling under CLP (<60 or <75 °C).

XTJ 568 is concluded not to be explosive as based on the chemical structure the substance does not contain any chemically instable or highly energetic groups that might lead to an explosion. XTJ 568 does not fulfil the classification criteria in order to be considered as explosive substance.

The self-igniting property was examined in a study according to EEC-method A.15 and resulted in a self-ignition temperature of 255°C at 1013 hPa, which is below 400°C.

Considering the chemical structure of the substance, XTJ 568 is considered not to contain any chemical groups indicating oxidising properties as the oxygen present in the molecule is chemically only bounded to carbon. Further testing was omitted and the substance is concluded to be incapable of reacting exothermically with combustible materials.

3.1.3 Conclusions on classification and labelling

Based on the results of the performed physico-chemical studies the substance XTJ 568 is found to be not flammable, not explosive, not self-igniting and not oxidising and a classification for physic-chemical hazards following CLP-criteria is judged not necessary.

4 HUMAN HEALTH HAZARD ASSESSMENT

Study	Batch n°	Purity %
Toxicological studies	8191-34 ((if otherwise, the batch is mentioned in the summary table)	97.8%
	OG704	97% (primary amine)
	AD31GF	97%
	8666-080C	~ 100%

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 10: Summary table of experimental studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
Qualitative judgement on the toxicokinetic behaviour based on physico-chemical characteristics	Low bioaccumulation	2 (reliable with restrictions) Key study Qualitative judgement Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	Anonymous 29 (2004)

4.1.2 Human information

No information available.

4.1.3 Summary and discussion on toxicokinetics

Following a qualitative judgement based on physico-chemical characteristics of the submitted substance (Anonymous 29, 2004), the substance will show a relatively high absorption after oral administration, mainly because of its high water solubility. If absorption occurs, the test substance will be extensively metabolised in the liver and rapidly excreted via bile and/or urine. Therefore, accumulation in the body during prolonged exposure will be very low. Dermal absorption is expected to be significant, following the logPow and skin corrosive properties of the submission substance.

Basic toxicokinetics

The qualitative judgement is based on physico-chemical characteristics. Some of these are determined under conditions which are not biologically relevant (like the octanol/water partition coefficient at pH 11). Furthermore, the submission substance is a multi-constituent substance while physico-chemical characteristics in general refer to pure substances. The assessment should thus be treated with care.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral : Rat (Sprague-dawley), 1female/dose (except for 1000 mg : 3 animals). Gavage Dose levels : 320, 550, 1000 and 2000 mg/kg bw OECD Guideline 425 (Acute oral toxicity : Up-and-down procedure) Deviations : - test article not characterized for stability - characterization for composition and purity of the test article not performed - Relative humidity from 18 to 69% and normally from 30 to 70% Batch n° OG704 Purity : 97% (primary amine)	Mortality observed in two of three animals receiving the test article at 1000 mg/kg bw and in the animal receiving the test article at 2000 mg/kg bw. Clinical signs observed : - at dose level of 320 mg/kg bw, the animal showed a piloerection at 30 minutes. Same reaction in the surviving animal at 1000 mg/kg bw (no clinical signs prior to death in the 2 other animals at 1000 mg/kg bw). - at dose level of 550 mg/kg bw, the signs observed are piloerection, decreased activity, decreased body tone and abnormal gait and stance at 4 hours. - at dose level of 2000 mg/kg bw, decreased body tone and abnormal gait were observed at 30 min and death was observed at 4 hours. Bodyweight : no biologically significant effect observed. Necropsy : no visible lesions in the surviving animals whereas dark red fluid-filled intestines observed in the animals found dead. LD50 : 1000 mg/kg bw	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl }propoxy)but-2-ylamine	Anonymous 19 (2012)
Oral : Rat (Wistar strain Crl:(WI) BR), 3/sex/dose (except for 2000 mg/kg bw just female) Dose levels : 200, 2000 mg/kg bw EU Method B.1 tris (Acute Oral Toxicity – Acute Toxic Class Method) OECD guideline 423 (Acute Oral toxicity – Acute Toxic Class Method) Batch n° 8191-34 Purity : 97,8%	Mortality observed in 2 females at 2000 mg/kg bw. No mortality at 200 mg/kg bw. Bodyweight gain : normal (except for 1 female at 200 mg between days 8 and 15 : reduced bodyweight). Clinical signs : - 200 mg/kg bw : displayed hunched posture and/or piloerection. Lethargy in males on day 1. - 2000 mg/kg bw : hunched posture, piloerection, lethargy, uncoordinated movements, ptosis, shallow respiration and/or chromodacryorrhoea. The surviving female had recovered from the symptoms by day 5. Necropsy of the females found dead : dark red discolouration or foci on the glandular mucosa of the stomach, and one showed signs of beginning autolysis, and fluid in the uterus and dark red foci were noted on the thymus. No other macroscopic effects observed on the other animals. LD50 : 200 – 2000 mg/kg bw (male/female) based on : test mat.	1 (reliable without restriction) Supportive study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl }propoxy)but-2-ylamine	Anonymous 17 (2003)

Dermal : Rat (Sprague-Dawley), 5/sex Dose level : 2000 mg/kg bw Coverage : semiocclusive OECD Guideline 402 (Acute dermal toxicity) Deviations : - test article not characterized for stability - information on the characterization for composition, strength or purity of the test article not provided - Relative humidity from 29 to 74% and normally from 30 to 70% EU Method B.3 (Acute toxicity (dermal)) Batch n° OG704 Purity : 97% (primary amine)	Two females were found dead, one at day 3 and one at day 5. Abnormal gait and stance, hunched posture, decreased activity, decreased body tone, piloerection, black fur around eyes, yellow wet fur of the lower ventral area were observed in the majority of animals. All animals exhibited necrosis at the application sites. A general decline in body weight was seen on day 8, however, by day 15 body weight were comparable to day 1. Necropsy : no visible lesions except for the animal died at the day 5 (dark red intestines). LD50 : >2000 mg/kg bw (male/female) based on : test material	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl }propoxy)but-2-ylamine	Anonymous 18 (2011)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In the 2012 study (Anonymous 19), Sprague-Dawley rats were exposed to XTJ-568 at dose levels of 320, 550, 1000 and 2000 mg/kg bw. One female by group was exposed except for the dose level of 1000 mg/kg bw for which the number of animals is 3. A mortality has been observed in two of three animals receiving the test article at 1000 mg/kg bw and in the animal receiving this substance at 2000 mg/kg bw. Some clinical signs are observed as at 550 mg/kg bw piloerection (also observed at 320 and 1000 mg/kg bw after 30 min), decreased activity, decreased body tone, abnormal gait and stance at 4 hours, and at 2000 mg/kg bw decreased body tone and abnormal gait after 30min. Terminal necropsy revealed no visible lesion in the animals at 320, 550 or in the surviving animal at 1000mg/kg bw. Necropsy of the two animals found dead at 1000 and 2000mg/kg bw revealed dark red fluid-filled intestines. The third animal found dead had no visible lesion. The determined LD50 is 1000 mg/kg bw.

In the 2003 study (Anonymous 17), Wistar rats were exposed to 200 and 2000 mg/kg bw. Mortality occurred in 2 out of 3 females at 2000 mg/kg bw (no other mortality observed). Bodyweights were normal except for 1 female at 200 mg/kg bw. Clinical signs like displayed hunched posture and/or piloerection appeared at 200 mg/kg bw. Hunched posture, piloerection, lethargy, uncoordinated movements, ptosis, shallow respiration and/or chromodacryorrhoea were observed at 2000 mg/kg bw. The surviving female did recovered from the symptoms by day 5. Necropsy of dead females showed

a dark red discolouration or foci on the glandular mucosa of the stomach, 1 female had signs of beginning autolysis. The LD50 is between 200 and 2000 mg/kg bw (male/female).

4.2.1.2 Acute toxicity: inhalation

No data available

4.2.1.3 Acute toxicity: dermal

In the acute dermal toxicity study (Anonymous 18, 2011), five Sprague-Dawley rats are exposed to 2000 mg/kg bw of XTJ-568, following the OECD guidance 402. Two females were found dead (at day 3 and 5 of the treatment) and the median lethal dose was found exceed the relevant limit dose for classification of 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

Not applicable : no data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Table 12 : Summary table of relevant acute toxicity studies

Study type	Species	Results	Reference
Oral	Rat	LD50 = 1000 mg/kg bw	Anonymous 19 (2012)
Oral	Rat	LD50 = 200-2000 mg/kg bw	Anonymous 17 (2003)
Dermal	Rat	LD50 = >2000 mg/kg bw	Anonymous 18 (2011)

An oral LD50 of 1000 mg/kg bw was defined in the 2012 study. The 2003 study showed a LD50 in a range of 200-2000 mg/kg bw. There were no effect on body weight and body weight gain in any of the oral acute studies, but some clinical signs were observed such as piloerection, decreased activity,…

The dermal acute toxicity was very low with a LD50 found to be >2000 mg/kg bodyweight.

4.2.4 Comparison with criteria

Table 13 : Results of the acute toxicity studies in comparison with CLP criteria

Results	CLP criteria
Oral : Key study : LD50 : 1000 mg/kg bw	Category 4 : LD50 : > 300 and ≤ 2000 mg/kg bw → The criteria are fulfilled

Supportive study : LD50 : 200-2000 mg/kg bw	
Dermal : LD50 > 2000 mg/kg bw	Category 4 : LD50 : > 1000 and ≤ 2000 mg/kg bw → No classification warranted

4.2.5 Conclusions on classification and labelling

The acute toxicity of XTJ-568 through the oral route fulfils the CLP criteria for classification in **category 4, H302 (harmful if swallowed)**.

Based on the results of the dermal acute toxicity study (LD50 >2000 mg/kg bw), no classification is warranted.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Two acute toxicity studies via the oral route revealed LD_{50} values between 300 and 2000 mg/kg bw (1000 mg/kg bw for the first study and between 200 and 2000 mg/kg bw for the second study). The acute oral toxicity of XTJ-568 thus fulfils the CLP criteria for classification in category 4, H302 (harmful if swallowed).

Based on the results of a dermal acute toxicity study showing an LD_{50} > 2000 mg/kg bw, the DS proposed no classification for the dermal route of exposure.

There were no acute inhalation data.

Comments received during public consultation

No comments were received in relation to acute toxicity.

Assessment and comparison with the classification criteria

RAC notes that mainly female rats were studied in the acute oral studies. Although using only females for the testing is appropriate according to the test guidelines, the absence of data on male rats (other than the information that 3 male rats survived treatment with 200 mg/kg bw) and the small number of animals tested introduces some uncertainty to the assessment. Two out of three female rats died at 1000 mg/kg bw and 2000 mg/kg bw in the two studies, respectively, and none in the next lower doses (550 and 200 mg/kg bw, respectively). Based on these two studies, the oral LD_{50} in female rats seems to be below 1000 mg/kg bw, but above 550 mg/kg bw. Overall, the data support an LD_{50} value in the range of 300-2000 mg/kg bw for oral acute toxicity and RAC therefore concludes that the substance should be classified as **Acute Toxicity 4 via the oral route, H302**.

A limit dose study was performed for the dermal route (2000 mg/kg bw) and although 2 out 5 females died, all males survived, resulting in an LD_{50} > 2000 mg/kg bw for the dermal route, and thus RAC agreed with the DS that **no classification for the dermal route** was warranted.

Due to the substance being corrosive to the skin, IND waved the acute toxicity inhalation studies (Annex VII of the Reach Regulation). RAC agrees with the DS conclusion that no classification for the inhalation route of exposure was possible, since there was no data available.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the acute toxicity studies via oral and dermal routes, no specific effects on target organs were observed.

4.3.2 Comparison with criteria

The substance does not meet the criteria for classification.

4.3.3 Conclusions on classification and labelling

No classification is needed.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

4.4.2 Eye irritation

4.4.2.1 Non-human information

According to the REACH Regulation (Annex VII column 2), an in vivo eye irritation test does not need to be conducted if the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

4.4.2.4 Comparison with criteria

4.4.2.5 Conclusions on classification and labelling

A skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H314: causes severe skin burns and eye damage). Thus, in this case both classification (Skin Corr. and Eye Dam. 1) are required but the hazard statement H318 "causes serious eye damage" is not indicated on the label because of redundancy.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

There were no test data for eye damage or irritation. The DS noted that according to CLP, a skin corrosive substance is also considered to cause serious eye damage, which is indicated in the hazard statement for skin corrosion (H314: causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1B and Eye Dam. 1) are required but the hazard statement under labelling "H318, causes serious eye damage" is not needed on the label because of redundancy.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There is no specific data on eye irritation/corrosion, but RAC agrees that eye corrosion can be expected.

However, eye corrosion is covered by the proposed classification for skin corrosion (category 1B, H314 (causes severe skin burns and eye damage). RAC proposes, in line with the Commission clarifications, that there should be additional classification with **Eye Dam. 1, H318**, but no labelling (i.e. no use of H318 "Causes serious eye damage" under labelling).

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

Table 14: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White), 1 male Dose level : 0.5ml Coverage : semiocclusive (shaved) Exposure periods : 3 minutes and 1 hour. EU Method B4 (Acute toxicity : dermal irritation / corrosion)	Erythema score : For 3 minutes of exposure : 1 (immediately after removal of bandage) and 2 (1h after) For 1 hour of exposure : 4 (immediately after removal of dressing) (full thickness destruction of skin : wounds with serious exudate, grey discolouration, sign of necrosis) Edema score :	1 (reliable without restriction) Key study Experimental study Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)etho	Anonymous 20 (2003)

OECD Guideline 404 (Acute dermal irritation / corrosion) Batch n° 8191-34 Purity : 97,8%	For 3 minutes of exposure : 0 (immediately and after 1h) For 1 hour of exposure : 2 (immediately after removal of bandage)	xy]methyl}propoxy)but-2-ylamine	

4.5.1 Non-human information

In an OECD study (Anonymous 20, 2003), effects were already observed after 3 min of exposure (erythema : score of 1 immediately after removal of bandage and score of 2 after 1 hour of recovery). After 1 hour of exposure, effects became more severe with an attributed erythema score of 4 immediately after removal of bandage correlated with a full thickness destruction of the skin (wounds with serious exudate, grey discoloration, signs of necrosis). Furthermore, an oedema was also observed with a score of 2 immediately after removal. Based on the severity of the skin reactions, no further animals were exposed and the initially treated animal was sacrificed after a 1-hour exposure period.

4.5.2 Human information

No information available.

4.5.3 Summary and discussion of corrosivity

The irritation/corrosivity study indicates severe effects visible immediately after an exposure period of 1 hour. Erythema were present with signs of necrosis and oedema.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 404 rabbit study, erythema was observed after 3 min of exposure to the pure substance. After 1 hour of exposure, effects became more severe with a full thickness destruction of the skin (wounds with serious exudate, grey discolouration, signs of necrosis), and the initially treated rabbit was humanely sacrificed after the 1 hour exposure period. Therefore the DS proposed that the substance should be classified for skin corrosivity, category 1B, H314 (causes severe skin burns and eye damage).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As erythema, but no necrosis, was observed after 3 minutes exposure, category 1A is not appropriate. However, full thickness destruction of skin, wounds with serious exudate, grey discoloration and sign of necrosis were observed in the single exposed rabbit after 1 hour exposure, fulfilling the criteria for category 1B.

In addition, it is noted that dissolving XTJ-568 in aqueous media will result in a basic pH (pH 12.5 at 500 g/L), likely explaining the corrosivity observed in the rabbit and further supporting classification. Thus, RAC agrees with the proposal of the DS for classification for **skin corrosivity, 1B, H314 (causes severe skin burns and eye damage)**.

The dossier submitter did not address further labelling with EUH071 and RAC notes that there is no inhalation toxicity data available for XTJ-568. According to CLP (Article 25.1 and Annex II: 1.2.6), corrosive substances shall be labelled 'EUH071, Corrosive to the respiratory tract' if there is no acute inhalation toxicity data available and the substance may be inhaled. The substance is a liquid with low vapour pressure (3.4 Pa at 20°C) which decomposes at 148°C, before boiling. Given the physico-chemical nature of this substance and the possibility that it may be inhaled, RAC proposes additional labelling with **EUH071 (Corrosive to the respiratory tract)**.

4.5.4 Comparison with criteria

A corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. The substance is classified in category 1A if corrosive effects seen in ≥ 1 of 3 animals after an exposure period of ≤ 3 minutes and an observation of ≤ 1hour. If the effects seen after an exposure period of > 3 minutes and ≤ 1 hours and observation period of ≤ 14 days, then the category B is justified.

The rabbit exposed to the XTJ-568 substance showed a full thickness destruction of skin, wounds with serious exudate, grey discoloration and sign of necrosis immediately after an exposure of one hour and immediately after removal of dressing.

4.5.5 Conclusions on classification and labelling

According to the criteria mentioned above, the substance should be classified for **skin corrosivity, category 1B, H314 (causes severe skin burns and eye damage)**.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea pig (Dunkin-Hartley), 10 treated and 5 control Guinea pig maximisation test Induction : intradermal 0.1% and epicutaneous : 2% (in corn oil) Challenge (2 weeks after the epidermal application) : epicutaneous, semiocclusive : 1% (in corn oil) and the vehicle. EU Method B.6 (Skin sensitisation) OECD Guideline 406 (Skin sensitisation) Batch n° 8191-34 Purity : 97,8%	No. with positive reactions : 1st reading : 0 out of 10 (test group); 24h after chall.; dose 0% 1st reading : 0 out of 10 (test group); 24 after chall.; dose 1% 2nd reading : 0 out of 10 (test group); 48h after chall.; dose 0% 2nd reading : 0 out of 10 (test group); 48 after chall.; dose 1% 1st reading : 0 out of 5 (negative control); 24h after chall.; dose 0% 1st reading : 0 out of 5 (negative control); 24h after chall.; dose 1% 2nd reading : 0 out of 5 (negative control); 48h after chall.; dose 0% 2nd reading : 0 out of 5 (negative control); 48 after chall.; dose 1% No evidence of skin sensitization Signs of irritation during induction : erythema : Intradermal : 5 animals with grade 2 and 5 animals with grade 3 Epidermal : 5 animals with grade 1 and 5 animals with grade 2	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	Anonymous 21 (2003)

4.6.1.1 Non-human information

Based on the guinea pig maximisation test (Anonymous 21, 2003) conducted on 10 treated and 5 control animals, OECD 406, there was no evidence that XTJ-568 caused skin hypersensitivity in guinea pig since no responses were observed. This study indicates a sensitisation rate of 0%.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

XTJ-568 did not induce any change of sensitisation rate and neither it showed any sensitisation potential.

4.6.1.4 Comparison with criteria

Results	CLP criteria
Sensitisation rate of 0%	GPMT : Skin sensitizer 1B : if ≥30% responding at >1% intradermal induction dose or ≥30% to <60% responding at >0.1% to ≤1% intradermal induction dose

4.6.1.5 Conclusions on classification and labelling

Based on the study results, XTJ-568 does not warrant a classification as a skin sensitiser according to the CLP criteria.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data available.

4.6.2.2 Human information

No data available.

4.6.2.3 Summary and discussion of respiratory sensitisation

This information is not available.

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

No classification is required due to the absence of data.

4.7 Repeated dose toxicity

Table 16: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Rat (Wistar Crl:(WI) BR), 5/sex/dose Subacute by gavage 0, 50, 150 and 1000 mg/kg bw/d (actual ingested) Exposure : 28d, 7d/week EU Method B.7 (Repeated Dose (28 Days) Toxicity (Oral)) OECD guideline 407 (Repeated dose 28-day oral toxicity in rodents) Batch n° 8191-34 Purity : 97,8%	Mortality : at 1000 mg/kg bw/d : 3 males and 1 female were sacrificed on days 16-18 and the remaining animals were killed on day 19. Body weight : at 150 mg/kg bw/d : slightly reduced weight gain (males), stagnant growth in week 4 (2 females). At 1000 mg/kg bw/d : markedly reduced weight gain + reduced food intake. Clinical observations : - 150 mg/kg bw/d : piloerection and chromodacryorrhoea, with less frequent signs of hunched posture, leanness and general swelling of the skin (females). - 1000 mg/kg bw/d : hunched posture, piloerection, salivation, chromodacryorrhoea, swelling of the abdomen, lean appearance, alopecia (females), abnormal gait, rales, quick breathing, gasping, ptosis, squeaking and dehydration. Laboratory findings : - at 150 mg/kg bw/d : increased individual alanine / aspartate aminotransferase and alkaline phosphatase activities in females. - at 1000 mg/kg bw/d : slightly lower red blood cell counts, hemoglobin value, hematocrit level and/or mean corpuscular hemoglobin concentration level, increased alanine aminotransferase and alkaline phosphatase activities, increased glucose level. + changes less pronounced as increased urea, potassium, inorganic phosphate, chloride, calcium and cholesterol, and reduced creatinine, sodium, total protein and albumin. Effects in organs : - 1000 mg/kg bw/d : some changes are observed on stomach, lungs, thymus and caecum. The weight of liver, epididymides and thymus are modified.	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine	Anonymous 22 (2003)

Rat (Crl:CD(SD)), 10/sex/dose Subchronic by gavage 0, 15, 50 and 150 mg/kg bw/d Exposure : 13 weeks (daily, seven days per week) OECD Guideline 408 (Repeated dose 90-day oral toxicity in rodents) Batch n° OG704 Purity : 97% (primary amine)	No mortality and no clinical signs observed. Bodyweight gain and food consumption were unaffected. Hematology : 150 mg/kg bw/d : for females, slightly low neutrophil and lymphocyte counts and for males, slightly high platelet counts. Blood chemistry : 150 mg/kg bw/d : for males, slightly low creatinine concentration and for females, slightly high calcium concentration. For all group of females, slightly high potassium concentrations. Organ weights : 150 mg/kg bw/d : for males, slightly low heart and thymus weight and low brain weight. Sperm analysis : 50 and 150 mg/kg bw/d : slight reductions in % motile and progressively motile sperm, however these observations are largely due to one animal in each group. Necropsy : no abnormal macroscopic findings. 15 mg/kg bw/d : in 1 control and 1 treated females : adenocarcinoma of the mammary gland, considered to be spontaneously occurring. Motor activity : Scores for males and females were considered to be unaffected by treatment NOAEL : 150 mg/kg bw/d	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine Purity : 97%	Anonymous 23 (2011)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

In the 2003 study (Anonymous 22), exposure of rats to XTJ-568 at concentrations of 0, 50, 150 and 1000 mg/kg bw/d for 28 days by gavage was followed by the daily evaluation of clinical signs and the weekly evaluation of parameters such as functional observation tests, bodyweight and food consumption. The clinical pathology and macroscopy, organ weight and histopathology on a selection of tissues were conducted at termination. At dose levels of 150 mg/kg bw, slightly reduced body weight gain, piloerection and chromodacryorrhoea with less frequent signs of hunched posture, leanness and general swelling of the skin were observed. In addition, the blood analyse indicates an increase of individual alanine/aspartate aminotransferase and alkaline phosphatase. At the highest dose level, the effects were more discernible : markedly reduced body weight gain (statistically significant at 1% in males : 12 and 32% versus 33 and 62%, respectively at day 8 and 15) with reduced food intake along with clinical signs as hunched posture, piloerection, salivation, chromodacryorrhoea, swelling of the abdomen, lean appearance, alopecia (this one in females only), abnormal gait, rales, quick breathing, gasping, ptosis, squeaking and dehydration. Moreover, the blood analysis indicates more effects : the red blood cell counts, haemoglobin value, haematocrit levels and/or mean corpuscular haemoglobin concentration levels were slightly decreased together

with an increase of alanine aminotransferase, alkaline phosphatase, and glucose levels. Some less pronounced changes were present like an increase of urea, potassium, inorganic phosphate, chloride, calcium and cholesterol and a reduce creatinine, sodium, total protein and albumin. At the highest dose, different modifications occurred in the stomach and consisted of thickening of the limiting ridge (2/5 males) or stomach wall (1 male and 1 female), crateriform retractions of the glandular mucosa (1 male), red discolouration of the limiting ridge (1 male), glandular mucosa (1 female) or cardia (1 female), red foci on the glandular mucosa (1 female).

In the 13-weeks study, 2011 (Anonymous 23), no mortality and no clinical signs were recorded after oral exposure in rats. During the exposure, body weight, food consumption, behaviour, motor activity were unaffected. There were no ophthalmic lesions. The haematology and blood chemistry investigations revealed slightly low neutrophil (0.62 vs 0.70 in control) and lymphocyte (4.95 vs 6.37 in controls) counts with associated low total white cell counts, slightly high calcium concentrations (2.66 vs. 2.60 in controls) in females receiving 150 mg/kg bw/d. Slightly high potassium concentrations were observed in all treated group of female. Slightly high platelet counts (1304 compared to 1144 for controls) and slightly low creatinine concentrations (29 compared to 35 for controls) were apparent for males receiving 150 mg/kg bw/d. More data on haematology and blood chemistry can be found in Annex 8 (Table 35 to 38) of this document.

Necropsy showed a slightly low heart and thymus weights for all treated males and a slightly low brain weights in males receiving 150 mg/kg bw/d. These changes were minor and not considered to be of any toxicological significance. Data on organ weight in males can be found in Annex 8 (Table 39) of this document. No visible lesions were observed at gross necropsy. The only finding was an adenocarcinoma of the mammary gland in 1 control and 1 treated female at 15 mg/kg bw/d, however these results are considered to be spontaneously occurring.

A sperm analysis was conducted and the results showed a slight reductions in % motile and progressively motile sperm at 50 (88 and 54 respectively) and 150 mg/kg bw/d (85 and 52 respectively) compared to control (92 and 65 respectively), but these observations are largely due to one animal in each group. The sperm analysis can be found in Annex 8 (Table 40) of this document.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The repeated toxicity was investigated in subacute and subchronic oral studies in rats. Their results are summarised in table 17.

Table 17: summary of repeated dose toxicity studies

Species, duration, route, dose	LOAEL (mg/kg bw/d)	NOAEL (mg/kg bw/d)	Reference
Rat, 28-Days, gavage, 0, 50, 150 and 1000 mg/kg bw/d	150	50	Anonymous 22 (2003)
Rat, 13-weeks, gavage, 0, 15, 50 and 150 mg/kg bw/d	/	150	Anonymous 23 (2011)

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The NOAEL for systemic toxicity was 150 mg/kg bw/d in the 13-weeks oral toxicity study (Anonymous 23, 2011).

A substance should be classify in STOT-RE, category 2, on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were

produced at generally moderate exposure concentrations. Furthermore, the guidance value to classify for repeated dose toxicity in category 2 is between 10 and 100 mg/kg bw/d for the oral route of exposure when the exposition is of 90 days.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

The oral toxicity does not meet the CLP criteria and should not be classified for specific target organ toxicity (repeated exposure).

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
In vitro : Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Strains of bacteria used : S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 + E Coli WP2 uvr A. (with and without metabolic activation) Doses : 100, 333, 1000, 3330 and 5000 µg/plate OECD Guideline 471 (Bacterial reverse Mutation Assay) EU Method B. 13/14 (Mutagenicity – Reverse Mutation test Using Bacteria) Batch 8191-34 Purity : 97,8%	Evaluation of results : negative Test results : negative for Salmonella typhimurium : TA 1535, TA 1537, TA 100, TA 98 and also Escherichia coli WP2uvrA (with and without metabolic activation) Cytotoxicity : no	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine	Anonymous 24 (2003)
In vitro : Mammalian chromosome aberration test (chromosome aberration) Peripheral human lymphocytes With or without metabolism activation Doses : In the first main test : with and without met. act. : 333, 666 and 1000 µg/ml (3h expo, 24h fixation) In the second main test : - With met. act. : 100, 666 and 1250 µg/ml (3h expo, 48h fixation) - without met. act. : 100, 500 and 800 µg/ml (24h expo, 24h fixation) or 700, 850 and 875 µg/ml (48h expo, 48h fixation) OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test) EU Method B.10 (Mutagenicity In Vitro Mammalian Chromosome Aberration Test) Batch n° 8191-34 Purity : 97,8%	Evaluation of results : negative Test results : negative for lymphocytes: the substance didn't induce a statistically significant increase in the number of cells with chromosome aberrations in absence or in presence of S9-mix in both experiments Cytotoxicity : in the first main test: with or without met. act. : an increase of the number of polyploidy cells indicating that the substance has the potential to disturb mitotic processes and cell cycle progression.	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine	Anonymous 25 (2003)

In vivo : Micronucleus assay (chromosome aberration) Mouse (male and female) ICR, 5/sex/group Oral : gavage Doses : 0, 125, 250 and 500 mg/kg OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) ICH S2A and ICH S2B Batch N°AD31GF Purity: 97%	Clinical signs : 500 mg : all mice exhibited piloerection and lethargy. No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the dose groups. No reductions in the ratio of polychromatic erythrocytes to total erythrocytes (PCEs/ECs) in the test article dose groups.	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine	Anonymous 26 (2011)

4.9.1 Non-human information

4.9.1.1 In vitro data

In the bacterial reverse mutation assay (Anonymous 24, 2003), XTJ-568 did not produce an increase in the number of revertants with any of the different strains TA 98, TA 100, T1535 and TA1537, at any concentration levels, in the absence or presence of S9-metabolic activation. No concentration-related effect was observed.

The aberration test (Anonymous 25, 2003) tested the possible clastogenicity of XTJ-568 in two independent experiments. In the second cytogenetic assay, the substance did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations in the absence and in the presence of S9-mix. The substance is therefore not considered to be a clastogen. However, in the first cytogenic assay, the test substance increased the number of polyploid cells both in the absence and presence of S9-mix and then may have the potential to disturb mitotic processes and cell cycle progression, thereby inducing numerical chromosome aberrations.

4.9.1.2 In vivo data

The in vivo micronucleus assay (Anonymous 26, 2011) did not reveal evidence of genotoxicity. There were no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes and no reduction in the ratio of polychromatic erythrocytes to total erythrocytes (PCEs/ECs) in the different dose groups. In this test, piloerection and lethargy were observed in all mice receiving a dose of 500 mg/kg.

4.9.2 Human information

No information available.

4.9.3 Other relevant information

No information available.

4.9.4 Summary and discussion of mutagenicity

An ames test, two *in vitro* aberration tests and one *in vivo* mutagenicity study were conducted with XTJ-568. Some effects (increase number of polyploidy cells) were seen in one *in vitro* mammalian chromosome aberration test (Anonymous 25 (2003)). However, the substance was not considered as being a clastogen under the condition of that aberration test. As all other studies showed negative outcomes, the global result was seen as being negative and XTJ-568 was not considered to be a germ cell mutagen.

4.9.5 Comparison with criteria

The classification in Category 2 is based on:
– Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
– Somatic cell mutagenicity tests in vivo, in mammals; or
– Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

4.9.6 Conclusions on classification and labelling

The global result of the different mutagenicity studies does not meet the above CLP criteria and XTJ-568 should therefore not be classified for the mutagenicity endpoints.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

An Ames test, two *in vitro* aberration tests and one *in vivo* mutagenicity study were conducted with XTJ-568. Some effects (increased number of polyploid cells) were seen in one *in vitro* mammalian chromosome aberration test. However, the substance was not considered by the DS to be a clastogen under the conditions of the test. As all other studies showed negative outcomes, overall the results were seen as being negative and XTJ-568 was not considered by the DS to be a germ cell mutagen.

Comments received during public consultation

Three MSCA commented that classification is not warranted. However, one commented that the lack of data made an independent assessment difficult, and another that the available database was not sufficient to evaluate the mutagenicity (no *in vitro* gene mutation test was available, there was no evidence that the substances had reached the bone marrow in the *in vivo* micronucleus assay) and that the no classification conclusion should therefore be based on lack of data rather than on the absence of mutagenic potential.

Assessment and comparison with the classification criteria

The substance was negative in the Ames tests and no chromosome aberrations were observed in two *in vitro* chromosome aberration tests. An increased number of polyploid cells was observed in one of the studies. In contrast, no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed in an *in vivo* mouse micronucleus assay. Although some uncertainty in evaluating the study was caused by there being no reduction in the PCE/EC-ratio, and thus no evidence that the substance reaches the bone marrow, clinical signs of lethargy and piloerection likely indicated systemic exposure to XTJ-568. Thus, based on lack of *in vivo* signs of mutagenicity, RAC agrees with the DS that **no classification is warranted for germ cell mutagenicity**.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

No data available.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

The reproductive toxicity studies and the 90-day oral toxicity study do not indicate systemic toxicity or pre-neoplastic lesions at the time of necropsy.

4.10.5 Comparison with criteria

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B,

based on strength of evidence together with additional considerations (see section 3.6.2.2 of Regulation (EC) N° 1272/2008). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

4.10.6 Conclusions on classification and labelling

Regarding the observations made in the 90-day study and in the reproductive toxicity studies, a classification is not warranted.

4.11 Toxicity for reproduction

Table 19: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference

Fertility : Two-generation study Rat (Wistar), 24/sex/group Oral : gavage Doses : 0, 150, 450 and 1000 mg/kg bw/d Exposure : - F0-Generation : a minimum of 70days prior to mating and continued until euthanasia - F1-Generation : potentially exposed in utero, through nursing during lactation and directly following weaning. After weaning, pups were treated for a minimum of 70days prior to mating and continued until euthanasia - F2-generation : potentially exposed in utero and through nursing during lactation OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) EU Method B.35 (Two-generation Reproduction Toxicity Test) Batch n° 8666-080C Purity : ~ 100%	F0 generation : Mortality : 1 female at the highest dose was found with a severe wound in the abdominal (probably an attack by the other animals) and was sacrificed . Clinical signs : at the highest dose in male, a slightly lethargy and a fluffy flur were noted. In 1 male and 1 female at 150 mg/kg bw/d and in all animals at 450 and 1000 mg/kg bw/d, slight to severe salivation was observed. Incidental findings such as pale appearance and/or piloerection (1female in control and 1 female at 1000 mg/kg bw/d), rales (1 male at 450 mg) and hunched posture (1 male at 1000 mg) were observed. Bodyweight : at the highest dose, a statistically significant decrease bodyweight and bodyweight gain were noted . No treatment-related changes in the other dose group. Macroscopic examination : no treatment related effect. At 450 mg, 1 female showed two early resorptions and 1 with dark red fluid in the left uterus horn. At 1000 mg, 1 female with a late resorption. Organ weight : statistically significant changes but these modifications were slight and in the normal range of biological variation noted for rats of this strain. Microscopic examination : - minimal or slight multifocal seminiferous epithelial degeneration in testis and minimal or slight seminiferous cell debris in epididymis in 3 animals at the highest dose ($p \leq 0.01$). - minimal to moderate attenuated myometrium (uterus) in 1 animal at 0mg, 2 at 150mg, 2 at 450mg and 6 at 1000mg ($p \leq 0.01$ at 1000mg) - vagina : minimal to moderate epithelial mucification in 2 animals at 0mg, 5 at 150mg, 8 at 450mg ($p \leq 0.05$) and 4 at 1000mg, and slight to moderate attenuated epithelium in 2 at 150mg, 1 at 450mg and 5 at 1000mg ($p \leq 0.01$). Estrous cycle : 2 females at 150 and 2 at 1000 mg/kg bw/d showed an irregular cycle together with 1 female at each two groups showed an extended estrus. In the highest group, an acyclic cycle was noted in 1 female. Sperm examination : a statistically significant decrease in left epididymis organ weight (group 4), sperm concentration of the left epididymis (group 3 and 4) and left testis (group 3) were noted. Moreover, in group 3 and 4, the quality of sperm was affected (motility score and percentage of sperm cells with normal morphology were statistically significant decreased and the number of spermatocytes with separated head was marked increased). At 150mg/kg bw/d, there was a trend towards lower sperm motility (but considered within the normal range of biological variation). Female reproduction parameters : unaffected. No signs of difficult parturition, abortion or premature	1 (reliable without restriction) Key study Experimental result Test material (Common name) : XTJ-568 dihydrochloride	Anonymous 28 (2010)

	birth and all fertility parameters were in the normal range. Breeding parameters : unaffected. F1 generation : pups : Mortality : 24/5, 6/4, 5/5 and 7/5 pups per litter found dead, respectively at 0, 150, 450 and 1000 mg/kg bw/d. these pups present some incidental findings Clinical signs : incidental findings but relationship with treatment Bodyweight : at birth : within same range. At 1000mg/kg bw/d : significantly decrease from lactation days 7 in males and days 14 in females Developmental observations : at 1000 mg/kg bw/d : day of balanopreputial separation (44.6 versus 42.5 in control) and vaginal opening (34.7 compared to 33.8 in the control group) was delayed Macroscopic examinations : an ureter dilation and pale discolouration of the right kidney were noted in 1 pup of the control group. Organ weight : at 1000 mg/kg bw/d : statistically significant decrease in spleen weight + decrease absolute weight of thymus and brain and increase brain/bodyweight ratio. At 450 mg/kg bw/d : decrease thymus weight in males. F1 generation : Mortality : at 450mg/kg bw/d, one male found dead on day 49 of premating period (necropsy : autolysis and many dark-red foci on thymus) and one female died spontaneously and at 1000 mg/kg bw/d, one female died. Clinical signs : slight to moderate salivation (in males : 0/24, 1/24, 21/24 and 24/24 and in females : 0/24, 0/24, 15/23 and 22/23, respectively at 0, 150, 450 and 1000 mg/kg bw/d). In control group, 1 female showed piloerection and hunched posture. At 150 mg/kg bw/d, one male showed a wound in the neck. + other incidental findings. Bodyweight : males : significant decrease bodyweight from days 29-57 of the premating period and during all evaluation period, respectively at 450 and 1000 mg/kg bw/d. For females, the decrease bodyweight was significantly observed during days 1-8 of the premating period and from day 20 post-coitum to day 7 lactation. Food consumption : in males, at 1000 mg/kg bw/d, absolute food consumption was significantly lower during the premating and days 1-29 of the postmating period and a similar trend was noted at 450 mg/kg bw/d. For females, at 1000 mg/kg bw/d, a trend towards higher food consumption was observed during the premating and from days 11 to 14 of post-coitum (also observed at 450mg/kg bw/d) whereas a significantly lower food intake was noted during the lactation period.		

	Macroscopic examination : 3 males in the highest dose group showed reduced size of testis, epididymides and/or seminal vesicles together with oligospermia epididymis, multifocal seminiferous epithelial degeneration testis and/or hypotrophic acini seminal vesicle. In females, uterus containing fluid was observed in all groups, respectively 2, 1, 2 and 0 at 0, 150, 450 and 1000 mg/kg bw/d (correlated with the stage of oestrus cycle). Organ weight : Terminal bodyweight was significantly lower in males at 1000mg/kg bw/d. And some organs had weight differences. (See table 23) Microscopic examination : Some modifications were noted : minimal multifocal seminiferous epithelial degeneration and minimal seminiferous cell debris (1 in control and 5 at 1000 mg/kg bw/d), minimal to slight attenuated myometrium (2/24, 1/24, 3/23 and 4/23), minimal to moderate epithelial mucification in vagina (2/24, 2/24, 1/23 and 7/23, significant ($p \leq 0.01$) at 1000 mg/kg bw/d) and minimal to slight attenuated epithelium in vagina (1/24, 2/24, 2/23 and 1/23) Estrous cycle : Percentage with a regular cycle : 95.8, 100.0, 87.0 and 65.2%, respectively at 0, 150, 450 and 1000 mg/kg bw/d (number females with irregular cycle : 1, 0, 2 and 5). Extended dioestrous during pairing was noted for one female at 150 mg/kg bw/d and 2 at 450 mg/kg bw/d and an acyclic cycle was observed in 1 female at 450 mg and in 3 at 1000 mg/kg bw/d. Sperm examination : significant decrease in sperm concentration and sperm quality (decrease motility, progressive motility and low percentage of sperm cells with normal morphology (also at 450mg) at 1000 mg. Reproduction parameters : no signs of difficult or prolonged parturition or signs of abortion Breeding data : at 1000 mg/kg bw/d, lower litter size was observed and the mean number of living pups was lower than control (7.9 vs 11.1). F2 generation : pups : Mortality : no treatment-related mortality Clinical signs : incidental findings but no relationship with treatment Bodyweight : on day 1of lactation, bodyweight comparable for all groups whereas on days 14 and 21, at 1000 mg/kg bw/d, bodyweight was significantly lower. Anogenital distance : significantly lower at 150 and 450 mg/kg bw/d (no modifications at 1000 mg/kg bw/d) Macroscopic examinations : no treatment related findings. Increased brain to bodyweight ratio was noted at 1000 mg/kg bw/d.		

	NOAEL (parental) : 450 mg/kg bw/d NOAEL (reproduction) : 450 mg/kg bw/d NOAEL (developmental) : 450 mg/kg bw/d		
Developmental : Prenatal developmental study Rat (Wistar), 24 female/group Oral : gavage Doses : 0, 150, 450 and 1000 mg/kg bw/d Exposure : from day 6 to day 19 post-coitum OECD Guideline 414 (prenatal developmental toxicity study) EU Method B.31 (prenatal developmetal toxicity study) Batch n° 8666-080C Purity : ~ 100%	Maternal findings : Mortality : / Clinical signs : one animal at 1000 mg/kg bw/d showed hunched posture, piloerection and salivation from day 7. + incidental findings (salivation, alopecia, scabbing, broken tail apex). Body weight : 1000 mg/kg bw/d : lower bodyweight and bodyweight gain from the second day of treatment (not significant) 450 mg/kg bw/d : slightly lower bodyweight gain during the first week (not significant) Food consumption : 1000 mg/kg bw/d : lower food intake (not significant) more pronounced in the first week. 450 mg/kg bw/d : lower during the first week of the treatment. Macroscopic examination : incidental findings (uterus containing fluid) Maternal pregnancy data : no significant differences. Fetal findings : Sex ratio : no effect Bodyweight : not affected External malformations : No test substance related effects. At 1000mg/kg bw/d, one litter had a dead fetus with umbilical hernia. And one control fetus had cranio-rachischisis. Visceral malformations : No test substance related effects. An external hydrocephaly was observed in one fetus at 150 mg and multiple visceral malformations were noted in one fetus at 450mg. Absence of visceral malformations in the highest group. Skeletal malformations : No test substance related effects. Two fetuses in the highest group had the malformation of severely malaligned sternebrae. Other variations as 14th full ribs, reduced ossification of the skull, unossified sternebrae, …., observed in the test substance group but similar frequencies in the control group. NOAEL (maternal) : 450 mg/kg bw/d NOAEL (developmental) : 1000 mg/kg bw/d	1 (reliable without restriction) Key study Experimental result Test material (common name) : XTJ-568 dihydrochloride	Anonymous 27 (2010)

Repeated dose toxicity Rat (Crl :CD(SD), 10/sex/dose Subchronic by gavage 0, 15, 50 and 150 mg/kg bw/d Exposure : 13 weeks OECD Guideline 408 (repeated dose 90-day oral toxicity in rodents) Batch n° OG704 Purity : 97% (primary amine)	No mortality and no clinical signs observed Bodyweight gain and food consumption were unaffected Hematology : 150 mg/kg bw/d : for females, slightly low neutrophil and lymphocyte counts and for males, slightly high platelet counts. Blood chemistry : 150 mg/kg bw/d : for males, slightly low creatinine concentration and for females, slightly high calcium concentration. For all group of females, slightly high potassium concentrations. Organ weights : 150 mg/kg bw/d : for males, slightly low heart and thymus weight and low brain weight. Sperm analysis : 50 and 150 mg/kg bw/d : slight reductions in % motile and progressively motile sperm, however these observations are largely due to one animal in each group. Necropsy : no abnormal macroscopic findings. 15 mg/kg bw/d : in 1 control and 1 treated females : adenocarcinoma of the mammary gland, considered to be spontaneously occurring. NOAEL : 150 mg/kg bw/d	1 (reliable without restriction) Key study Experimental result Test material (IUPAC name) : Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine Purity : 97%	Anonymous 23 (2011)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

XTJ-568 dihydrochloride was given by gavage administration at concentrations of 0, 150, 150 and 1000 mg/kg bw/d to groups of 24 males and 24 females throughout 3 generations (F0, F1 and F2) (Anonymous 28, 2010). The premating period in F0 and F1 generation was a minimum of 70 days and exposure was continued until euthanasia. The pups were potentially exposed in utero, through nursing during lactation and directly following weaning.

During the F0-generation observation, a female was found dead with a severe wound in the abdominal region at the higher dose, probably due to an attack by the other animals present. The necropsy has revealed a missing jejunum and an alopecia on the left cheek. None of the clinical signs was considered toxicologically-relevant : a slightly lethargy and fluffy flur were observed at 1000 mg (however there were no similar observation in the F1-generation), a slight to severe salivation were also noted in 1 male and 1 female at 150 mg and in all animals at 450 and 1000 mg (the taste of the substance was considered as being the cause) and some incidental finding were observed in one or two animals.

The highest dose group revealed a significantly decrease in the bodyweight in males from day 36 of the premating period (289g versus 306g) and in females on day 64 of the premating period (207g versus 217g), day 1 of the mating period (323g vs 358g and 212g vs 221g, respectively in males and females), day 17-20 of the post-coitum period (296g vs 324g) and during the lactation (day 1, 228g vs 249g and day 21, 256g vs 274g). The bodyweight gain was also significantly decreased in males during the entire premating period (day 64, 95g vs 115g) and in females on the day 64 of the premating period (63g vs 70g) and day 20 post-coitum (37g vs 45g). The food intake had a trend towards decreasing at 1000 mg but this change was only significant during the lactation period (45g vs 59g at days 14-21).

At the necropsy, there were no gross and no treatment-related macroscopic changes. At 450 mg/kg bw/d, one female showed two early resorptions and another showed dark red fluid in the left uterus horn whereas at 1000 mg/kg bw/d, one female recorded one late resorption. Some slight changes, statistically significant, in organ weights are observed : decreased absolute brain weight (in males at 450 and 1000 mg) and increased to bodyweight ratio (males at 1000mg), increased liver to bodyweight ratio (both at 1000mg and females at 150mg), increased kidney to bodyweight ratio (males at 450 and 1000mg), increased absolute adrenal weight and adrenal to bodyweight ratio (females at 1000mg), decreased absolute spleen weight and spleen to bodyweight ratio (females at 1000mg), decreased absolute prostate weight (1000mg, 0.528g vs 0.649g), decreased seminal vesicle weight (150, 1.656g and 1000mg, 1.677g vs 1.885g), decreased absolute ovaries weight (1000mg 0.114g vs 0.132g) and increased testes to body weight ratio (1000mg, 1.00 vs 0.87g). These changes were in the normal range of biological variation and potentially caused by the relatively low terminal bodyweight.

The microscopic examination revealed some modifications. In 3 males of the highest group, a minimal to slight multifocal seminiferous epithelial degeneration ($p \leq 0.01$) was observed in testis. A minimal to slight seminiferous cell debris ($p \leq 0.01$) was also noted in the epididymis. Whereas, in females, a minimal to moderate attenuated myometrium in uterus was noted in 1, 2, 2 and 6 animals, respectively at 0, 150, 450 and 1000 mg/kg bw/d. In vagina, a minimal to moderate epithelial mucification and a minimal to moderate attenuated epithelium were observed, respectively, in 2 and 0 animals at 0mg, in 5 and 2 animals at 150mg, in 8 ($p \leq 0.05$) and 1 animals at 450 mg and in 4 and 5 ($p \leq 0.01$) animals at 1000mg.

The oestrous cycle evaluation showed 2 females each at 150mg/kg bw/d and 1000mg/kg bw/d with an irregular cycle and 1 females at each 2 doses with an extended oestrous.

The XTJ-568 dihydrochloride treatment revealed a statistically significant decrease in the left epididymis organ weight (1000mg/kg bw/d), sperm concentration of the left epididymis (450 and 1000 mg/kg bw/d) and left testis (450mg/kg bw/d). The quality of sperm was also affected in these 2 groups : the motility score and the percentage of sperm cells were decreased and the number of sperm with separated head was increased. At 150mg/kg bw/d, there was a trend towards lower sperm motility scores however this modification was considered to be within the normal range of biological variation.

Table 20 : Sperm motility, concentration and morphology

	GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
MOTILITY				
MEDIAN	3	3 $	3 $$	2 $$
N	23	24	24	24
LEFT EPIDIDYMIS WEIGHT				
MEAN	0.19	0.19	0.18	0.16 *
ST.DEV.	0.03	0.04	0.04	0.03
N	24	23	24	24
LEFT EPIDIDYMIS CONCENTRATION				
MEAN	470.6	390.6	365.8 *	336.3 **
ST.DEV.	137.5	157.5	110.0	130.4
N	24	23	24	24
LEFT TESTIS WEIGHT				
MEAN	1.36	1.33	1.31	1.37
ST.DEV.	0.10	0.18	0.13	0.16
N	24	23	24	24
LEFT TESTIS CONCENTRATION				
MEAN	82.1	78.8	66.4 *	71.2
ST.DEV.	15.4	22.6	19.8	19.9
N	24	23	24	24
NORMAL MORPHOLOGY				
MEAN	87	85	63 $$	63 $$
ST.DEV.	9	8	24	13
N	23	23	20	23

Units Motility (Score)
Weight (Grams)
Concentration (Millions/Gram)
Normal Morphology (%)

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level
$/$$ Wilcoxon test significant at 5% ($) or 1% ($$) level

The F0-female reproduction parameters did not show any sign of difficult parturition, abortion or premature birth and all fertility parameters (number of implantation sites, conception rate, …) were well within the normal range of variation.

The F1-pup generation did not reveal treatment-related mortality, indeed the number of pups per litter which were found dead was 24/5, 6/4, 5/5 and 7/5, respectively in the control, low dose, mid dose and high dose group. The pups dead showed cannibalism or autolysis. At 1000 mg/kg bw/d, the pups showed a significantly lower bodyweight in male and female from lactation days 7 and 14, respectively (day 7, 12.2g vs 13.7g and day 14, 24.1g vs 30.4g), whereas this parameter was in range of the control animals at birth (5.9g vs 5.8g).

Moreover, in the highest group, the day of balanopreputial (statistically significant) and vaginal opening was delayed, respectively 44.6d vs 42.5d and 34.7d vs 33.8d.

The F1-pup show, also in the high dose group, a statistically significant decrease in the weight of spleen together with a decrease of the thymus and the brain weights and an increase brain/body weight ratio. The decrease of thymus weight was already observed at 450 mg/kg bw/d in males.

Table 21 : Organ weight in male and female pups

MALE PUPS

		GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/K
BODY WEIGHT (GRAM)	MEAN	48.43	49.15	44.66	34.65 **
	ST.DEV.	6.30	4.44	4.38	4.81
	N	20	22	15	16
BRAIN (GRAM)	MEAN	1.44	1.45	1.40	1.35 **
	ST.DEV.	0.05	0.07	0.09	0.07
	N	20	22	15	16
BRAIN (%)	MEAN	3.01	2.97	3.15	3.95 **
	ST.DEV.	0.34	0.18	0.32	0.43
	N	20	22	15	16
SPLEEN (GRAM)	MEAN	0.228	0.238	0.202	0.132 **
	ST.DEV.	0.055	0.058	0.037	0.038
	N	20	22	15	16
SPLEEN (%)	MEAN	0.467	0.481	0.450	0.377 **
	ST.DEV.	0.083	0.082	0.057	0.069
	N	20	22	15	16
THYMUS (GRAM)	MEAN	0.228	0.224	0.197 *	0.156 **
	ST.DEV.	0.040	0.030	0.043	0.036
	N	20	22	15	16
THYMUS (%)	MEAN	0.473	0.458	0.439	0.447
	ST.DEV.	0.068	0.058	0.066	0.065
	N	20	22	15	16
F0-GENERATION FEMALE PUPS					
BODY WEIGHT (GRAM)	MEAN	45.69	47.35	44.45	35.59 **
	ST.DEV.	5.32	3.78	5.39	6.50
	N	20	21	19	19
BRAIN (GRAM)	MEAN	1.38	1.40	1.37	1.31 **
	ST.DEV.	0.05	0.05	0.05	0.08
	N	20	21	19	19
BRAIN (%)	MEAN	3.05	2.97	3.11	3.77 **
	ST.DEV.	0.31	0.20	0.27	0.48
	N	20	21	19	19
SPLEEN (GRAM)	MEAN	0.233	0.246	0.225	0.152 **
	ST.DEV.	0.057	0.056	0.063	0.040
	N	20	21	19	19
SPLEEN (%)	MEAN	0.505	0.518	0.500	0.423 **
	ST.DEV.	0.081	0.092	0.100	0.056
	N	20	21	19	19
THYMUS (GRAM)	MEAN	0.227	0.229	0.215	0.167 **
	ST.DEV.	0.038	0.036	0.046	0.047
	N	20	21	19	19
THYMUS (%)	MEAN	0.499	0.482	0.481	0.464
	ST.DEV.	0.076	0.062	0.078	0.061
	N	20	21	19	19

At necropsy, incidental findings were observed : no milk in the stomach, small size. In the control group, one pup showed an ureter dilatation and a pale discolouration of the right kidney.

1 female and 1 male were found dead at 450 mg/kg bw/d and 1 female at 1000 mg/kg bw/d in the F1-generation. The male found dead did show a beginning of autolysis and many dark-red foci on the thymus.

Excessive salivation was seen in 0/24, 1/24, 21/24 and 24/24, respectively in the different groups, and were considered to be related to the taste of the formulation. Other incidental clinical signs, such as piloerection and/or hunched posture were noted in one female in the control group and in 2 females in the highest dose group. A wound in the neck was observed in one male at 150 mg/kg bw/d. The study revealed also different slight effect such as alopecia, scabbing, scales and/or wounds, chromodacryorrhoea,…

The bodyweight was lower in males treated with 450 and 1000 mg/kg bw/d than the controls during the whole observation period correlated with a lower food consumption. This decrease was significant for 450 mg/kg bw/d from day 29 to day 57 (281g vs 299g at d29 and 342g vs 362g at d57, $p \leq 0.05$) of the premating period and for 1000 mg/kg bw/d during the entire premating (at day 64, 312g vs 373g in control), mating (at day 15, 329g vs 396g) and post-mating (at day 36, 365g vs 422g) period. In females, the highest dose group showed a trend towards, statistically significant, lower bodyweight and this decrease was significant during days 1-8 of pre-mating period (109g vs 122g at day 1 and 131g vs 142g at day 8) and from day 20 post-coitum (281g vs 306g in control) until day 7 of lactation (241g vs 258g).

In the high dose group, 3 males had macroscopic modifications as reduced size of testes (animal number 285, 288), epididymides (no. 285), and/or seminal vesicles (nos. 286, 288). These modifications were correlated with oligospermia epididymis (no. 285), multifocal seminiferous epithelial degeneration testis (no. 285), hypotrophic acini seminal vesicle (no. 286). Some incidental findings seen among control or treated animals were observed (enlarged spleen, bent tail apex, black-brown and hard nodule in the liver, black-brown foci on the thymus, and alopecia of various body parts). Fluid in uterus was noted in 2/24, 1/24, 2/23 and 0/23 females treated, respectively, at 0, 150, 450 and 1000 mg/kg bw/d, this observation was due to the stage of the oestrous cycle.

Table 22 : Macroscopic findings in the F1-generation

MALES

	GROUP 1 CONTROL	GROUP 2 150MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
INTERCURRENT DEATH				
Animals examined			1	
Animals affected			1	
General observations				
Beginning autolysis			1	
Thymus				
Focus/foci			1	
NECROPSY				
Animals examined	24	24	23	24
Animals without findings	24	23	21	21
Animals affected	0	1	2	3
Testes				
Reduced in size	0	0	0	2
Epididymides				
Reduced in size	0	0	0	1
Seminal vesicles				
Reduced in size	0	0	0	2
Spleen				
Enlarged	0	0	0	1
Skin				
Alopecia	0	0	2	0
Bone				
Tail bent	0	1	0	0

FEMALES

NECROPSY				
Animals examined	24	24	23	23
Animals without findings	16	21	19	21
Animals affected	8	3	4	2
Liver				
Nodule(s)	0	1	0	0
Uterus				
Contains fluid	2	1	2	0
Thymus				
Focus/foci	1	0	0	0
Skin				
Alopecia	5	1	4	2

Some statistically significant changes in organ weight were observed (see table 23). Some variations were observed in reproductive organs : decreased absolute testes weight and increased testes to body weight ratios in males at 1000 mg/kg bw/d, decreased absolute prostate weight in males at 1000 mg/kg bw/d, decreased absolute epididymides weight in males at 450 and 1000 mg/kg bw/d, decreased absolute seminal vesicle weight in males at 150 mg/kg bw/d and at 1000 mg/kg bw/d.

Table 23 : Organ weight in the F1-generation

MALES

		GROUP 1 CONTROL	GROUP 2 150MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
NECROPSY					
BODY W.	MEAN	424	427	404	348 **
(GRAM)	ST.DEV	34	35	35	32
	N	24	24	23	24
BRAIN	MEAN	2.03	2.04	1.98	1.90 **
(GRAM)	ST.DEV	0.06	0.08	0.07	0.09
	N	24	24	23	24
PITUITARY	MEAN	0.015	0.017	0.015	0.016
(GRAM)	ST.DEV	0.003	0.004	0.004	0.004
	N	24	24	23	24
LIVER	MEAN	12.10	12.35	12.19	10.86 **
(GRAM)	ST.DEV	1.61	1.18	1.39	1.42
	N	24	24	23	24
THYROIDS	MEAN	0.033	0.035	0.033	0.031
(GRAM)	ST.DEV	0.007	0.007	0.008	0.006
	N	23	24	23	24
KIDNEYS	MEAN	2.62	2.61	2.63	2.34 **
(GRAM)	ST.DEV	0.31	0.15	0.31	0.28
	N	24	24	23	24
ADRENALS	MEAN	0.051	0.050	0.049	0.047
(GRAM)	ST.DEV	0.007	0.008	0.006	0.006
	N	24	24	23	24
SPLEEN	MEAN	0.655	0.643	0.624	0.578 *
(GRAM)	ST.DEV	0.086	0.093	0.079	0.138
	N	24	24	23	24
TESTES	MEAN	3.61	3.71	3.62	3.23 **
(GRAM)	ST.DEV	0.30	0.36	0.32	0.44
	N	24	23	23	24
PROSTATE	MEAN	0.721	0.674	0.690	0.594 **
(GRAM)	ST.DEV	0.169	0.136	0.142	0.081
	N	24	24	23	24
EPIDIDYMIDES	MEAN	1.257	1.267	1.168 *	1.033 **
(GRAM)	ST.DEV	0.106	0.128	0.111	0.133
	N	24	24	23	24
SEMINAL VES	MEAN	2.011	1.794 *	1.823	1.675 **
(GRAM)	ST.DEV	0.272	0.186	0.341	0.283
	N	24	24	23	24

FEMALES

		GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
NECROPSY					
BODY W.	MEAN	261	270	270	248
(GRAM)	ST.DEV	16	19	28	19
	N	24	24	23	23
BRAIN	MEAN	1.82	1.85	1.83	1.76 *
(GRAM)	ST.DEV	0.09	0.13	0.09	0.07
	N	24	24	23	23
PITUITARY	MEAN	0.016	0.017	0.017	0.016
(GRAM)	ST.DEV	0.003	0.002	0.004	0.003
	N	24	24	23	23
LIVER	MEAN	11.20	11.73	12.44 *	11.24
(GRAM)	ST.DEV	1.10	1.42	1.54	1.82
	N	24	24	23	23
THYROIDS	MEAN	0.031	0.031	0.027	0.028
(GRAM)	ST.DEV	0.006	0.005	0.007	0.006
	N	24	24	23	23
KIDNEYS	MEAN	1.94	2.02	2.03	1.86
(GRAM)	ST.DEV	0.14	0.13	0.21	0.20
	N	24	24	23	23
ADRENALS	MEAN	0.067	0.071	0.072	0.070
(GRAM)	ST.DEV	0.010	0.008	0.012	0.013
	N	24	24	23	23
SPLEEN	MEAN	0.509	0.532	0.514	0.484
(GRAM)	ST.DEV	0.052	0.052	0.054	0.066
	N	24	24	23	23
OVARIES	MEAN	0.124	0.131	0.127	0.118
(GRAM)	ST.DEV	0.019	0.019	0.028	0.021
	N	24	24	23	23
UTERUS	MEAN	0.508	0.562	0.511	0.498
(GRAM)	ST.DEV	0.127	0.151	0.156	0.167
	N	24	24	23	23

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

The microscopic examination of females revealed a minimal to slight attenuated myometrium in uterus, a minimal to moderate epithelial mucification and a minimal to slight attenuated epithelium in vagina, respectively in 2, 2 and 1 in control group, in 1, 2 and 2 at 150mg, in 2, 1 and 2 at 450mg and in 4, 7 and 1 at 1000mg/kg bw/d. In males, a minimal multifocal seminiferous epithelial degeneration in testis and a minimal seminiferous cell debris in 1 control and 5 at the highest group were observed.

The percentage of females per group that were classified as having a regular estrous cycle (number of females with irregular cycle) was 95.8 (1 with extended di-estrous), 100.0 (0), 87.0 (2 with extended di-estrous) and 65.2% (5) in the different groups. Furthermore, one female at 450 and 3 females at 1000 mg/kg bw/d showed an acyclic cycle. One female in the high dose group showed red discoloured vaginal secretion on days 14 and 15 post-coitum, the reason is not established

A significant decrease of concentration and quality (motility, progressive motility) was seen during the sperm evaluation of the high dose group. A low percentage of sperm cells with normal morphology was also noted at 450 and 1000 mg/kg bw/d.

Table 24 : Sperm motility, concentration and morphology in the F1-generation

	GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
MOTILITY				
MEAN	54	64	46	30 $$
ST.DEV.	25	21	27	24
N	22	21	19	20
PROGRESSIVE MOTILITY				
MEAN	28	29	18	13 $$
ST.DEV.	16	17	17	12
N	22	21	19	20
LEFT EPIDIDYMIS WEIGHT				
MEAN	0.20	0.21	0.20	0.18 *
ST.DEV.	0.03	0.04	0.02	0.03
N	24	24	23	24
LEFT EPIDIDYMIS CONCENTRATION				
MEAN	451.7	381.0	399.2	307.6 **
ST.DEV.	134.1	159.4	155.4	110.8
N	24	24	23	24
LEFT TESTIS WEIGHT				
MEAN	1.48	1.53	1.41	1.29 **
ST.DEV.	0.18	0.17	0.19	0.21
N	24	24	23	24
LEFT TESTIS CONCENTRATION				
MEAN	60.9	62.7	50.6	52.1
ST.DEV.	27.3	26.7	28.5	32.0
N	24	24	23	24
NORMAL MORPHOLOGY				
MEAN	92	90	65 $$	64 $$
ST.DEV.	8	10	25	16
N	23	24	23	24

Units Motility (%)
Progressive motility (%)
Weight (Grams)
Concentration (Millions/Gram)
Normal Morphology (%)

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level
$/$$ Wilcoxon test significant at 5% ($) or 1% ($$) level

Following treatment at 1000 mg/kg bw/d, litter size was significantly lower than controls. The mean number of living pups at first litter check was 7.9 in high group as compared to 11.1 in the control group.

Table 25 : Breeding data for the F1-generation

	GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
LITTERS				
TOTAL	24	23	22	20
DURATION OF GESTATION				
MEAN (+)	21.3	21.2	21.1	21.4
ST.DEV.	0.5	0.4	0.3	0.5
N	24	23	22	20
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED (#)	1	0	1	1
TOTAL	1	0	2	1
MEAN (+)	0.0	0.0	0.1	0.1
ST.DEV.	0.2	0.0	0.4	0.2
N	24	23	22	20
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES (#)	53 / 47	49 / 51	53 / 47	48 / 52
TOTAL	266	263	255	158
MEAN (+)	11.1	11.4	11.6	7.9 ++
ST.DEV.	3.0	1.9	2.3	2.8
N	24	23	22	20
POSTNATAL LOSS				
% OF LIVING PUPS	0.8	0.8	1.2	1.3
LITTERS AFFECTED (#)	2	2	3	2
TOTAL (#)	2	2	3	2
MEAN (+)	0.1	0.1	0.1	0.1
ST.DEV.	0.3	0.3	0.4	0.3
N	24	23	22	20
CULLED PUPS				
TOTAL	81	78	77	20
LIVING PUPS DAY 4 P.P.				
TOTAL	183	183	175	136
MEAN (+)	7.6	8.0	8.0	6.8 +
ST.DEV.	1.2	0.2	0.2	2.0
N	24	23	22	20
BREEDING LOSS DAYS 5 – 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	0.0	0.0	0.6	0.0
LITTERS AFFECTED (#)	0	0	1	0
TOTAL (#)	0	0	1	0
MEAN (+)	0.0	0.0	0.0	0.0
ST.DEV.	0.0	0.0	0.2	0.0
N	24	23	22	20
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES (#)	51 / 49	49 / 51	50 / 50	49 / 51
TOTAL	183	183	174	136
MEAN (+)	7.6	8.0	7.9	6.8 +
ST.DEV.	1.2	0.2	0.3	2.0
N	24	23	22	20
VIABILITY INDEX (#)	99.2	99.2	98.8	98.7
WEANING INDEX (#)	100.0	100.0	99.4	100.0

The evaluation of the F2-generation did not revealed any gross modifications. The mortality observed during the first days of lactation was 3 pups (from 3 litters), 2 (2), 6 (3) and 3 (3), respectively at 0, 150, 450 and 1000 mg/kg bw/d.

The body weight were statistically significant decreased at 1000 mg/kg bw/d in both sexes on days 14 (28.1g vs 31.4 in control group) and 21 (44.3g vs 49.1g) post-partum. Some incidental findings, with no relationship with treatment, were noted in the pups as small size, pale or cold appearance, no milk in stomach, missing tail apex, alopecia of various body parts, cannibalism and/or autolysis.

At necropsy, an increased, statistically significant, brain to bodyweight ratio was noted in females at 1000 mg/kg bw/d (3.2% vs 2.96% in control). The anogenital distance was significantly lower in males at 150 mg/kg bw/d and 450 mg/kg bw/d and in females at 150 mg/kg bw/d.

Table 26: Anogenital distance of pups F2-generation

		GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
anogenital distance M	MEAN	2.71	2.51	2.57	2.63
	MEDIAN (+)	2.64	2.54 ++	2.60 ++	2.65
	N	24	23	21	18
anogenital distance F	MEAN	0.97	0.93	0.95	1.03
	MEDIAN (+)	0.95	0.93	0.94	1.04
	N	23	23	22	18

Those findings on sperm toxicity were not confirmed by the 13 weeks study described under section 4.7 (Anonymous 23, 2011), were a special examination on sperm motility was conducted. The results only showed slight reductions in % motile and progressively motile sperm at 50 (88 and 54 respectively) and 150 mg/kg bw/d (85 and 52 respectively) compared to control (92 and 65 respectively), but these observations are not statistically significant and largely due to one animal in each group. The data on sperm analysis are summarised in table 27.

Table 27: Sperm analysis

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group		Motile sperm (%)	Progressively motile sperm (%)	-------- Cauda epididymis-------- Weight (g)	Sperm count (millions/g)	Total (million)	---------- Testis ---------- Weight (g)	Sperm count (millions/g)	Total (million)
Statistical test:		Sh	Wi	Sh	Wi	Wi	Wi	Wi	Wi
1	Mean	92	56	0.255	946	241	1.86	135	249
	SD	5	10	0.028	230	73	0.18	23	30
	n	10	10	10	10	10	10	10	10
2	Mean	95	59	0.312	870	248	1.81	138	251
	SD	2	10	0.205	167	80	0.21	13	45
	n	9	9	10	10	10	10	10	10
3	Mean	88	54	0.259	950	246	1.92	131	250
	SD	9	10	0.026	152	48	0.11	26	48
	n	10	10	10	10	10	10	10	10
4	Mean	85	52	0.241	928	223	1.82	134	244
	SD	30	22	0.025	176	47	0.09	19	39
	n	10	10	10	10	10	10	10	10

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The developmental toxicity of XTJ-568 (Anonymous 27, 2010) has been investigated in 96 mated female wistar rats. The substance was administered once daily by oral gavage from day 6 to 19 post-coitum. The rats were assigned to four dose groups of 0, 150, 450 and 1000 mg/kg bw/d.

The maternal examination revealed no mortalities and no relevant clinical signs. One animal in the highest dose group showed a hunched posture, piloerection and salivation from day 7 of treatment. However these findings were limited to one animal and the toxicological significance was doubted. In the highest dose group, a lower body weight and body weight gain together with a lower food

intake were observed, but these findings were not always statistically significant. At day 20 post coitum, body weight was 295g in the high dose group compared to 315g in the control group, the body weight gain was also modified (27g vs 38g).

The macroscopic examination did not reveal any alterations considered as a result of treatment. Incidental findings as uterus containing fluid, alopecia and scabbing were observed in treated and control groups.

Table 28 : Macroscopic examination

	GROUP 1 CONTROL	GROUP 2 150 MG/KG	GROUP 3 450 MG/KG	GROUP 4 1000 MG/KG
POST COITUM				
Animals examined	24	24	24	24
Animals without findings	22	23	22	23
Animals affected	2	1	2	1
Uterus				
Contains fluid	1	0	0	0
Skin				
Alopecia	1	1	1	1
Scab formation	1	1	0	0
Bone				
Tail bent	0	0	1	0

The number of pregnant females were 21, 20, 24 and 20 in groups 1, 2, 3 and 4, respectively. No abortion or early deliveries were noted and no significant differences were observed regarding the number of corpora lutea, implantation sites, viable or dead foetuses, early or late resorptions, or pre- and post-implantation loss.

Table 29 : Fetal data

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	111	123	234	0	9	1	10	244	282	38	NA	21
MEAN	5.3	5.9	11.1	0.0	0.4	0.0	0.5	11.6	13.4	1.8	3.5	
S.D.	2.03	1.65	1.56	0.00	0.75	0.22	0.87	1.63	1.66	1.54	0.24	
2 TOTAL	114	106	220	0	14	0	14	234	274	40	NA	20
MEAN	5.7	5.3	11.0	0.0	0.7	0.0	0.7	11.7	13.7	2.0	3.6*	
S.D.	1.56	1.72	2.43	0.00	0.80	0.00	0.80	2.49	1.75	2.08	0.18	
3 TOTAL	133	150	283	0	18	0	18	301	337	36	NA	24
MEAN	5.5	6.3	11.8	0.0	0.8	0.0	0.8	12.5	14.0	1.5	3.5	
S.D.	1.64	1.70	1.96	0.00	1.11	0.00	1.11	1.25	1.83	1.82	0.22	
4 TOTAL	103	133	236	1	15	0	16	252	297	45	NA	20
MEAN	5.2	6.7	11.8	0.1	0.8	0.0	0.8	12.6	14.9	2.3	3.3	
S.D.	2.16	1.93	1.70	0.22	0.91	0.00	0.95	1.70	2.06	1.74	0.26	

* = Significantly different from the control group at 0.05
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG 2- 150 MG/KG 3- 450 MG/KG 4- 1000 MG/KG

The fetal findings did not reveal a difference in the mean litter size (11.1, 11.0, 11.8 and 11.8 respectively at 0, 150, 450 and 1000 mg/kg bw/d), in the sex ratio and in the fetal body weight. There were no test substance-related effects on fetal external, fetal visceral and fetal skeletal morphology. A litter in the high dose group had a dead fetus showing umbilical hernia, not considered to be induced by the test substance, and a control fetus had cranio-rachischisis (open cranial vault and vertebral column). During the visceral observation, an external hydrocephaly was observed in one fetus in the low dose group and multiple visceral malformations (dextrocardia, lobular agenesis and dysgenesis

of the lungs, and displacement of the left adrenal) were noted in one fetus at 450 mg/kg bw/d. Due to the absence of malformations in the highest dose group, these findings were considered to be of spontaneous origin and not attributed to the test substance administration. One fetus, in the low dose group, had a dark red area on a liver lobe. The skeletal examination revealed malformation of severely malaligned sternebrae in two fetus of the highest dose group. Skeletal variations that occurred in the all test substance groups were 14th full ribs, 14th rudimentary ribs, 27 presacral vertebrae, cervical centrum1 ossified, reduced ossification of the skull, bent rib, slightly to moderately malaligned sternebrae, 7th cervical rib, unossified sternebrae, ossified tarsals, 7th sternebra and accessory skull bones. However, all of the these variations occurred at similar frequencies in the control group.

Table 30 : Fetus and litter malformations

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	234	220	283	236	21	20	24	20
CRANIORACHISCHISIS	1	0	0	0	1	0	0	0
NUMBER EXAMINED VISCERALLY	116	112	143	119	21	20	24	20
ADRENAL DISPLACED	0	0	1	0	0	0	1	0
LUNGS- LOBULAR AGENESIS	0	0	1	0	0	0	1	0
LUNGS- LOBULAR DYSGENESIS	0	0	1	0	0	0	1	0
DEXTROCARDIA	0	0	1	0	0	0	1	0
HYDROCEPHALY	0	1	0	0	0	1	0	0
NUMBER EXAMINED SKELETALLY	118	108	140	117	21	20	24	20
STERNEBRA(E) MALALIGNED (SEVERE)	0	0	0	2	0	0	0	2
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	1	0	0	0	1	0	0	0
SOFT TISSUE :	0	1	1	0	0	1	1	0
SKELETAL :	0	0	0	2	0	0	0	2
COMBINED :	1	1	1	2	1	1	1	2

1- 0 MG/KG 2- 150 MG/KG 3- 450 MG/KG 4- 1000 MG/KG

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	234	220	283	236	21	20	24	20
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	116	112	143	119	21	20	24	20
LIVER- ACCESSORY LOBULE(S)	11	14	13	16	6	8	6	9
HEMORRHAGIC ADRENAL(S)	2	0	0	4	2	0	0	2
ACCESSORY SPLEEN(S)	0	1	0	0	0	1	0	0
HEART- EXTRA PAPILLARY MUSCLE	0	0	0	1	0	0	0	1
NUMBER EXAMINED SKELETALLY	118	108	140	117	21	20	24	20
14TH FULL RIB(S)	8	3	4	1	4	3	2	1
14TH RUDIMENTARY RIB(S)	55	55	55	50	18	18	20	17
27 PRESACRAL VERTEBRAE	5	0	2	1	3	0	1	1
CERVICAL CENTRUM #1 OSSIFIED	40	45	30	34	15	17	16	16
REDUCED OSSIFICATION OF THE SKULL	11	22	17	21	6	8	10	10
BENT RIB(S)	8	10	15	21	6	8	10	9
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	6	4	5	2	5	4	5	2
7TH CERVICAL RIB(S)	16	19	19	25	10	12	13	13
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	16	6	13	17	9	4	9	8
HYOID UNOSSIFIED	3	0	0	0	2	0	0	0
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	1	1	2	1	1	1	2	1
TARSAL(S) OSSIFIED	0	0	0	1	0	0	0	1
7TH STERNEBRA	0	0	0	1	0	0	0	1
ACCESSORY SKULL BONE(S)	1	1	2	0	1	1	2	0
ENTIRE STERNUM UNOSSIFIED	1	0	0	0	1	0	0	0
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	1	0	0	0	1	0	0	0
VERTEBRAL CENTRA UNOSSIFIED	1	0	0	0	1	0	0	0
METACARPAL(S) AND/OR METATARSAL(S) UNOSSIFIED	1	0	0	0	1	0	0	0

1- 0 MG/KG 2- 150 MG/KG 3- 450 MG/KG 4- 1000 MG/KG

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

No other information available.

4.11.4 Summary and discussion of reproductive toxicity

The two-generation study (Anonymous 28, 2010) was performed in rats. The oral exposure did not revealed severe modifications in mortality and clinical signs. Some variations were noted in fertility parameters in the F0-generation. Microscopic examination showed, in the highest male group (1000mg/kg bw/d), statistically significant minimal or slight multifocal seminiferous degeneration in testis and minimal or slight seminiferous cell debris in epididymis. The sperm evaluation confirmed these results and revealed a statistically significant decrease at 1000mg/kg bw/d of sperm concentration of the left epididymis, left testis and the percentage of sperm cells with normal morphology and the number of sperm with separated head was increased (also observed at 450 mg/kg bw/d). A slight lower sperm mobility was observed at 150 mg/kg bw/d. In females, a minimal to moderate attenuated myometrium and epithelial mucification of vagina were observed in all treated groups ($p \leq 0.01$ at 1000mg/kg bw/d and $p \leq 0.05$ at 450mg/kg bw/d for vagina). An irregular cycle was also noted at 150 and 1000 mg/kg bw/d, however the female reproduction parameters were unaffected. The evaluation of the followed generation revealed the same variations. In pups, the modifications were less pronounced. In the F1-generation, the highest dose group showed a delay in the day of balano-preputial separation and in the vaginal opening. A lower anogenital distance was observed in F2-generation.

This information was not confirmed by the 13-week study (Anonymous 23, 2011) where sperm motility and sperm count were unaffected.

The prenatal study (Anonymous 27, 2010) was conducted in rats, assigned in 4 groups (0, 150, 450 and 1000mg/kg bw/d). At 1000mg/kg bw/d, a lower food intake as compared to the control group was recorded in mated females during the whole treatment period (not always statistically significant). Effects were most pronounced in the first week. Slight body weight loss occurred during the first two days of treatment. Lower body weight and body weight gain as compared to controls were noted from the second day of treatment onwards. In addition, for uterus weight corrected body weight gain was statistically significant decreased for the high dose group as compared to control animals. No mortality occurred, and clinical signs and macroscopic examination were unaffected. No maternal toxicity was observed in the 150 and 450 mg/kg bw/day group. And no developmental toxicity was observed following treatment up to 1000mg/kg bw/day.

4.11.5 Comparison with criteria

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. 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.

For XTJ-568, the results of the two-generation study reveal some variations in the fertility parameters at a dose lower than the one causing a decrease in body weight. The effects are observed already at 150 mg/kg bw/d however there are more pronounced and statistically significance at 450 mg/kg bw/d

and mainly at 1000 mg/kg bw/d. Negative results of the 13-week study (Anonymous 23, 2011) cannot disregard those findings.

Some effects on development were observed in the 2-generation study. Balanopreputial (statistically significant) and vaginal opening were delayed in the F1 generation. The anogenital distance was significantly lower in males at 150 mg/kg bw/d and 450 mg/kg bw/d and in females at 150 mg/kg bw/d in the F2 generation.

4.11.6 Conclusions on classification and labelling

A classification as reprotoxicity in category 2 is warranted for fertility and development.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

In a 2-generation toxicity study in rats, treatment with XTJ-568 dihydrochloride resulted in a statistically significant decrease in the left epididymis weight (at the high dose, 1000 mg/kg bw/d). Microscopic examination showed, in the male high dose group, statistically significant minimal or slight multifocal seminiferous degeneration in the testis and minimal or slight seminiferous cell debris in the epididymis. The sperm evaluation confirmed these results and revealed a statistically significant decrease at 1000 mg/kg bw/d of sperm concentration in the left epididymis and left testis. The percentage of sperm cells with normal morphology was decreased and the number of sperm with separated head was increased (also observed at 450 mg/kg bw/d). A slightly lower sperm mobility was observed already at 150 mg/kg bw/d. The lack of effects on the testis in the repeated dose 13-week toxicity study at doses up to 150 mg/kg bw/d were not considered to negate those findings. Therefore classification for effects on fertility in category 2 was proposed (Repr. 2, H361f).

Developmental toxicity

In a standard OECD TG 414 developmental toxicity study, no effects were observed at up to the highest dose of 1000 mg/kg bw/d. However, delayed postnatal development was observed in the 2-generation study, expressed as a statistically significant delay in balanopreputial separation and vaginal opening in the F1 generation. The anogenital distance was significantly lower in males at 150 and 450 mg/kg bw/d and in females at 150 mg/kg bw/d in the F2 generation. A classification for developmental toxicity in category 2 was therefore proposed (Repr. 2, H361d).

The overall reproductive toxicity classification, proposed by the dossier submitter, was Repr. 2, H361fd.

Comments received during public consultation

Comments were received from 2 MSCAs and one industry organisation. They were all supportive of classification in Cat. 2 for effects on fertility, but questioned classification for developmental toxicity on the basis that the effects on development were considered to be most likely caused by a slower growth of the pups.

Assessment and comparison with the classification criteria

Fertility

For effects on fertility, the 2-generation study is the key study and effects on the testis the key toxicological effect. At the top dose (1000 mg/kg bw/d), salivation (perhaps caused by bad taste of XTJ-568) and a statistically decreased body weight gain were observed. It was very difficult to get a clear picture about the magnitude of the decreased weight gain. Palatability may have been an issue, as indicated by slightly lower food consumption, but the extent was not clear. In F0 adults, the decreased body weight gain seemed less than 10%, and if so, this constituted only limited general toxicity.

Substantially decreased body weights (-28%) were observed in male top dose F1 pups (of unclear age, perhaps day 21) and 18% lower body weight at necropsy of the adult F1 males. In F1 female the effects on body weight at the top dose were lower (-22% and -5%, for pups and adults respectively). At the mid dose (450 mg/kg bw/d), the F1 body weights were decreased by < 8%.

Testis-related findings in the F0 generation

1000 mg/kg bw/d

In the CLH dossier, minimal or slight multifocal seminiferous epithelial degeneration in testis and minimal or slight seminiferous cell debris in epididymis in 3 animals at the top dose ($p \leq 0.01$) were reported. Other findings at the top dose included a statistically significant decrease in absolute left epididymis organ weight (possibly caused by the decreased body weight; relative weight not given), a decreased sperm concentration of the left epididymis (-29%; $p < 0.01$) and left testis (-13%; but not statistically significant), decreased sperm motility score of 2 (vs 3 in controls), and decreased percentage of sperm cells with normal morphology (63% vs 87% in the controls).

450 mg/kg bw/d

Some effects were also observed at the mid dose, including a statistically significant decreased sperm concentration of the left epididymis (-22%; $p < 0.05$) and left testis (-19%; $p < 0.05$), decreased sperm motility score (2 vs 3 in controls), and decreased percentage of sperm cells with normal morphology (63% vs 87% in controls).

Testis-related findings in the F1 generation

1000 mg/kg bw/d

Upon macroscopic examination, 3 males in the top dose group showed reduced size of testis, epididymides and/or seminal vesicles together with oligospermia in the epididymis, multifocal seminiferous epithelial degeneration in the testis or hypotrophic acini in the seminal vesicles. Other findings at the top dose included statistically significant decreases

in absolute weight of the epididymis, testes, prostate, and seminal vesicles (likely affected by the decreased body weight; relative weights not given). Microscopic examination revealed minimal multifocal seminiferous epithelial degeneration and minimal seminiferous cell debris in 5 top dose animals vs 1 in controls. The sperm examination showed a statistically significant decrease in sperm concentration (-32%) in the left epididymis and sperm quality (motility was decreased; 30% vs 54% in controls), progressive motility (13 vs 28% in controls), and low percentage of sperm cells with normal morphology (64 vs 92% in controls). The litter size was decreased (7.9 pups/litter vs 11.1 in the controls). According to information received during the Public Consultation (Huntsman BVBA, see the RCOM), fewer implantation sites were also observed in the group with the decreased litter size, perhaps supporting a relationship to the testis toxicity. However, similar effects were not observed in F0 animals.

450 mg/kg bw/d

The sperm examination revealed a statistically significant decrease in percentage of sperm cells with normal morphology (65% vs 92% in controls). The other sperm parameters were decreased (by 8%-17%), but the decreases were not statistically significant.

In a range-finding 28 day study in Wistar rats, all animals in the 1000 mg/kg bw/d dose group were sacrificed before the end of the study because of extensive toxicity. On the other hand, rather limited toxicity was observed in Wistar rats exposed to 1000 mg/kg bw/d during days 6-19 in a developmental toxicity study (no statistical effects on body weight gain but some clinical signs) and in Wistar rats of the 2-generation study (effects on body weight gain). In these three studies showing very different levels of toxicity, the same strain of rats was used and all involved gavage exposure. However, in the 2-generation and developmental toxicity studies with low general toxicity in adult rats, the substance was administered as a dihydrochloride salt of XTJ-568, whereas another batch of XTJ-568 (non-salt) was used for the range-finding 28 day (Anonymous 22, 2003) repeated dose toxicity study where extensive toxicity was observed (at 1000 mg/kg bw/d). This may indicate that the inherent toxicity of the salt vs the non-salt differs, which is most likely related to the pH of the dosing solution and the rate of release of the free diamine. The chemical composition of the different batches of substance is otherwise not expected to differ (see RCOM; Huntsman BVBA). Although differences in toxicity were indicated by the available studies, it is generally considered acceptable to perform studies using the salt of a substance (vs pure substance) to avoid problems with pH, and RAC is therefore of the opinion that the above developmental toxicity and 2-generation studies can be used to assess the reproductive toxicity of XTJ-568.

Repeated dose toxicity studies usually provide additional information on testes toxicity, but the available studies for this substance used lower exposure levels (≤ 150 mg/kg bw/d) than the 2-generation study, therefore the lack of testicular toxicity in those studies did not contradict the findings in the 2-generation study.

The 2-generation study showed a relatively consistent picture of testicular toxicity between the generations. The major effects on body weight in the F1 animals was a concern when evaluating the study, but the findings in F0 animals and the indications in the mid dose of F1 occurred without concomitant effects on body weights. Although it

could be a possible borderline case, RAC is of the view that there is sufficient evidence to classify based on direct effects on the testes caused by XTJ-568.

As no human data is available, category 1A is not relevant. Category 1B can be applied when animal studies provide clear evidence of effects on fertility. However, there are some uncertainties arising from maternal toxicity occurring in the F1 generation at the top dose, effects mainly occurring at very high exposure levels which may be not be easily encountered for a substance with such a high pH, and the fact that the 2-generation study has been performed on a salt of XTJ-568 and not the pure substance. Still, the 2-generation study is considered to provide "some evidence" of effects on fertility, thus **RAC concludes that classification in category 2 (H361f)** is warranted.

The CLH report did not propose setting a specific concentration limit (SCL), but RAC notes that based on the available data (ED_{10} > 400 mg/kg bw/d) the substance could be considered to belong to the low potency group, where an SCL of 3-10% could be considered. The generic concentration limit (GCL) for category 2 is 3%, but based on the CLP guidance there is not sufficient reason for deviating from the GCL in this case.

Developmental toxicity

Significantly delayed balanopreputial separation and delayed vaginal opening in the F1 generation by 2 and 1 days, respectively, were observed in the 2-generation study. However, similar delays were not reported for F2 pups. In contrast, whereas no effects on anogenital distance was reported for F1 pups, significantly lower anogenital distance values were reported in F2 pups at 150 (both sexes) and 450 mg/kg bw/d (males). No effects were found in the top dose animals. At the top dose in F1 and F2 pups, decreased body weights were observed during the latter part of the weaning period. Because of lack of consistency between the generations, small effects, and likely correlation to lower body weight gain, RAC is of the opinion that the observed effects are not sufficiently adverse according to the criteria to warrant classification for developmental toxicity.

RAC concludes that the resulting overall classification for reproductive toxicity should be **Repr. 2, H361f**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No data available.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

Study	Batch n°	Purity %
Ecotoxicological studies	8191-34 (if otherwise, the batch is mentioned in the summary table)	97.8%
	OG704	~ 100%
	DR32630507	90%

5.1 Degradation

Table 31: Summary of relevant information on degradation

Property	Method	Results	Remarks	Reference
Hydrolysis Batch n° : 8191-34, purity : 97.8%	EU Method C.7 (Degradation: Abiotic Degradation: Hydrolysis as a Function of pH) OECD 111 GLP	Half-life (DT50): t1/2 (pH 4): > 8760 h at 50 °C (Temp.: CA 50 °C) t1/2 (pH 7): > 8760 h at 50 °C (Temp.: CA 50 °C) t1/2 (pH 9): > 8760 h at 50 °C (Temp.: CA 50 °C) Transformation products: no	2 (reliable with restrictions) key study experimental result	Anonymous 30, (2004)
Dissociation constant	Calculated from structure of main component	pKa of 9.9 and 9.3 for both of the R-NH3+ groups	For complex mixtures (e.g. UVCBs) containing ionisable components the assessment of pKa is clearly complicated. Estimation of the pKa values of the main component is therefore considered as an alternative. In addition to the pKa values for the R-NH3+ groups, pKa values were calculated for the R-O-R groups to be -4.7 and -5.3	Anonymous 16, 2004
Test type: ready biodegradability activated sludge, domestic, non-adapted Batch n° : 8191-34, purity : 97.8%	EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test) OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test) GLP	not readily biodegradable % Degradation of test substance: 1 — 3 after 28 d (CO2 evolution) (average = 2%)	1 (reliable without restriction) key study experimental result	Anonymous 31, (2003a)
Test type: inherent biodegradability mixture of sewage, soil and natural water Batch n° OG704Purity : 100%	OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) GLP	not inherently biodegradable % Degradation of test substance: 3.88 after 28 d (Test mat. analysis)	1 (reliable without restriction) supporting study experimental result	Anonymous 32, (2011)

5.1.1 Stability

Hydrolysis

A single study following test method C.7 (OECD 111) is available on hydrolysis (Anonymous 30, 2004). As XTJ 568 appeared to be hydrolytically stable in the preliminary test at pH4,5 and 7 and at 50°C, the extrapolated half-life time at 25 °C is concluded to be > 1 year. This study is a well performed GLP study in accordance with accepted test guidelines (EEC C.7). Validation details for the analytical method applied were not reported and therefore the study was assigned a Klimisch 2 score.

Dissociation constant

The dissociation constant of XTJ 568 was calculated from the structure of the main component. For complex mixtures containing ionisable components the assessment of pKa is clearly complicated. Estimation of the pKa values of the main component is therefore considered as an alternative. For both of the R-NH3+ groups a pKa of 9.9 and 9.3 is calculated.
In addition to the pKa values for the R- NH3+ groups, pKa values were calculated for the R-O-R groups to be -4.7 and -5.3

Photolysis in water

No data available.

Photolysis in soil

No data available.

Photodegradation

No data available.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Estimations on biodegradability are not performed as experimental results are available.

5.1.2.2 Screening tests

Ready biodegradability

The ready biodegradability of the substance XTJ 568 was examined in a CO2 Evolution test, performed according to OECD guideline 301B. Only 1-3%, with an average of 2%, CO2 was formed after 28 days. The pass level of 70% was not achieved and the substance is considered not readily biodegradable.

Inherent biodegradability

The potential of XTJ 568 to ultimately biodegradation was examined in a study of inherent biodegradation performed according to OECD Guideline 302C (modified MITI II). After 28 days only 3.88% of the test substance was degraded and the pass level of more than 70% was not reached, demonstrating that XTJ 568 is not ultimately degraded.

5.1.2.3 Simulation tests

No such studies were performed as it was already concluded from the CO2 evolution study that the substance is not rapidly biodegradable

5.1.3 Summary and discussion of degradation

XTJ 568 was demonstrated to be hydrolytically stable at pH 4, 7 and 9 (extrapolated half-life time at 25 °C > 1 year) in a GLP study according to test method EC C.7 (Anonymous 30, 2004).

In the study on ready biodegradability it was demonstrated that within 28 days only up to 3% (with an average of 2%) of it underwent mineralization. An inherent biodegradation study also showed no significant biodegradation of the substance. XTJ 568 does not meet the biodegradability criterion of at least 70% mineralization within 28 days and is concluded to be not readily biodegradable.

Based on the results of the abiotic and biotic degradation of XTJ 568 it can be concluded that the substance is not rapidly degradable in the environment.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 32: Summary of relevant information on adsorption/desorption

Property	Method	Results	Remarks	Reference
Study type: adsorption/desorption (soil) batch equilibrium method Batch n° : 8191-34: purity : 97.8%	equivalent or similar to OECD Guideline 106 (Adsorption - Desorption Using a Batch Equilibrium Method) Guidelines for the testing of chemicals (HJ/T 153-2004, SEPA)	Adsorption coefficient: Koc (24 h): 60.3 (% Org. C: 0.99) (#1 Jiangxi red soil) Koc (24 h): 61.4 (% Org. C: 1.21) (#2 Nanjing yellow brown soil) Koc (24 h): 33.2 (% Org. C: 2.6) (#3 Shanxi grey desert soil)	2 (reliable with restrictions) key study experimental result	Anonymous 35, (2012)
Study type: adsorption (soil) QSAR estimate Batch n° : 8191-34, purity : 97.8%	TGD Part III on QSARs, 2003	Adsorption coefficient: log Koc: 2.06 (QSAR estimate)	3 (not reliable) supporting study (Q)SAR	Anonymous 33 (2003)
Study type: adsorption (no final test performed) QSAR calculation	OECD Guideline 121 (Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High	Adsorption coefficient: log Koc: 2.8 — 3.2 (Values for component 1	3 (not reliable)	Anonymous 34 (2011)

Property	Method	Results	Remarks	Reference
Batch n° DR 32630507 Purity : 90%	Performance Liquid Chromatography (HPLC)) technical guidance documents in support of Commission Directive 93/67/EEC on risk assessment for new substances	and 2, corrected for underestimation.)	supporting study (Q)SAR	

Based on a calculation of the pKa's and the results of the determination of the log Kow, it is clear that the test substance is ionized for at least 10% in the range pH 5.5 -7.5. Therefore both the ionized and the unionized form should be tested in appropriate buffer solutions. Since the highest pKa was calculated to be 9.9, the test substance must be tested both at a pH of > 10.9 (neutral form) and at a pH < 8.9 (ionized form). Due to the limited pH range of the cyanopropyl column required for the OECD 121 method, testing at pH > 10.9 is impossible. Testing at pH < 8.9 is in principle possible, but due to the very unfavorable interaction of the protonated form of the test substance with residual silanol groups of the stationary phase, test substance peak shape and retention time reproducibility will be extremely poor. Therefore it is not possible to determine the partition constant according to the OECD 121 method (HPLC method).

Alternatively, a batch equilibrium test was performed in 2012 according to a guideline similar to OECD guideline 106. In this study, the adsorption of XTJ 568 was studied in three different Chinese soils with variable properties (pH, organic carbon, CEC, % clay). The log Koc values obtained in these soils range from 1.52 to 1.79. A key Koc value of 49.7 (geometric mean of three Koc values) was selected as key value. This corresponds to a log Koc value of 1.7. Although for this substance it would have been interesting to investigate pH dependency of adsorption, no information on this is obtained since pH was not monitored in the aqueous phase. Therefore this study was scored as Klimisch 2.

As supporting information, several QSAR estimates can be used. The QSAR for nonhydrophobics reported by Sabljic and Güsten (1995) and reported in the TGD (2003) of the European Commission yields a log Koc of 2.06 (using a linear regression equation based on the log Kow). This value may be underestimated since the model may underestimate the log Koc of aliphatic amines by 1-2 log units. Therefore, the value should be used with caution.

In another QSAR prediction (also based on log Kow), the final values were (worst case) corrected for the possible underestimation for aliphatic amines. The resulting values were log Koc = 2.8 and 3.2 for two components, respectively. Here too, these values should be used with caution. Therefore both QSAR estimates represent supporting information.

5.2.2 Volatilisation

Based on a MW of 220, a water solubility of 500 g/L and a vapour pressure of 3.4 Pa (20°C) (i.e. 4.8 Pa at 25°C) the Henry’s Law constant at 25°C is 1.97E-03 Pa.m3/mol (i.e. 9.44E-04 Pa.m3/mol at environmental temperature, 12°C), indicating slow volatilisation from water to air

5.2.3 Distribution modelling

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5.3 Aquatic Bioaccumulation

Table 33: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
The partition coefficient A GLP study according to test methods EC A.8 and OECD 117 to be 2.0 at 24 °C, following the HPLC method. Batch n° : 8191-34, purity : 97.8%	Log Kow (Pow): 2 at 24 °C	The pH of the mobile phase was 11 at which >90% of the substance is in its non ionised form. In addition to the log Pow of the main component, additional log Pow values could be calculated for two impurities (log Pow = 1.9 and 3.2).	Anonymous 9, 2004

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

A Log Kow was experimentally derived using the OECD 117 test guideline (Anonymous 9, 2004) and resulted in a Log Kow =2 at 24°C. In comparison with the threshold values of CLP (log Kow≥4), the substance shows low potential to bioaccumulate.

5.3.1.2 Measured bioaccumulation data

No measured data are available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The bioaccumulation potential of XTJ 568 was estimated from the Log Kow. The experimentally derived log Kow of 2 is lower than the threshold values of CLP and therefore the substance is considered to show low potential for bioaccumulation.

5.4 Aquatic toxicity

Table 34: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Acute fish test : *Cyprinus carpio* freshwater static ISO 7346-1 (Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static Method)	LC50 (96 h): > 100 mg/L test mat. (nominal)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy**	Anonymous 36, (2004a)

EU Method C.1 (Acute Toxicity for Fish) OECD Guideline 203 (Fish, Acute Toxicity Test) GLP Batch n° : 8191-34, purity : 97.8%		**]methyl}propoxy)but-2-ylamine**	
Acute fish test : *Danio rerio* freshwater semi-static OECD Guideline 203 (Fish, Acute Toxicity Test) GLP Batch n° OG704 Purity : 100%	LC50 (96 h): > 117.37 mg/L test mat. (meas. (arithm. mean))	1 (reliable without restriction) supporting study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**	Anonymous 37 (2011a)
Chronic fish test : *Danio rerio* freshwater juvenile fish: growth flow-through OECD Guideline 215 (Fish, Juvenile Growth Test) GLP Batch n° OG704 Purity : 100%	NOEC (28 d): >= 110.09 mg/L test mat. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**	Anonymous 38, (2011b)
Acute invertebrate test : *Daphnia magna* freshwater static ISO 6341 15 (Water quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea)) EU Method C.2 (Acute Toxicity for Daphnia)	EC50 (48 h): 88 mg/L test mat. (nominal) based on: mobility	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy**	Anonymous 39 (2004b)

OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) GLP Batch n° : 8191-34, purity : 97.8%		**]methyl}propoxy)but-2-ylamine**	
Chronic invertebrate test : *Daphnia magna* freshwater semi-static OECD Guideline 211 (Daphnia magna Reproduction Test) GLP Batch n° OG704 Purity : 100%	NOEC (21 d): 7.95 mg/L test mat. (nominal) based on: reproduction (: live offspring per surviving parent)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**	Anonymous 40, (2011)
Algae growth inhibition test : *Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae)* freshwater static ISO 8692 (Water Quality - Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum) EU Method C.3 (Algal Inhibition test) OECD Guideline 201 (Alga, Growth Inhibition Test) GLP Batch n° : 8191-34, purity : 97.8%	EC50 (72 h): > 100 mg/L test mat. (nominal) based on: growth rate NOEC (72 h): 4.6 mg/L test mat. (nominal) based on: growth rate	1 (reliable without restriction) key study experimental result Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	Anonymous 41, (2004c)
Toxicity to aquatic micro-organisms activated sludge of a predominantly domestic sewage freshwater aerobic	EC50 (30 min): > 100 mg/L test mat. (nominal) based on: respiration rate	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy**	Anonymous 42, (2003b)

OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) EU Method C.11 (Biodegradation: Activated Sludge Respiration Inhibition Test) GLP Batch n° : 8191-34, purity : 97.8%		**]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but -2-ylamine**	

5.4.1 Fish

Method	Results	Remarks
Acute fish test : *Cyprinus carpio* freshwater static ISO 7346-1 (Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static Method) EU Method C.1 (Acute Toxicity for Fish) OECD Guideline 203 (Fish, Acute Toxicity Test) GLP Batch n° : 8191-34, purity : 97.8%	LC50 (96 h): > 100 mg/L test mat. (nominal)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**
Acute fish test : *Danio rerio* freshwater semi-static OECD Guideline 203 (Fish, Acute Toxicity Test) GLP Batch n° OG704 :Purity : 100%	LC50 (96 h): > 117.37 mg/L test mat. (meas. (arithm. mean))	1 (reliable without restriction) supporting study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**
Chronic fish test : *Danio rerio* freshwater	NOEC (28 d): >= 110.09 mg/L test mat. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) key study experimental result

juvenile fish: growth flow-through OECD Guideline 215 (Fish, Juvenile Growth Test) GLP Batch n° OG704 :Purity : 100%		**Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**

5.4.1.1 Short-term toxicity to fish

An acute toxicity study with XTJ 568 was performed in Cyprinus carpio according to ISO Guideline 7346-1, EU Method C.1 and OECD Guideline 203. An 96h-EC50 of > 100 mg/L for the endpoint mortality and a 96h-NOEC of 100 mg/L for other clinical effects (not further specified) were observed.

A supporting study (Anonymous 37, 2011) on Danio rerio confirmed the findings (96hLC50 > 117.37 mg/L test mat. (meas. (arithm. mean)).

96h Acute toxicity in Cyprinus carpio with XTJ 568, Anonymous 36, (2004a)

Key study

Guidelines:
ISO 7346-1 (Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static Method)
EU Method C.1 (Acute Toxicity for Fish)
OECD Guideline 203 (Fish, Acute Toxicity Test)

GLP : Yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
Batch n° 8191-34, purity : 97.8%
Test species : Carp (Cyprinus carpio)
Type of test : 96h static toxicity test
Number of organisms, age, weight, length : 26 fish with a mean length of resp. 2.3 ±0.2 cm and 2.1±0.1cm and a mean weight of resp. 0.33±0.09g and 0.33±0.04g in the range-finding- and limit test.
Biological loading: 0.46g fish/L, i.e. 7 fish per 5L of test medium
Applied and measured concentrations :

- Nominal concentrations: Range finding test : 0.1, 1.0, 10 and 100 mg/L
  Limit test: 100 mg/L
- Measured concentrations: Range finding :10 mg/L: ranged from 9.52 to 10.2mg/L;

Limit test : 100 mg/L: ranged from 103 to 108 mg/L; (103-108% of nominal concentration)

Test conditions :
Dissolved oxygen : 6.5-8.7 mg/L.
pH : 7.9-8.1 (final test)
Water medium type : Fresh water
Water hardness : 250 mg CaCO3/L
Temperature : 20.3-21.2°C (final test) : maintained within the limits of 20-24°C as prescribed
Photoperiod : 16h daily
Vehicle : none
Analytical methods : concentrations were measured by using HPLC
Validity of test :

- No mortality seen in control group
- Oxygen concentration maintained at at least 60% of the air saturation value throughout the test
- Actual test concentrations maintained within 80% of the initial concentrations throughout the duration of the test
- pH did not vary by more than 1 unit
- temperature maintained within the limits of 20-24°C as prescribed by the protocol

Findings :
Mortality : no mortality in carp at 100 mg/L
Clinical conditions : no other clinical effects observed in carp at 100 mg/L

Conclusions :
LC50 (96 h): > 100 mg/L test mat. (nominal)

Acute toxicity in Danio rerio with XTJ 568, Anonymous 37, (2011a)

Supporting study
Guidelines: OECD Guideline 203 (Fish, Acute Toxicity Test)

GLP : yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine,
Test species : Danio rerio
Type of test : 96h semi-static toxicity test
Applied and measured concentrations :
Final (limit) test:

- Nominal concentrations: control + 120 mg/L
- Mean measured concentrations: < LOQ + 117.37 mg/L

Test conditions :
Dissolved oxygen : 66.4-97.1% of ASV
pH : 7.26-7.93
Type of water : fresh water

Temperature : 22.0-24.1°C
Water Hardness : 165 mg CaCO3/L
*Analytical methods :*HPLC
Validity of test :

- No mortality seen in control group
- Oxygen concentration maintained at at least 60% of the air saturation value throughout the test
- Actual test concentrations maintained within 80% of the initial concentrations throughout the duration of the test
-

Findings :
Mortality : the 96-h LC50 for zebra fish (Danio rerio) was concluded to be > 120 mg/L (nominal test concentration) as no mortalities were observed at this concentration level.

Conclusions :
LC50 (96 h): > 117.37 mg/L test mat. (meas. (arithm. mean))

5.4.1.2 Long-term toxicity to fish

Chronic toxicity in Danio rerio with XTJ 568, Anonymous 38, (2011b)

Key study

Guidelines : OECD Guideline 215 (Fish, Juvenile Growth Test)

GLP : yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
Test species : Danio rerio
Type of test : 28 d flow through toxicity test
Number of organisms, age, weight, length : 5 fish, 2 vessels per concentration, 2 vessels per control
Biological loading: 0.2-0.3g fish/L
Applied and measured concentrations :

- Nominal: control + 1.6-5.0-16-50-120 mg/L
- Mean measured: < LOQ + 1.32-4.78-14.63-42.92-110.09 mg/L

Test conditions :
Temperature : 22.0-22.9°C
Water hardness : 165-170 mg CaCO3/L
pH : 7.23-7.95
Dissolved oxygen : 60.3-100.0% of ASV
Photoperiod :12h light, 12h dark
Vehicle : none
*Analytical methods :*HPLC
Validity of test :

- No mortality seen in control group

- Oxygen concentration maintained at at least 60% of the air saturation value throughout the test
- Actual test concentrations maintained within 80% of the initial concentrations throughout the duration of the test

Findings :
Mortality : No mortalities were observed throughout the test (Li et al., 2011)
Behavioral observations : A 28-d juvenile growth inhibition test with zebra fish (Danio rerio) did not reveal any adverse effects on fish growth at any of the concentrations tested.

Conclusions :
When expressed as mean measured test substance concentration, the NOEC, LOEC and EC50 can be considered >= 110.09, > 110.09 and > 110.09 mg/L, respectively.

5.4.2 Aquatic invertebrates

Method	Results	Remarks	Reference
Acute invertebrate test : *Daphnia magna* freshwater static ISO 6341 15 (Water quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea)) EU Method C.2 (Acute Toxicity for Daphnia) OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) GLP Batch n° : 8191-34, purity : 97.8%	EC50 (48 h): 88 mg/L test mat. (nominal) based on: mobility	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**	Anonymous 39 (2004b)
Chronic invertebrate test : *Daphnia magna* freshwater semi-static OECD Guideline 211 (Daphnia magna Reproduction Test) GLP Batch n° OG704 :Purity : 100%	NOEC (21 d): 7.95 mg/L test mat. (nominal) based on: reproduction (: live offspring per surviving parent)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy**	Anonymous 40, (2011)

		]methyl}propoxy)but -2-ylamine	

5.4.2.1 Short-term toxicity to aquatic invertebrates

Acute toxicity in Daphnia magna with XTJ 568, Anonymous 39, (2004b)

Key study

Guidelines :
ISO 6341 15 (Water quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea))
EU Method C.2 (Acute Toxicity for Daphnia)
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)

GLP : yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
Test batch : 8191-34, purity : 97.8%
Test species : Daphnia magna
Type of test : 48h static toxicity test
Number of organisms, age : 20 per concentration, age of <24h from parental daphnids of more than 2 weeks old
Loading : 5 per vessel containing 80 ml medium
Applied and measured concentrations :

- Nominal concentrations: Control + 10, 18, 32, 56 and 100 mg/L (final test)
- Measured concentrations: 10 mg/L: ranged from 10.0 to 10.2; 32 mg/L: ranged from 32.1 to 33.5 mg/L; 100 mg/L: ranged from 103 to 106 mg/L; overall 100-106% of nominal concentrations

Test conditions :
Temperature : 20.3-21.2°C (final test)
pH : 7.9-8.1 (final test)
Dissolved oxygen : 8.7-9.3 mg/L
Water hardness : 250 mg CaCO3/L
Photoperiod : 16h daily
Analytical methods : HPLC
Validity of test :

- In the control, no daphnia became immobilised or trapped at the surface of the water
- The concentration of dissolved oxygen remained above 3mg/L
- The concentration of the substance was maintained to more than 80% of the initial concentration throughout the duration of the test
- pH did not vary by more than 1 unit
- Test temperature between 18 and 22°C as prescribed by protocol

Findings :

Acute immobilisation of daphnia after 24 and 48h in the final EC50-test

Concentration XTJ568 (mg/L)	Vessel number	Number Daphnia exposed	Response at 24h		Response at 48h	
			Number of daphnids	TOTAL %	Number of daphnids	TOTAL %
Blank-control	A B C D	5 5 5 5	0 0 0 0	0	0 0 0 0	0
10	A B C D	5 5 5 5	0 0 0 0	0	0 0 1 0	5
18	A B C D	5 5 5 5	0 0 0 0 (1)	0	0 0 0 1 (2)	5
32	A B C D	5 5 5 5	0 0 0 0	0	0 0 0 0	0
56	A B C D	5 5 5 5	1 1 0 0	10	1 1 0 0	10
100[1]	A B C D	5 5 5 5	2(1) 1 3 3	45	3 3 3 4	65

[1] pH adjusted to 7.9 at the start of the test
Between brackets : number of daphnia observed trapped at the surface of the test solutions. These organisms were reimmersed before recording the mobility.

XTJ 568 did not induce acute immobilisation at 32 mg/L after 48 hours of exposure (NOEC). The 24-h EC50 approximated the maximum concentration tested and was estimated at 110 mg/L based on nominal exposure concentrations (95% confidence interval between 85 and 180 mg/L). The 48-h EC50 was 88 mg/L based on nominal concentrations (95% confidence interval between 73 and 110 mg/L).

Conclusions : EC50 (48 h): 88 mg/L test mat. (nominal) based on mobility

5.4.2.2 Long-term toxicity to aquatic invertebrates

Chronic toxicity in Daphnia magna with XTJ 568, Anonymous 40, (2011)

Key study

Guidelines : OECD Guideline 211 (Daphnia magna Reproduction Test)

GLP : yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine
Test species : Daphnia magna
Type of test : 21d semi-static toxicity test
Number of organisms, age : 10 female Daphnia aged less than 24h per concentration

Applied and measured concentrations :

- Nominal: control + 0.5, 1.26, 3.16, 7.95, 20 mg/L
- Measured: control (< LOQ) + within 20% deviation from nominal

Test conditions :

Temperature : 19.1-20.7°C (water temperature)
Water hardness : 195-210 mg CaCO3/L
pH : 6.81-7.41
Dissolved oxygen : 7.22-10.58 mg/L
Photoperiod : light : 16h, dark : 8h
Light intensity : 505 lux
Analytical methods : HPLC
Validity of test :

- Mortality of parents does not exceed 20% at the end of test
- The mean number of live offspring produced per parent animals surviving at the end of the test is not lower than 60%

Findings : It is a slight disadvantage that the EC50 lies between the NOEC and the LOEC (NOEC, LOEC and EC50 of 7.95, 20, and 13.51 mg/L, respectively), but the results of the test can be considered reliable. Although some mortality was observed, no concentration response relationship was obtained; mortality may have been affected by handling stress in some of the lower test concentrations. The fact that only the reproduction of the parent animals surviving the complete test were taken into account avoids any effect of this mortality on the reproduction-based EC50

Conclusions : NOEC(21d): 7.95 mg/L test mat. (nominal) based on reproduction (living offspring per surviving parent)

5.4.3 Algae and aquatic plants

Method	Results	Remarks	Reference
Algae growth inhibition test : *Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae)* freshwater static ISO 8692 (Water Quality - Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum) EU Method C.3 (Algal Inhibition test) OECD Guideline 201 (Alga, Growth Inhibition Test) GLP	EC50 (72 h): > 100 mg/L test mat. (nominal) based on: growth rate NOEC (72 h): 4.6 mg/L test mat. (nominal) based on: growth rate	1 (reliable without restriction) key study experimental result Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy] but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy] methyl}propoxy)but-2-ylamine	Anonymous 41, (2004c)

Batch n° 8191-34, purity : 97.8%			

Fresh water algal growth inhibition test with XTJ 568, Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae), Anonymous 41, (2004c)

Key study

Guidelines : ISO 8692 (Water Quality - Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum)

GLP : yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine,
Batch number : 8191-34, purity : 97.8%
Test species : Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata)
Type of test : 72h static toxicity test
Number of replicates, initial cell density :
Range finding test : 3 replicates per concentration and 3 replicates in the control group
Final test :
3 replicates of each test concentration
6 replicates of blank-control
1 replicate of the highest concentration without algae

Initial cell density of 1x 10^4 cells/ml

Mean cell density during the final test

Nominal Conc. XTJ 568 (mg/l)	Exposure time (hours)			
	0	24	48	72
Blank-control	1.0	4.9	30.4	122.8
2.2	1.0	5.0	28.1	104.7
4.6	1.0	4.2	25.9	109.8
10	1.0	3.8	22.7	93.4
22	1.0	3.4	19.9	85.2
46	1.0	3.2	14.1	57.9
100	1.0	3.0	11.0	42.7

Applied and measured concentrations :
Range finding test : range of 0.1 to 100 mg/l increasing by a factor of 10
Final test :

- Nominal concentrations: 2.2, 4.6, 10, 22, 46 and 100 mg/L
- Measured concentrations: 98-107% of nominal, at a nominal concentration of 2.2 mg/l the actual measured concentration at the start of the test was only 44% of nominal, but at the end of the test it remained stable at 47-51% of nominal. There is no explanation for the low recovery

at 2.2 mg/l. Since this concentration was toxicological not relevant it was not used to calculate the EC and all results were therefore related to nominal concentrations (4.6-100 mg/l)

Test conditions :

Temperature : 23.5-24°C (measured in temperature control vessel)

pH : 8.0-10.0 (measured at the beginning and at the end of the test).

pH of the control, 2.2 and 10 mg/L test groups was > 9.0 and in addition pH of the 2.2 mg/L test group increased > 1.5 unit during the test period. Since this was related to a relatively high rate of algal growth this deviation was not considered to have affected the validity of the test.

Water hardness : 24 mg CaCO3/L

Light regime : continuously

Light intensity : 80-115µE/m^2/s

*Analytical methods :*HPLC

Validity of test :

- Cell density increased by an average factor of >16 within 3 days
- Temperature remained in the range described by the protocol
- pH of the control, 2.2 and 10 mg/L test groups was above 9.0 and in addition pH of the 2.2 mg/L test group increased with more than 1.5 unit during the test period. Since this was related to a relatively high rate of algal growth this deviation was not considered to have affected the validity of the test.
- Concentration of test substance was maintained to within 80% of the initial concentration (98-107% of nominal). At the start of the test, at a nominal concentration of 2.2 mg/l the actual measured concentration was only 44% of nominal, At the end of the test it remained stable at 47-51% of nominal. There is no explanation for the low recovery at 2.2 mg/l. Since this concentration was toxicological not relevant it was not used in the EC- calculation and all results were therefore related to nominal concentrations (4.6-100 mg/l)

Findings :

Percentage inhibition of cell growth during the final test

Nominal Conc. XTJ 568 (mg/l)	Cell growth (0-72h)	
	Mean Area (A)	Inhibition (%)
Blank-control	2259.14	
2.2	1991.12	11.9
4.6	1980.04	12.4
10	1694.72	25.0
22	1520.84	32.7
46	1048.60	53.6
100	788.48	65.1

Percentage reduction of growth rate at different time intervals during the final test

Nominal Conc. XTJ 568 (mg/l)	Mean growth rate					
	µ(0-24hrs)	Reduction (%)	µ(0-48hrs)	Reduction (%)	µ(0-72hrs)	Reduction (%)
Blank-control	0.06607		0.07109		0.06673	
2.2	0.06725	-1.8	0.06946	2.3	0.06459	3.2
4.6	0.05975	9.6	0.06775	4.7	0.06522	2.3
10	0.05533	16.3	0.06498	8.6	0.06299	5.6
22	0.05073	23.2	0.06202	12.7	0.06161	7.7
46	0.04753	28.1	0.05491	22.8	0.05624	15.7
100	0.04609	30.2	0.04986	29.9	0.05212	21.9

XTJ 568 reduced algal growth rate as well as biomass significantly at nominally 10 mg/L and higher. The 72-h NOEC, LOEC, EC10 and EC50 based on algal biomass were 4.6, 10, 4.4 and 44 mg/L, respectively, whereas the 72-h NOEC, LOEC, EC10 and EC50 based on algal growth rate were 4.6, 10, 20 and > 100 mg/L, respectively.

Conclusion : NOEC for freshwater algae: 4.6 mg/L; the EC50 (based on growth rate reduction) is > 100 mg/L.

5.4.4 Other aquatic organisms (including sediment)

Method	Results	Remarks	Reference
Toxicity to aquatic micro-organisms activated sludge of a predominantly domestic sewage freshwater aerobic OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) EU Method C.11 (Biodegradation: Activated Sludge Respiration Inhibition Test) GLP Batch n° : 8191-34, purity : 97.8%	EC50 (30 min): > 100 mg/L test mat. (nominal) based on: respiration rate	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine**	Anonymous 42, (2003b)

5.4.5

Toxicity to aquatic micro-organisms

Activated sludge respiration inhibition test with XTJ568, Anonymous 42, (2003b)

Guidelines : OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)
EU Method C.11 (Biodegradation: Activated Sludge Respiration Inhibition Test)

GLP : Yes

Reliability : 1 (reliable without restriction)

Material and methods :
Test substance: Reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine,
Batch number : 8191-34, purity : 97.8%
Test species: micro-organisms in activated sludge of a predominantly domestic sewage
Type of test : 30 min contact activated sludge respiration inhibition test

Applied and measured concentrations :

- Nominal concentrations: Control + 100 mg/L
- Concentrations not measured, but test substance can be considered stable during test period.

Test conditions :
Temperature : 19.3 - 20.0 °C.
pH : 6.8 (control) / 7.3-7.4 (100 mg/L)
Dissolved oxygen : Measured at the start of oxygen consumption measurement.
8.3 mg/L (control) / 8.2-8.6 (100 mg/L)

Findings : the effect of XTJ 568 on respiration was investigated through 10-min measurements of oxygen consumption after a 30-min contact period. A control and a single concentration of 100 mg/L were tested in duplicate. In one of the duplicates at 100 mg/L no inhibition of the respiration rate of the sludge was recorded, whereas in the other a very slight inhibition (11%) was observed compared to the control. Therefore, no further testing was needed. The EC50 is > 100 mg/L.

Conclusions :
EC50 (30 min): > 100 mg/L test mat. (nominal) based on: respiration rate

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Degradation

XTJ 568 was demonstrated to be hydrolytically stable at pH 4, 7 and 9.

In a ready biodegradability and an inherent biodegradation study it was demonstrated that XTJ 568 did not meet the biodegradability criterion of at least 70% mineralization within 28 days. In the ready biodegradability study only up to 3% (with an average of 2%) of XTJ 568 underwent mineralization within 28 days. It is concluded that XTJ 568 is not readily biodegradable.

Based on the results of the abiotic and biotic degradation of XTJ 568 it can be concluded that the substance is not rapidly degradable in the environment.

Bioaccumulation

The bioaccumulation potential of XTJ 568 was estimated from the Log Kow. The experimentally derived log Kow of 2 is lower than the threshold value of 4 (CLP) . No measured BCF is available.

The substance is considered to show low potential for bioaccumulation.

Aquatic toxicity

Both acute and chronic toxicity studies were conducted on XTJ 568 for the three trophic levels.

- the most sensitive species for acute aquatic toxicity was Daphnia magna, with an 48hEC50 of 88 mg/L test mat. (nominal), not meeting the acute toxicity criterion of ≤1mg/l
- the most critical chronic endpoint was the NOEC of the algae Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) : 72h NOErC of 4.6 mg/L test mat. (nominal), not meeting the aquatic chronic toxicity criterion for a non-degradable substance of ≤ 0.1 mg/L

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Endpoint	Classification criteria	Results	conclusion
	CLP		
Degradation	The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydroysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of > 70 % within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation	hydrolytically stable, the extrapolated half-life time at 25 °C is concluded to be > 1 year 1 — 3% degradation after 28 d (CO_2 evolution) (average = 2%)	XTJ 568 is not rapidly degradable
Bioaccumulation	log Kow ≥ 4: The substance meets the criterion	Log Kow (Pow): 2 at 24 °C	XTJ 568 does not meet the criterion
Acute Aquatic toxicity	LC/EC50≤1 mg/l	Most sensitive species : Daphnia magna, EC50 (48 h): 88 mg/L test mat. (nominal)	EC50 > 1 mg/l : no classification
Chronic toxicity	Non rapidly degradable substance : Category Chronic 1: Chronic NOEC or ECx ≤0,1 mg/l Category Chronic 2 : Chronic NOEC or ECx ≤1 mg/l	Chronic toxicity studies available for all three trophic levels Most sensitive species : Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata), NOErC (72 h): 4.6 mg/L test mat. (nominal)	NOEC >1 mg/l : no classification
SUMMARY			**NO CLASSIFICATION**

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

There is currently no entry for XTJ-568 in Annex VI of CLP, but when the substance was evaluated in 2004 under the Dangerous Substances Directive (Dir. 67/548/EEC), an environmental hazard classification (R52/53) was proposed by the evaluating MSCA. New aquatic toxicity tests and degradation studies have since been conducted, requiring a new assessment of the need for classification for environmental hazards.

A ready biodegradability test (OECD TG 301B) and an inherent biodegradability test (OECD TG 302C) both showed that XTJ-568 is not readily biodegradable, with only a few percent degradation within 28 days. An experimentally derived log K_{OW} of 2 (OECD TG 117) indicated a low potential for bioaccumulation. Acute and chronic aquatic toxicity tests were available for all trophic levels, with the lowest EC_{50} being 88 mg/L and the lowest NOEC 4.6 mg/L, both being above the criteria for classification. The DS concluded that the substance should therefore not be classified for environmental hazards.

Comments received during public consultation

One MSCA commented and agreed with the proposal for no classification for environmental hazards.

Assessment and comparison with the classification criteria

XTJ-568 is hydrolytically stable.Reliable ready and inherent degradability tests (OECD TG 301B and 302C, respectively) show only a few percent mineralisation within 28 days, leading to the conclusion that the substance is not rapidly degradable.

There is no measured BCF available, but the measured log K_{OW} was 2 (OECD TG 117), indicating a low potential for bioaccumulation.

There are acute and chronic toxicity data for fish (*Cyprinus carpio, Danio rerio*), invertebrates (*Daphnia magna*), as well as a 72h static algal growth inhibition test (*Pseudokirchneriella subcapitata*). In many tests, the EC_{50}/NOEC were higher than the highest tested concentrations. Some of the chronic studies were not conducted according to GLP, and this may decrease their reliability. However, RAC evaluated the available information and concluded that the studies could still been used in the assessment. For acute toxicity, *Daphnia magna* was the most sensitive species with a 48h EC_{50} of 88 mg/L (nominal). For chronic toxicity, the most sensitive species was the algae *Pseudokirchneriella subcapitata (former Selenastrum capricornutum)* with a 72h NOE_rC = 4.6 mg/L (nominal).

Classification for acute aquatic toxicity would be relevant if the EC_{50} had been < 1 mg/L, and since the lowest EC_{50} is 88 mg/L, RAC agrees with the DS that **no classification for acute aquatic toxicity** is warranted.

For a substance which is not rapidly degradable and which has chronic toxicity data available for all three trophic levels, classification for chronic aquatic toxicity would be

relevant if the NOEC would be < 1 mg/L. Since the lowest NOAEC is 4.6 mg/L, RAC concludes that XTJ-568 **should not be classified for chronic aquatic toxicity**.

6 OTHER INFORMATION

No other relevant information available for classification purposes.

7 REFERENCES

7.1 Physical and chemical properties of the substance

Author(s)	**Year**	***Title*** ***Source (where different from the Company),*** ***Company, Report Number,*** ***GLP or GEP status (where relevant),*** **Published or not**	***Test Facility***
Anonymous 1	2003	Spectral data of XTJ-568	Austin Research Laboratories, Huntsman LLC
Anonymous 2	2003	Development of an analytical method for the analysis of XTJ 568 in milli-q water	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 3	2003	Determination of the freezing temperature of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 4	2003	Determination of the boiling temperature of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 5	2003	Determination of the density (liquid) of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 6	2003	Determination of the vapour pressure of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 7	2003	Determination of the surface tension of an aqueous solution of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 8	2003	Determination of the water solubility of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 9	2003	Determination of the partition coefficient of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 10	2003	Determination of the flash-point of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 11	2003a	Determination of the flammability (contact with water) of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands

Author(s)	**Year**	***Title*** ***Source (where different from the Company),*** ***Company, Report Number,*** ***GLP or GEP status (where relevant),*** **Published or not**	***Test Facility***
Anonymous 12	2003b	Statement of the pyrophoric properties of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 13	2003	Statement on the explosive properties of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 14	2003	Determination of the auto-ignition temperature (liquids) of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 15	2011	XTJ 568 : Determination of viscosity	Harlan Laboratories, Derbyshire, UK
Anonymous 16	2004	Determination of the dissociation constant	Notox B.V., 's-Hertogenbosch, The Netherlands

7.2 Toxicology and metabolism of the substance

Author(s)	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**	**Test Facility**
Anonymous 17	2003	Assessment of acute oral toxicity with XTJ 568 in the rat (acute toxic class method)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 18	2011	Acute Dermal Toxicity Study in Rats with XTJ-568	Calvert Laboratories, Inc., Scott Township, USA
Anonymous 19	2012	Acute oral toxicity study in rat –up and down procedure with XTJ 568	Calvert Laboratories, Inc., Scott Township, USA
Anonymous 20	2003	Primary skin irriation/corrosion study with XTJ 568 in the rabbit (semi-occlusive application)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 21	2003	Assessment of contact hypersensitivity of XTJ 568 in the albino guinea pig (maximisation-test)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 22	2003	Subacute 28-day oral toxicity with XTJ 568 by daily gavage in the rat	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 23	2011	XTJ568: Toxicity Study by Oral Administration to CD Rats for 13 Weeks	Huntingdon Life Sciences
Anonymous 24	2003	Evaluation of the mutagenic activity of XTJ 568 in the Salmonella typhimurium reverse mutation assay and the Echerichia coli reverse mutation assay (with independent repeat)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 25	2003	Evaluation of the ability of XTJ 568 to induce chromosome aberrations in cultured peripheral human lymphocytes	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 26	2011	Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of XTJ-568	Bioreliance, Rockville,

Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Test Facility
Anonymous 27	2010	A prenatal development toxicity of XTJ 568 in dihydrochloride in rats by oral gavage	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 28	2010	A two-generation reproduction toxicity of XTJ 568 in dihydrochloride in rats by oral gavage	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 29	2004	Toxicokinetic assessment of XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands

7.3 Environmental fate and behaviour of the substance

Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Test Facility
Anonymous 30	2004	Determinatio of the hydrolysis of XTJ 568 as a function of pH	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 31	2003	Determination of 'ready biodegradability' : carbon dioxide (CO2) evolution test (modified sturm test) with XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 32	2011	Inherent biodegradation test of XTJ 568	Bioassay and Safety Assessment laboratory, Shanghai, China
Anonymous 33	2003	Statement on the estimation of the adsorption coefficient (Koc) of XTJ 568 on soil and on sewage sludge using high performance lisuid chromatography (HPLC)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 34	2011	XTJ 568 : determination of adsorption coefficient	Harlan Laboratories Ltd, Derbyshire, UK
Anonymous 35	2012	Adsorption/desorption test on XTJ-568	Shangai Academy of Environmental Testing laboratory, Shanghai, China

7.4 Ecotoxicology of the substance

Author(s)	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**	**Test Facility**
Anonymous 36	2004a	96-hour acute toxicity study in carp with XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 37	2011a	Acute toxicity test of XTJ 568 to zebrafish (Danio rerio)	Bioassay and Safety Assessment laboratory, Shanghai, China
Anonymous 38	2011b	Fish, juvenile growth test of XTJ 568 to zebrafish (Danio rerio)	Bioassay and Safety Assessment laboratory, Shanghai, China
Anonymous 39	2004b	Acute toxicity study in Daphnia magna with XTJ 568 (static)	Notox B.V., 's-Hertogenbosch, The Netherlands
Anonymous 40	2011	Daphnia magna reproduction test of XTJ 568	Bioassay and Safety Assessment laboratory, Shanghai, China
Anonymous 41	2004c	Fresh water algal growth inhibition test with XTJ 568	Notox B.V., 's-Hertogenbosch, The Netherlands
Confidential 42	2003b	Activated sludge respiration inhibition test with XTJ 568 (contact time : 30 minutes)	Notox B.V., 's-Hertogenbosch, The Netherlands

8 ANNEXES

Recapitulative on the main findings of the XTJ568: Toxicity Study by Oral Administration to CD Rats for 13 Weeks (Anonymous 23, 2011)

<u>Haematology:</u>

Table 35 : haematology in males. At 150 mg/kg bw/d, slightly high platelet counts was observed.

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group /Sex		WBC x10^9/L	N x10^9/L	L x10^9/L	E x10^9/L	B x10^9/L	M x10^9/L	LUC x10^9/L	Plt x10^9/L	PT sec	APTT sec
Statistical test:		Wi	Wi	Wi	lWi	Wi	Wi	Wi	Wi	Sh	Wi
1M	Mean	13.22	2.09	10.33	0.21	0.05	0.47	0.07	1144	19.0	17.5
	SD	3.791	0.947	3.044	0.240	0.030	0.156	0.038	159.5	4.07	3.03
	N	10	10	10	10	10	10	10	10	10	10
2M	Mean	11.94	2.02	9.26	0.14	0.04	0.41	0.07	1174	19.9	16.9
	SD	3.209	0.807	2.484	0.052	0.020	0.175	0.030	141.7	1.21	2.34
	N	10	10	10	10	10	10	10	10	10	10
3M	Mean	13.55	2.81	9.88	0.18	0.05	0.56	0.07	1228	20.6	14.8
	SD	6.706	1.788	4.807	0.120	0.034	0.346	0.050	164.0	0.87	2.27
	N	10	10	10	10	10	10	10	10	10	10
4M	Mean	10.93	1.63	8.59	0.13	0.04	0.49	0.06	1304*	20.8	16.0
	SD	3.285	0.653	2.767	0.100	0.026	0.184	0.026	108.7	0.98	1.85
	N	10	10	10	10	10	10	10	10	10	10

Table 36: haematology in females. At 150 mg/kg bw/d, slightly decrease neutrophil and lymphocyte counts.

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group /Sex		WBC x10^9/L	N x10^9/L	L x10^9/L	E x10^9/L	B x10^9/L	M x10^9/L	LUC x10^9/L	Plt x10^9/L	PT sec	APTT sec
Statistical test:		Wi	lWi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	7.44	0.70	6.37	0.09	0.03	0.17	0.09	1053	22.2	14.0
	SD	1.336	0.232	1.340	0.033	0.012	0.041	0.030	97.4	1.22	1.37
	N	10	10	10	10	10	10	10	10	10	10
2F	Mean	7.90	0.85	6.65	0.10	0.03	0.18	0.10	1043	22.4	14.4
	SD	1.586	0.307	1.504	0.037	0.015	0.053	0.048	96.8	1.17	1.96
	N	10	10	10	10	10	10	10	9	10	10
3F	Mean	6.90	0.95	5.63	0.08	0.02	0.17	0.06	970	22.2	14.0
	SD	2.000	0.765	1.321	0.031	0.014	0.068	0.024	93.5	1.00	1.97
	N	10	10	10	10	10	10	10	10	10	9
4F	Mean	5.89*	0.62	4.95*	0.08	0.02	0.16	0.06	1124	22.3	14.5
	SD	1.156	0.384	1.111	0.026	0.010	0.042	0.035	107.6	0.65	2.25
	N	10	10	10	10	10	10	10	10	10	10

Blood Chemistry:

Table 37: Blood chemistry in males : at 150 mg/kg bw/d : slightly low creatinine concentration.

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group /Sex		ALP U/L	ALT U/L	AST U/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	Trig mmol/L
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	100	41	69	5.33	35	7.55	1.73	0.91
	SD	18.2	6.2	7.1	0.997	4.9	0.477	0.464	0.430
	N	10	10	10	10	10	10	10	10
2M	Mean	88	47	70	5.59	37	7.57	1.79	1.04
	SD	16.3	7.0	5.7	0.958	4.7	0.602	0.436	0.661
	N	10	10	10	10	10	10	10	10
3M	Mean	94	44	73	4.98	36	7.67	1.71	1.46
	SD	17.5	9.0	13.5	1.135	4.0	0.660	0.339	0.640
	N	10	10	10	10	10	10	10	10
4M	Mean	103	47	72	5.21	29*	7.29	1.80	0.86
	SD	22.1	8.9	9.0	0.610	5.1	0.594	0.353	0.260
	N	10	10	10	10	10	10	10	10

Table 38 : Blood chemistry in females : all treated females reveal a slightly increase in potassium concentration. Moreover, a slightly decrease in phosphore concentration at 15 mg/kg bw/d and a slow high calcium concentration are observed at 150 mg/kg bw/d.

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group /Sex		Na mmol/L	K mmol/L	Cl mmol/L	Ca mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	A/G Ratio
Statistical test:		Wi	Wi	Wi	Wi	Du	Wi	Wi	Wi
1F	Mean	142	4.1	104	2.60	1.70	70	40	1.38
	SD	0.9	0.22	1.7	0.074	0.178	3.5	2.5	0.162
	N	10	10	10	10	10	10	10	10
2F	Mean	142	4.4*	104	2.59	1.49*	70	40	1.37
	SD	1.5	0.31	1.7	0.061	0.130	4.1	2.3	0.091
	N	10	10	10	10	10	10	10	10
3F	Mean	142	4.5*	103	2.63	1.67	71	41	1.37
	SD	0.8	0.24	1.0	0.057	0.216	2.3	2.0	0.086
	N	10	10	10	10	10	10	10	10
4F	Mean	142	4.3*	103	2.66*	1.69	72	42	1.41
	SD	1.3	0.44	1.2	0.028	0.172	3.0	1.9	0.055
	N	10	10	10	10	10	10	10	10

Necropsy:

Table 39 : Organ weight in males :

Group	:	1	2	3	4
Compound	:	Control	XTJ568	XTJ568	XTJ568
Dose (mg/kg/day)	:	0	15	50	150

Group /Sex		Terminal Bodyweight	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
Unadjusted Means											
Statistical test:		Wi			Sh				Sh		
1M	Mean	535	0.051	2.276	1.343	1.554	3.388	19.687	0.907	3.720	0.292
	SD	60	0.008	0.107	0.076	0.160	0.391	3.189	0.217	0.342	0.054
	N	10	10	10	9	10	10	10	10	10	10
2M	Mean	505	0.048	2.193	1.345	1.381	3.040	18.774	0.736	3.608	0.208
	SD	38	0.006	0.090	0.267	0.083	0.230	2.166	0.104	0.394	0.046
	N	10	10	10	10	10	10	10	10	10	10
3M	Mean	541	0.049	2.267	1.362	1.491	3.296	19.984	1.116	3.823	0.230
	SD	55	0.009	0.093	0.070	0.169	0.348	4.058	0.896	0.217	0.078
	N	10	10	10	10	10	10	10	10	10	10
4M	Mean	500	0.053	2.149	1.297	1.398	3.151	18.545	0.811	3.612	0.228
	SD	52	0.008	0.108	0.132	0.087	0.397	1.958	0.251	0.202	0.067
	N	10	10	10	10	10	10	10	10	10	10
Adjusted Means											
Statistical test:			Wi	Wi		Wi	Wi	Wi		Wi	Wi
1M	Mean		0.050	2.259		1.524	3.306	19.105		3.685	0.286
2M	Mean		0.050	2.211		1.411*	3.123	19.370		3.644	0.213*
3M	Mean		0.048	2.244		1.449*	3.181	19.168		3.774	0.223*
4M	Mean		0.055	2.172*		1.439*	3.264	19.347		3.660	0.235*

Sperm analysis

Table 40 : Sperm analysis

Group	:	1	2	3	4				
Compound	:	Control	XTJ568	XTJ568	XTJ568				
Dose (mg/kg/day)	:	0	15	50	150				

Group		Motile sperm (%)	Progressively motile sperm (%)	-------- Cauda epididymis-------- Weight (g)	Sperm count (millions/g)	Total (million)	--------- Testis --------- Weight (g)	Sperm count (millions/g)	Total (million)
Statistical test:		Sh	Wi	Sh	Wi	Wi	Wi	Wi	Wi
1	Mean	92	56	0.255	946	241	1.86	135	249
	SD	5	10	0.028	230	73	0.18	23	30
	n	10	10	10	10	10	10	10	10
2	Mean	95	59	0.312	870	248	1.81	138	251
	SD	2	10	0.205	167	80	0.21	13	45
	n	9	9	10	10	10	10	10	10
3	Mean	88	54	0.259	950	246	1.92	131	250
	SD	9	10	0.026	152	48	0.11	26	48
	n	10	10	10	10	10	10	10	10
4	Mean	85	52	0.241	928	223	1.82	134	244
	SD	30	22	0.025	176	47	0.09	19	39
	n	10	10	10	10	10	10	10	10

9 CONFIDENTIAL DATA

Composition of substance : see confidential annex