Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted
9 December 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON REACTION MASS OF 1-[2-(2-AMINOBUTOXY)ETHOXY]BUT-2-YLAMINE AND 1-({[2-(2-AMINOBUTOXY)ETHOXY]METHYL}PROPOXY)BUT-2-YLAMINE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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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: -
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2016	Belgium	Huntsman (Europe) BVBA	Company-Manufacturer	1
Comment received				
PLEASE SEE ATTACHMENT: The attached document ("Huntsman XTJ 568 PC report reprotox final_2016 04 20.pdf") is extremely important to be considered as it provides a full review of four toxicity reports that are relevant for reproductive toxicity. Therefore we highly encourage to consider this attached document as the provided comments for reproductive toxicity only summarize the findings of this review study. ECHA note: The following attachment was submitted with the comment above: *Huntsman XTJ 568 PC report reprotox final_2016 04 20*				
Dossier Submitter's Response				
BE thanks Huntsman (Europe) BVBA for providing the document.				
RAC's response				
RAC appreciates receiving the document which has been very helpful.				

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2016	Netherlands		MemberState	2
Comment received				
MSCA comments for Mutagenicity and Reproductive toxicity only. - NL agrees with no classification for mutagenicity. - NL agrees with classification in Category 2 for fertility, but requests further discussion on the classification for developmental effects.				

- Some studies were performed using the dichloride of XTJ 568 which will probably not have the strong corrosive effect of XTJ 568 itself. Please explain which studies were performed with the XTJ 568 and which with the dichloride and how this affects the MTD in the studies and possibly the classification.
Dossier Submitter's Response
BE thanks NL for its support to BE proposal to classify for toxicity to reproduction in category 2 for fertility and to not classify for germ cell mutagenicity. The prenatal developmental toxicity study (OECD 414) and the two-generation reproductive toxicity study (OECD 416) are performed with XTJ 568 dihydrochloride.
RAC's response
The support for classifying for effects on fertility is noted, as well as questioning of the proposed classification for developmental toxicity. The RAC also agrees with no classification for mutagenicity. RAC appreciates the clarification regarding the use of free diamine versus a dihydrochloride, and further notes that it would be helpful to have information as to the rate of dissociation of the dihydrochloride at different conditions to support the read across between these two forms of the substance. It seems that the diamine is more toxic than the dihydrochloride, but RAC accepts using the dihydrochloride studies as basis for the reproductive toxicity classification.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.04.2016	France		MemberState	3
Comment received				
As no carcinogenic study has been performed we should be more in favor not to conclude on carcinogenicity rather to make a conclusion based on the observations coming from the 90 days or reproductive toxicity studies. Therefore it should be clearly stated that the classification is not warranted due to the absence of relevant data.				
Dossier Submitter's Response				
BE thanks FR for its comment and agree to specify that the classification as carcinogen is not warranted due to the absence of relevant data although the CLH report will not be updated by BE at this stage as mentioned in the process.				
RAC's response				
We agree with the French comment but also note that this endpoint was not open to comment in the PC and therefore will not be dealt with in the opinion.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2016	Sweden		MemberState	4
Comment received				
Based on the arguments presented regarding germ cell mutagenicity, the Swedish CA supports that the available information does not meet the requirements of the CLP criteria for classification of 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 No. 447-920-2) as a germ cell mutagen. Accordingly, classification for germ cell mutagenicity is not warranted.				

We would also like to take the opportunity to comment that the results of the studies are merely presented as concluding statements, for example negative or no effect, without reporting any data. For that reason it is not possible for the reader to evaluate the studies thoroughly and to judge if the conclusion made by the DS is acceptable. A higher level of detail is indeed desirable.
Dossier Submitter's Response
BE thanks SE for its support. We provide more information as required by Sweden in the Annexes of this document
RAC's response
The support and the further information in the Annexes are noted.

Date	Country	Organisation	Type of Organisation	Comment number
21.04.2016	France		MemberState	5
Comment received				
There is no in vitro mammalian gene mutation test and in the in vivo mutagenicity test, there is no evidence that the compound has reached the target organ (no modification of the PCEs/ECs). Therefore it should be clearly stated that the classification is not required due to lack of data.				
Dossier Submitter's Response				
BE thanks FR for its comment and agree to specify that the classification as mutagen is not warranted due to the lack of data although the CLH report will not be updated by BE at this stage as mentioned in the process				
RAC's response				
RAC agrees with the proposal for no classification. The lack of data is noted in the opinion.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2016	Germany		MemberState	6
Comment received				
We agree with the proposed classification Repr 2, H361 for effects on fertility based on findings on sperm toxicity in the two-generation study (Anonymous 28, 2010). Furthermore the proposed classification Repr 2, H361 for effects on development needs more scrutiny. This proposal is based on, firstly, delayed balanopreputial (statistically significant) and vaginal opening in the high exposure group (1000 mg/kg bw/d) in the F1 generation. From our point of view, this observation is related to a significantly decreased pup body weight from lactation days 7 in males (29%) and lactation days 14 in females (22%) and could be a non-specific effect with no relevance for classification. Furthermore, these effects were not observed in the F2 generation. Secondly, a lower anogenital distance in males (statistically significant) 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 was listed as justification for classification. However, no effects on anogenital distance were observed in the high exposure group (1000 mg/kg bw/d) in the F2 generation or in any of the exposure group in the F1 generation. Therefore, we are not sure whether these single findings might be sufficient for classification for developmental toxicity. This should be discussed by RAC.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON REACTION MASS OF 1-[2-(2-AMINOBUTOXY)ETHOXY]BUT-2-YLAMINE AND 1-({[2-(2-AMINOBUTOXY)ETHOXY]METHYL}PROPOXY)BUT-2-YLAMINE

Dossier Submitter's Response
BE thanks DE for its support to BE proposal to classify as Repr. 2 H361 for fertility. Regarding the developmental toxicity, BE CA acknowledges that the effects are relatively limited. Indeed, the only effects we see are : • statistically significant delayed balanopreputial at high dose level (1000 mg/kg bw/d) in the F1 generation. • vaginal opening at high dose level (1000 mg/kg bw/d) in the F1 generation. • lower anogenital distance in males (statistically significant) 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. Although those effects are limited, BE CA was of the opinion to consider it relevant in the frame of a classification process. Morevover, the complete picture was not available in the full study report since data on delayed balanopreputial and vaginal opening were only described for the high dose group (1000 mg/kg bw/d). It is therefore not possible to determined a potential trend for those effects. No information on deleyaed balanopreputial and vaginal opening was described in the F2 generation. The anogenital distance is also not reported for the F1 generation.
RAC's response
The support for classifying for effects on fertility is noted. Based on the likely relationship to a slower growth of the pups and a lack of consistency between generations, RAC does not agree that classification for developmental toxicity is warranted. The lack of data for some effects in some dose groups/generations is a concern but not a reason for classification.

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2016	Belgium	Huntsman (Europe) BVBA	Company-Manufacturer	7
Comment received				
Section 4.11.5 of the Belgian Authority's CLH report for XTJ 568 states that substances are classified in Category 2 for reproductive toxicity when there is some evidence from experimental animals of effects on fertility, development, or sexual function occurring in the absence of other toxic effects or, if occurring with other toxic effects, the adverse effects on reproduction are not considered secondary to that toxicity. It is Penman Consulting's (PC) view that the results of the OECD TG 416 study with XTJ 568 dihydrochloride satisfy these conditions with regard to effects on male and female fertility. It should be noted that since no OECD TG 416 study is available for XTJ 568 free amine, the CLP assessment is based on read-across from the dihydrochloride salt of XTJ 568 to the XTJ 568 free diamine. The Belgian Authority's proposal to classify XTJ 568 in Rep Cat 2 was partially based on developmental effects in the OECD TF416 study. We do not concur, for the following reasons: 1. No pre-natal developmental effects were seen in the OECD TG 414 study, in which the NOAEL for developmental effects was 1000 mg/kg/d. 2. On page 56 (last para) of the CLH document, the Belgian Authority refers to: a. Statistically significant delays in balanopreputial and vaginal openings in F1 pups at 1000 mg/kg/d. However, this finding was not observed in F2 pups and it occurred in the presence of parental toxicity. b. Significantly lower anogenital distance in F2 males at 150 and 450 mg/kg/d and in females at 150 mg/kg/d. However, this finding was not seen in F2 high dose males or				

females, nor was it seen in F1 pups.

Conclusion:
In PC's judgment, the results of the OECD TG 416 oral (gavage) 2-generation reproductive toxicity study with XTJ 568 dihydrochloride supports a reproductive Category 2 classification according to EU CLP guidance, since NOAEL for reproductive effects (150 mg/kg/d) is below the NOAEL for parental toxicity (450 mg/kg/d), and several reproductive effects were observed at 1000 mg/kg/d in the presence of some parental toxicity. However, it should be noted that test item in this study was the dihydrochloride salt of XTJ 568 diamine and no OECD TG 416 study is available for XTJ 568 free diamine. Therefore, the assessment is based on read-across from dihydrochloride salt of XTJ 568 to the XTJ 568 free diamine. Although PC concurs with the Belgian Authority's proposal to classify XTJ 568 in Reproductive Category 2 based on fertility effects in the OECD TG 416 study, we do not concur with their proposal to classify XTJ 568 in Rep Cat 2 based on developmental effects in the OECD TG 416 study, for the reasons outlined above.

Dossier Submitter's Response

BE thanks Hunstman (Europe) BVBA for its comment and for its support to BE proposal to classify for toxicity to reproduction in category 2 for fertility.
More comments on developmental toxicity are to be found in the answer to comment 6.

RAC's response

The support for fertility and non-support for developmental toxicity is noted. RAC shares this view and refers to the response to comment #6 above.

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2016	Netherlands		MemberState	8

Comment received

NL agrees with classification for fertility. However, the reasoning why Cat 2 is proposed rather than 1B is not very clear in the report. The main argument to classify as Cat 2 seems to be that most effects are only significant at the highest dose, at which also systemic toxicity occurs. In addition, some effects show no clear dose-response relationship, such as the testis sperm concentration (Table 20) and the irregular oestrous cycle (p45) of the P0 generation, which also occurred in the controls.
The main argument for classification as 1B would be the histopathological findings in the testis at the highest dose and the decreased motility and normal morphology of the sperm cells in the P0 and F1 generation (Tables 20 and 24). The latter effects are also seen at the mid dose level, at which only some slight systemic toxicity is observed (salivation and decrease of thymus weight in males), which does not explain the effects on the reproductive organs.

The stated developmental effects were:
• Delayed balanopreputial separation and vaginal opening. However, this effect was observed together with reduced post-natal body weight gain at the highest dose which also showed reduced maternal body weight. This developmental effect is considered likely to be secondary to the maternal toxicity.
• Reduction in male anogenital distance at 150 and 450 mg/kg bw/day. However, the relevance of this effect can be doubted as it showed no dose effect relation and the average anogenital distance at the highest dose was even above the controls.

The only argument to include development in the classification is that it cannot be

excluded that some fertility effects in the second generation are related to development. This applies in particular to the reduced number of pups in second generation as this effect was not found in the first generation. If this is the reason for suggesting both classification for effects on fertility and development, a classification without specifying this could be considered.

In addition, we would like to make the following remarks:

On page 43 a reproductive NOAEL of 450 mg/kg bw/d is given. As several effects, particularly on sperm parameters, were significant at this concentration, we would consider this the LOAEL and 150 mg/kg bw/d the NOAEL for this endpoint.

On pag. 46, the sperm motility is marked as significantly different in the mid- and high dose groups, but the median scores are the same or one point lower than the controls respectively. Also, the values for median motility are very low and the st. dev. is missing. Please check whether this is correct.

On the same page, it is unclear what is meant with the dead pups per litter. For example, the mortality of the controls is 24/5. Are these the numbers of males/females? And what was the total number pups? Please clarify this.

On pag. 48, at the end of the second paragraph, there seems to be information missing. Please provide the remaining information in the RCOM.

Dossier Submitter's Response

BE thanks NL for its support to classify for fertility. BE CA decided to propose a classification as cat 2 instead of 1B since the effects are observed in one species but we acknowledge that the effects are seen at doses that can lead to a classification in cat. 1B.

More comments on developmental toxicity are to be found in the answer to comment 6.

BE thanks NL for their remarks although the CLH report will not be updated by BE at this stage as mentioned in the process.

1) Concerning the choice of the reproductive NOAEL in the 2-generation study, BE agree to consider 450 mg/kg bw/d as the LOAEL and not as the NOAEL as indicated in the CLH report.
2) Concerning the sperm motility in the 2-generation study, the table reported in the CLH dossier is the one indicated in the full study report.
3) Concerning the request to clarify the meaning of dead pups per litter indicated in the CLH report :
   A total of 24 dead pups were found among the 5 examined litters in the control group. In the same way, for the lowest dose group, a total of 6 pups were found dead across the 4 examined litters (1 male in one litter, 1 female in one litter, 1 male in one litter and 1 male and 2 female in one litter).
4) Concerning the missing information in page 48, the end of the sentence was "chromodacryorrhea of the eye and/or periorbital region and broken tail apex".

RAC's response

The support for fertility is noted. Althought Cat 1B could be discussed for fertility, the testicular findings come from a single study and mainly from a dose level also causing some maternal toxicity. The findings constitute "some evidence" but are not sufficient for Cat 1B according to the RAC evaluation. Regarding developmental toxicity, see the response to comment number 6.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
21.04.2016	France		MemberState	9
Comment received				
As the concentration used for the challenge test is low (1%) and the number of animals tested limited, to confirm the reliability score of 1, it would be useful to have more information about the preliminary range finding test that should have been done to justify that 1% is indeed the maximum tolerated concentration. Furthermore could you please confirm that challenge was performed by topical application and not epidermal application as stated in the CLH report.				
Dossier Submitter's Response				
BE thanks FR for its comment on the skin sensitisation hazard. 1) Concerning the necessity to have more information about the preliminary range finding test and the choice of the challenge's concentration, the registration dossier indicates that the maximum non-irritant concentration was selected. The preliminary range finding test examines a series of different dose level (100% (undiluted), 50%, 20%, 10%, 5%, 2%, 1% and if needed further lower concentration). However the results of the preliminary range finding test was not mentioned in the available report. 2) Concerning the application route of the challenge, the Chemical Safety report and the registration dossier indicate that challenge was performed by epicutaneous/epidermal and semi-occlusive application.				
RAC's response				
RAC supports no classification but the endpoint was not open for comenting and will therefore not be discussed in the opinion.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
21.04.2016	France		MemberState	10
Comment received				
We agree that the substance Reaction mass of XTJ568 does not fulfill criteria to be classified for environmental hazards				
Dossier Submitter's Response				
Thanks for your support.				
RAC's response				
The support is noted.				

NON-CONFIDENTIAL ATTACHMENTS

1. *Huntsman XTJ 568 PC report reprotox final_2016 04 20*. Submitted on 20/04/2016 by Huntsman (Europe) BVBA. [Please refer to comments No 1, 7]

ANNEXES

Results of the Salmonella Thyphimurium reverse mutation assay and Escherichia Coli reverse mutation assay :

TABLE 1 MUTAGENIC RESPONSE OF **XTJ 568** IN THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY AND IN THE *ESCHERICHIA COLI* REVERSE MUTATION ASSAY

Experiment 1
Day of performance:
TA100 and WP_2uvrA: 04 March 2003
TA1535, TA1537 and TA98: 07 March 2003

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium* and one *Escherichia coli* strain				
	TA1535	TA1537	TA98	TA100	WP_2uvrA
	Without S9-mix				
positive control	416 ± 21	451 ± 54	262 ± 44	800 ± 20	502 ± 31
solvent control	9 ± 4	6 ± 2	24 ± 2	121 ± 8	16 ± 3
3				112 ± 14	13 ± 1
10				107 ± 9	10 ± 2
33				110 ± 11	15 ± 3
100	8 ± 1	6 ± 3	22 ± 5	115 ± 14	12 ± 3
333	10 ± 5	5 ± 1	24 ± 8	108 ± 2	16 ± 3
1000	13 ± 4	8 ± 2	25 ± 4	104 ± 2	16 ± 3
3330	11 ± 5	6 ± 2	21 ± 6	119 ± 10	11 ± 2
5000	12 ± 2	6 ± 2	17 ± 2	116 ± 7	13 ± 3
	With S9-mix[1]				
positive control	128 ± 20	415 ± 41	423 ± 11	1133 ± 31	184 ± 8
solvent control	9 ± 5	9 ± 1	30 ± 6	129 ± 4	13 ± 2
3				115 ± 9	12 ± 3
10				128 ± 10	10 ± 3
33				133 ± 10	14 ± 2
100	11 ± 5	6 ± 2	28 ± 7	112 ± 7	13 ± 2
333	8 ± 3	6 ± 3	32 ± 5	119 ± 7	16 ± 2
1000	11 ± 5	9 ± 1	32 ± 1	130 ± 10	16 ± 2
3330	11 ± 3	6 ± 5	30 ± 7	130 ± 10	12 ± 1
5000	9 ± 3	4 ± 2	23 ± 4	120 ± 13	16 ± 1

Solvent control: 0.1 ml Milli-Q water
1 The S9-mix contained 5% (v/v) S9 fraction

TABLE 2 MUTAGENIC RESPONSE OF **XTJ 568** IN THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY AND IN THE *ESCHERICHIA COLI* REVERSE MUTATION ASSAY

Experiment 2
Day of performance: 11 March 2003

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium* and one *Escherichia coli* strain				
	TA1535	TA1537	TA98	TA100	WP_2uvrA
Without S9-mix					
positive control	453 ± 9	537 ± 75	809 ± 47	888 ± 31	815 ± 35
solvent control	11 ± 4	6 ± 2	22 ± 4	116 ± 5	20 ± 4
100	10 ± 3	4 ± 1	24 ± 3	114 ± 12	19 ± 3
333	9 ± 3	4 ± 1	24 ± 4	97 ± 1	17 ± 1
1000	9 ± 2	6 ± 2	21 ± 2	121 ± 9	18 ± 5
3330	9 ± 2	4 ± 1	25 ± 2	114 ± 4	19 ± 4
5000	7 ± 1	5 ± 2	23 ± 4	104 ± 6	15 ± 2
With S9-mix[1]					
positive control	111 ± 14	107 ± 5	471 ± 81	1061 ± 69	192 ± 33
solvent control	6 ± 3	5 ± 1	27 ± 1	107 ± 15	20 ± 2
100	9 ± 3	5 ± 1	33 ± 4	111 ± 17	22 ± 3
333	9 ± 3	5 ± 1	24 ± 4	91 ± 11	14 ± 2
1000	8 ± 2	6 ± 3	30 ± 3	109 ± 15	21 ± 6
3330	8 ± 4	4 ± 1	30 ± 4	109 ± 5	24 ± 5
5000	6 ± 1	5 ± 1	25 ± 3	104 ± 10	17 ± 2

Solvent control: 0.1 ml Milli-Q water
1 The S9-mix contained 10% (v/v) S9 fraction

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON REACTION MASS OF 1-[2-(2-AMINOBUTOXY)ETHOXY]BUT-2-YLAMINE AND 1-({[2-(2-AMINOBUTOXY)ETHOXY]METHYL}PROPOXY)BUT-2-YLAMINE

Results of the Chromosome aberration in cultured peripheral human lymphocytes :

TABLE 1 MITOTIC INDEX OF DONOR CULTURES TREATED WITH XTJ 568
Dose range finding
Period of treatment: From: 19-03-2003 To: 21-03-2003

XTJ 568 concentration (µg/ml)	Number of metaphases per 1000 cells Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
3 h exposure time, 24 h fixation time		
Control [a]	24	100
100	26	108
333	20	83
1000	16	67
3330	- [b]	- [b]
5000	- [b]	- [b]
24 h exposure time, 24 h fixation time		
Control [a]	16	100
100	25	156
333	33	206
1000	3	19
3330	- [b]	- [b]
5000	- [b]	- [b]
48 h exposure time, 48 h fixation time		
Control [a]	25	100
100	30	120
333	28	112
1000	9	36
3330	- [b]	- [b]
5000	- [b]	- [b]
With metabolic activation (+S9-mix)		
3 h exposure time, 24 h fixation time		
Control [a]	34	100
100	28	82
333	31	91
1000	26	76
3330	- [b]	- [b]
5000	- [b]	- [b]

[a] F10 medium buffered with 20 mM HEPES.
[b] Cell lysis

TABLE 2 MITOTIC INDEX OF DONOR CULTURES TREATED WITH XTJ 568
First cytogenetic assay
Period of treatment: From: 26-03-2003 To: 27-03-2003

XTJ 568 concentration (µg/ml)	Number of metaphases per 1000 cells [a] Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
3 h exposure time, 24 h fixation time		
Control [b]	69 - 75	100
333	63 - 59	85
666	67 - 61	89
1000	29 - 25	38
1250	1 - 1	1
1500	- [c] - - [c]	- [c]
2000	- [c] - - [c]	- [c]
MMC-C; 0.5 µg/ml	34 - 48	57
With metabolic activation (+S9-mix)		
3 h exposure time, 24 h fixation time		
Control [b]	65 - 51	100
333	66 - 62	110
666	70 - 60	112
1000	28 - 22	43
1250	2 - 1	3
1500	- [c] - - [c]	- [c]
2000	- [c] - - [c]	- [c]
CP; 15 µg/ml	23 - 31	47

[a] Duplicate cultures
[b] F10 medium buffered with 20 mM HEPES.
[c] Cell lysis

TABLE 3 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH XTJ 568
(Without S9-mix) [a)]
3 h exposure time, 24 h fixation time
First cytogenetic assay

Conc µg/ml	F10-HEPES (1.0% v/v)			333 µg/ml			666 µg/ml			1000 µg/ml			MMC-C 0.5 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			85			89			38			57		
No. of Cells scored	100	100	200	100	100	200	100	100	200	76[b)]	97[b)]	173	100	100	200
No. of Cells with aberrations (+ gaps)	0	0	0	0	5	5	1	1	2	2	0	2	18	18	36 ***
No. of Cells with aberrations (- gaps)	0	0	0	0	4	4	1	1	2	1	0	1	18	17	35 ***
g'					1					1			1	1	
g"															
b'					2		1	1					3	5	
b"										1			2	2	
m'															
m"					2		1								
exch.													10	10	
dic															
d'															
misc.	poly						poly	7poly		poly endo	2poly		5intra	intra	
total aberr (+ gaps)	0	0		0	5		2	1		2	0		21	19	
total aberr (- gaps)	0	0		0	4		2	1		1	0		20	18	

a) Abbreviations used for various types of aberrations are listed in appendix 1.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.

b) No more scorable metaphases available.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 4 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH XTJ 568
(With S9-mix) [a)]
3 h exposure time, 24 h fixation time
First cytogenetic assay

Conc µg/ml	F10-HEPES (1.0% v/v)			333 µg/ml			666 µg/ml			1000 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			110			112			43			47		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps)	0	0	0	0	0	0	0	0	0	5	1	6	24	26	50 ***
No. of Cells with aberrations (- gaps)	0	0	0	0	0	0	0	0	0	3	1	4	21	24	45 ***
g'										2			4	4	
g"															
b'										1	1		11	20	
b"										2			4	4	
m'															
m"													2	1	
exch.													8	6	
dic													1		
d'															
misc.				poly			2poly			3poly	3poly				
total aberr (+ gaps)	0	0		0	0		0	0		5	1		30	35	
total aberr (- gaps)	0	0		0	0		0	0		3	1		26	31	

a) Abbreviations used for various types of aberrations are listed in appendix 1.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
The numerical variation polyploidy (poly) was not counted as an aberration.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 5 MITOTIC INDEX OF DONOR CULTURES TREATED WITH XTJ 568
Second cytogenetic assay
Period of treatment: From: 09-04-2003 To: 11-04-2003

XTJ 568 concentration (µg/ml)	Number of metaphases per 1000 cells [a] Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
24 h exposure time, 24 h fixation time		
Control [b]	46 - 39	100
100	37 - 57	111
333	42 - 30	85
500	32 - 32	75
600	25 - 31	66
800	20 - 26	*54
1000	4 - 0	5
MMC-C; 0.2 µg/ml	13 - 20	39
48 h exposure time, 48 h fixation time		
Control [b]	35 - 42	100
100	41 - 37	101
333	55 - 41	125
500	42 - 52	122
600	38 - 36	96
800	44 - 34	101
1000	2 - 2	5
MMC-C; 0.1 µg/ml	16 - 30	60
With metabolic activation (+S9-mix)		
3 h exposure time, 48 h fixation time		
Control [b]	53 - 64	100
100	70 - 57	109
333	66 - 60	108
666	54 - 66	103
1000	100 - 89	162
1250	14 - 40	46
1500	0 - 0	0
CP; 15 µg/ml	26 - 28	– [c]

[a] Duplicate cultures
[b] F10 medium buffered with 20 mM HEPES.
[c] CP was fixed after 24 hours. Therefore, the mitotic index could not be calculated as percentage of control.
*) The quality control for the mitotic index, counted by two different persons revealed that this concentration was cytotoxic enough (inhibition of the mitotic index greater than 50%) to choose for the scoring of chromosome aberrations.

TABLE 6 MITOTIC INDEX OF DONOR CULTURES TREATED WITH XTJ 568
Cytogenetic assay 2A
Period of treatment: From: 07-05-2003 To: 09-05-2003

XTJ 568 concentration (µg/ml)	Number of metaphases per 1000 cells [a] Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
48 h exposure time, 48 h fixation time		
Control [b]	25 - 26	100
700	32 - 21	104
850	19 - 12	61
875	17 - 7	47
900	8 - 6	27
925	8 - 0	16
950	2 - 0	4
975	0 - 0	0
MMC-C; 0.1 µg/ml	24 - 15	76

[a] Duplicate cultures
[b] F10 medium buffered with 20 mM HEPES.

TABLE 7 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH XTJ 568
(Without S9-mix) [a]
24 h exposure time, 24 h fixation time
Second cytogenetic assay

Conc µg/ml	F10-HEPES (1.0% v/v)			100 µg/ml			500 µg/ml			800 µg/ml			MMC-C 0.2 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			111			75			54			39		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps)	1	0	1	0	2	2	0	0	0	1	2	3	17	20	37 ***
No. of Cells with aberrations (- gaps)	0	0	0	0	1	1	0	0	0	1	1	2	17	20	37 ***
g'	1				1						1		1		
g"													2		
b'					1						1		9	13	
b"										1			6	4	
m'													1		
m"															
exch.													3	3	
dic															
d'															
misc.	endo						2poly			poly	poly		ma		
total aberr (+ gaps)	1	0		0	2		0	0		1	2		22	30	
total aberr (- gaps)	0	0		0	1		0	0		1	1		19	30	

[a] Abbreviations used for various types of aberrations are listed in appendix 1.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ o- *** $P < 0.001$.

TABLE 8 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH XTJ 568
(Without S9-mix) [a)]
48 h exposure time, 48 h fixation time
Cytogenetic assay 2A

Conc µg/ml	F10-HEPES (1.0% v/v)			700 µg/ml			850 µg/ml			875 µg/ml			MMC-C 0.1 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			104			61			47			76		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps)	2	2	4	2	4	6	2	3	5	4	2	6	37	34	71 ***
No. of Cells with aberrations (- gaps)	1	2	3	1	4	5	2	3	5	4	2	6	36	34	70 ***
g'	1			1									1	2	
g"															
b'	1	2			4		3	3		4	2		26	28	
b"				1									8	3	
m'															
m"															
exch.													7	6	
dic															
d'															
misc.								poly endo			endo				
total aberr (+ gaps)	2	2		2	4		3	3		4	2		42	39	
total aberr (- gaps)	1	2		1	4		3	3		4	2		41	37	

a) Abbreviations used for various types of aberrations are listed in appendix 1.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$.

TABLE 9 CHROMOSOME ABERRATIONS IN DONOR CULTURES TREATED WITH XTJ 568
(With S9-mix) [a]
3 h exposure time, 48 h fixation time
Second cytogenetic assay

Conc µg/ml	F10-HEPES (1.0% v/v)			100 µg/ml			666 µg/ml			1250 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			109			103			46			- [b]		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps)	1	1	2	1	1	2	2	1	3	0	0	0	20	22	***) 42
No. of Cells with aberrations (- gaps)	0	1	1	1	0	1	2	1	3	0	0	0	19	22	***) 41
g'	1												1		
g"					1										
b'		1		1			2	1					12	15	
b"													7	5	
m'															
m*															
exch.														3	
dic															
d'															
misc.	poly												ma		
total aberr (+ gaps)	1	1		1	1		2	1		0	0		30	23	
total aberr (- gaps)	0	1		1	0		2	1		0	0		29	23	

a) Abbreviations used for various types of aberrations are listed in appendix 1.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.
The numerical variation polyploidy (poly) was not counted as an aberration.

b) CP was fixed after 24 hours. Therefore, the mitotic index could not be calculated as percentage of control.

*) Significantly different from control group (Chi-square test), * P < 0.05, ** P < 0.01 or *** P < 0.001.

Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of XTJ-568:

The incidence of micronucleated polychromatic erythrocytes per 10,000 polychromatic erythrocytes scored (2000 PCEs/mouse) and the proportion of polychromatic erythrocytes per total erythrocytes are summarized and presented for each treatment group by sacrifice time

- No appreciable reductions in the ratio of polychromatic erythrocytes to total erythrocytes (PCEs/ECs) in the test article groups relative to the respective vehicle control groups were observed suggesting that the test article did not inhibit erythropoiesis.
- No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in test article groups relative to the respective vehicle control groups was observed in male or female mice at 24 or 48 hours after dose administration ($p > 0.05$, binominal disrtibution, Kastenbaum-Bowman Tables).
- CP, the positive control, induced a statistically significant increase in the incidence of micronucleated PCEs ($p \leq 0.05$, binominal distribution, Kastenbaum-Bowman Tables) in both male and female mice. The number of micronucleated PCEs in the vehicle control groups did not exceed the historical vehicle control range. Based upon this, all criteria for a valid test were met as specified in the protocol.

The results are summarised in the following table:

Table 13.4: Summary of Bone Marrow Micronucleus Analysis Following a Single Oral Administration of XTJ-568 in ICR Mice

Treatment (PO, 20 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Purified water	M	24	5	0.449 ± 0.03	---	0.3 ± 0.27	3 / 10000
	F	24	5	0.473 ± 0.03	---	0.5 ± 0.35	5 / 10000
XTJ-568							
125 mg/kg	M	24	5	0.457 ± 0.03	2	0.7 ± 0.27	7 / 10000
	F	24	5	0.469 ± 0.03	-1	0.4 ± 0.22	4 / 10000
250 mg/kg	M	24	5	0.455 ± 0.03	1	0.7 ± 0.27	7 / 10000
	F	24	5	0.452 ± 0.04	-4	1.2 ± 0.45	12 / 10000
500 mg/kg	M	24	5	0.447 ± 0.04	0	0.9 ± 0.74	9 / 10000
	F	24	5	0.467 ± 0.02	-1	0.8 ± 0.57	8 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.458 ± 0.02	2	41.5 ± 7.12	*415 / 10000
	F	24	5	0.473 ± 0.02	0	47.3 ± 7.68	*473 / 10000
Purified water	M	48	5	0.485 ± 0.05	---	0.7 ± 0.27	7 / 10000
	F	48	5	0.523 ± 0.03	---	0.8 ± 0.45	8 / 10000
XTJ-568							
500 mg/kg	M	48	5	0.550 ± 0.03	13	0.5 ± 0.00	5 / 10000
	F	48	5	0.521 ± 0.06	0	0.5 ± 0.00	5 / 10000

PCE: Polychromatic Erythrocytes; MPCEs (MNPCEs): Micronucleated Polychromatic Erythrocytes
*Statistically significant increase compared to vehicle control, $p \leq 0.05$ (Binomial Distribution, Kastenbaum-Bowman Tables)