Report for

Huntsman LLC

Review of Repeated Dose, Developmental and Reproductive Toxicity Studies with Derivatives of XTJ-568 to Determine whether the Results Satisfy the EU Criteria for Classification as a Reproductive Toxicant

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1 Introduction

Huntsman LLC (Mr. R. Papciak) requested Penman Consulting bvba (PC) to conduct an independent review of the following four GLP toxicity study reports with XTJ 568:

XTJ-568 (free diamine):

90-day oral toxicity – SD Rat, OECD Guideline 408 (Huntingdon)

28-day oral toxicity – Wistar Rat, OECD Guideline 407 (Notox)

XTJ-568 (dihydrochloride salt):

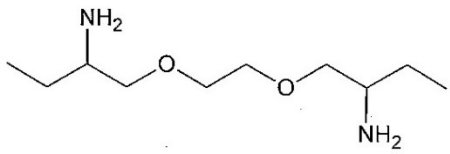
Prenatal Development Toxicity study in Wistar Rat, OECD Guideline 414 (Notox)

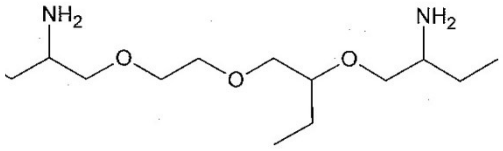
Two Generation Reprotox Study in Wistar Rat, OECD Guideline 416 (Notox)

Subsequent to the review of these studies, Huntsman requested PC to assess the results of each study in the context of the EU implementation of GHS a defined in Regulation (EC) No 1272/2008 and specifically for reproductive toxicity hazard classification.

2 Background on XTJ 568

XTJ 568, Reaction mass of 1-{2-(2-aminobutoxy)ethoxy]but-2ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine EC 447-920-2 is a polyetherdiamine, comprising of two structures:

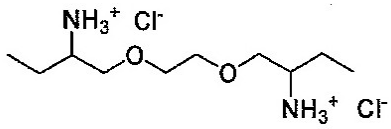


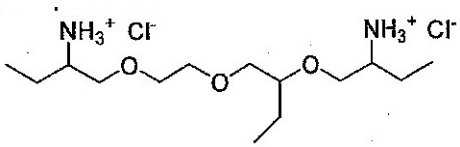


XTJ 568 free diamine is miscible with water in at least a 1:1 (w:v) ratio (i.e. water solubility of the test substance is > 1000 g substance/L water). As the water solubility is defined as units of mass per volume of solution, the water solubility is >500 g/L, at 20 °C. A pH of 12.5 was measured at this concentration.

A dihydrochloride salt was synthesized specifically for the OECD TG416 and OECD TG414 studies discussed below (personal communication B. Bossuyt to L. Segal April 15 2016). The water solubility of XTJ 568 dihydrochloride salt would be expected to be similar, since the salt will dissociate when in contact with water to release the free diamine.

XTJ 568 dihydrochloride





Molecular formula	$C_{10}H_{26}Cl_2N_2O_2$ (component A) $C_{14}H_{34}Cl_2N_2O_3$ (component B)
Molecular weight	277.23 (component A) 349.34 (component B)

3 XTJ 568 Study Review

3.1 28 Day Repeated Dose Study

Method: OECD TG 407

Test substance: Free Diamine

Study reference: NOTOX Project 375694

Date: 19 December 2003

As noted in an email dated 07 April 2016 (B. Bossuyt to L. Segal), this study was conducted in 2003 as part of the former EU Notification of New Substance (NONS) requirements under the Dangerous Substances Directive[1] 6th Amendment.

The following are PC comments and conclusions regarding this study:

1. The standard OECD TG 407 method was followed, with 3 test groups and 1 control comprised of 5 Wistar rats/sex/group.
2. Doses were selected based on a 5-day dose range finding study, in which no adverse effects were reported at 50, 250 or 1000 mg/kg/day.
3. Dose levels of 0, 50, 150 and 1000 mg/kg/day were selected for the 28-day study. The test item was dissolved in water to prepare dosing solutions; controls received water alone; dose volume administered was 5ml/kg.
4. Dosing solutions used in the study were <u>not</u> subjected to quantitative analysis prior to or during the study. The accuracy of dose solution preparation was evaluated <u>after</u> study completion on separately prepared samples.
    a. The analytical work (App 4) was dated 9th October 2003- to 25th November 2003 but the experimental studies were performed from 28th April 2003 to 25th May 2003.
    b. There was high variability in the concentrations of the dosing solutions (65-105%). Section 7.1 and Appendix 4 do not provide an adequate explanation for this, raising concerns about the accuracy of the dosing formulations used in the study.
    c. There was also a difference between dose groups [75-99% low, 84-87% mid and 68-105% high] which would yield potential dose variations of 37.5-49.5 mg/kg (low), 126-130.5 mg/kg(mid) and 680-1050 mg/kg (high).
5. Three early deaths (humane sacrifice) at 1000 mg/kg on days 16 and 18; all high dose rats were sacrificed on day 19 due to poor condition.
6. Significantly reduced weight gain and food consumption in both sexes at 1000 mg/kg; to a much lesser extent at 150 mg/kg.

[1] Directive 67/548/EEC

7. Tissues from all animals in mid and high dose groups were examined microscopically, due to high mortality in top dose group;
    a. Organ weight changes at 1000 mg/kg: increased absolute and relative liver weight (male and female); reduced absolute and relative epididymis (male); reduced abs and relative thymus weight (females).
    b. Histopathology at 1000 mg/kg: stomach irritation/erosion (male and female), atrophy thymic cortex. (m)
8. Stomachs examined from control, low, medium and high dose groups due to severe irritation at 1000 mg/kg; macroscopic pathology was consistent with stomach irritation in both sexes at the high dose
9. Mixed haematology findings suggestive of anaemia at 1000 mg/kg.

Overall Comments:

The dosing solutions used in this 28 day study were not quantitatively analysed during the study and dose solution accuracy was evaluated after study completion with separately prepared test item samples. There was high concentration variability (65-105%), raising concerns about the accuracy of the dosing formulations used in the 28 d oral gavage study. No similar issues with concentration variability of the dose formulations were seen in the subsequent studies with XTJ 568.

The LOAEL of 150 mg/kg/d, based on slight body weight. effect and clinical signs in this 28 d study, is inconsistent with the results from the 90 d study with XTJ 568 free diamine, in which 150 mg/kg/d was the NOAEL.

Data from this 28 d study are considered to be of limited value for the assessment of CLP Reproductive Toxicity Classification.

3.2 90 Day Repeated Dose Study

Method: OECD TG 408

Test substance: Free Diamine

Study reference: HLS study EJG0005

Date: 10 November 2011

As noted in an email dated 07 April 2016 (B. Bossuyt to L. Segal), this study was requested by the Chinese Authorities to support Chinese registration of XTJ 568. Because the Chinese Authorities would not accept a study with the XTJ 568 dihydrochloride salt, the 90-day study was conducted with the XTJ 568 free diamine.

The following are PC comments and conclusions regarding this study:

1. The doses for this 90-day oral gavage (OECD TG 408) study were selected based upon the results of the 28 d oral gavage study (Notox Project 375694; 19 Dec 2003) with XTJ 568 free amine, in which the NOAEL was 50 mg/kg/d and the LOAEL was 150 mg/kg/d (based on slight body weight depression and clinical signs in the absence of histopathological changes). The severe stomach irritation seen at 1000 mg/kg/d was also a factor in selecting the top dose.
2. Section 1.5 of the 90-day study report (HLS EJG0005) states that:

> "*Consequently doses of 15, 50 and 150 mg/kg/day were selected in conjunction with the Sponsor for this 13-week rat study. The high dose of 150 mg/kg/day was expected to result in some toxicity but was not anticipated to cause undue toxicity or mortality. The mid-dose of 50 mg/kg/day was expected to be well tolerated although it may have resulted in some non-adverse toxicity. No effects of treatment were anticipated at the low dose of 15 mg/kg/day.*"

Since 150 mg/kg/d was the NOAEL in the 90 d study, the expectation that "*The high dose of 150 mg/kg/day was expected to result in some toxicity*" was not achieved in this experiment.

3. Four groups of Crl:CD(SD) rats were dosed by gavage with 0 (water), 15, 50 or 150 mg/kg/d of XTJ 568 free amine in a dose volume of 10 ml/kg. Each study group was composed of 10 rats/sex. Animals were dosed orally by gavage 7 days/week for 13 weeks.
4. The homogeneity and stability of XTJ 568 in water was confirmed for formulations at nominal concentrations of 1.5 mg/mL and 15 mg/mL during magnetic stirring for 2 hours, ambient temperature storage for 24 hours and refrigerated storage for up to 15 days. The mean concentrations of XTJ 568 in test formulations were analysed on study weeks 4 and 13 and were within ±10% of nominal concentrations. All dosing formulations were prepared weekly and stored refrigerated (approximately 4C) before use.
5. There were no deaths or clinical signs of toxicity observed at any dose level. Motor activity scores, sensory reactivity and grip strength were unaffected by treatment. Bodyweight gain and food consumption was not affected at any dose level.
6. Haematology and clinical chemistry evaluations during the final week of study did not reveal any findings considered to be of toxicological significance. Females receiving 150 mg/kg/d had statistically significantly lower neutrophil and lymphocyte counts (with associated low total white cell counts) compared to control. However, since these changes were restricted to one sex, they were not considered to be of toxicological significance. All inter-group differences in clinical chemistry parameters that attained statistical significance lacked dose-relationship, or were restricted to one sex, and were not considered to be toxicologically significant.
7. Reductions (not statistically significant) in % motile and progressively motile sperm were noted at 50 and 150 mg/kg/d. However, these differences in mean values were largely due to low values in one animal in each group. Although the reason for the low values in single males in the 50 and 150 mg/kg/d groups was not established, they were not considered to be related to exposure to XTJ 568.
8. The macroscopic and microscopic examinations revealed no changes associated with treatment.
9. Daily oral (gavage) administration of XTJ-568 to male and female Crl:CD(SD) rats for 13 weeks at doses up to 150 mg/kg/day was well tolerated. No target organs were identified at histopathological examination. The NOAEL was considered to be 150 mg/kg/d.

<u>Overall Comments</u>:

PC considers this 90-day oral (gavage) toxicity study with XTJ 568 free diamine to have been adequately conducted and reported, and in general the results appear to be reliable. The

analytical data in this study supports the accuracy and homogeneity of the dosing formulations used in the study. We agree with the conclusion that the NOAEL in the study is 150 mg/kg/d.

However, one area of significant concern is the dose selection for the study. Based on the mild effects seen at 150 mg/kg/d in the 28 d study used to set the doses for this 90-day study, as well as the uncertainty of the accuracy of the dosing solutions used in the 28 d study, the assumptions made in the dose selection in this 90-day study can be questioned. Since 150 mg/kg/d was the NOAEL in the 90-day study, the expectation that "*The high dose of 150 mg/kg/day was expected to result in some toxicity*" was not achieved in the experiment. Consequently, this 90-day study may be considered deficient with respect to para 13 of OECD TG408, because the highest dose administered (150 mg/kg/d) failed to "*induce toxicity but not death or severe suffering*".

In our view, the highest dose used in the 90 day study was not the 'maximum tolerated dose' and hence, the lack of effects on sperm concentration, motility and/or morphology does not negate the observations seen in the two generation reproductive toxicity study (Notox Project 489273). We also question why data from the OECD TG 414 and OECD TG 416 studies were not used to inform dose selection for the 90 d study, notwithstanding the differences between XTJ 568 free diamine and XTJ 568 dihydrochloride salt.

Variables between this 90 d study and the previously conducted OECD TG414, OECD TG416 and OECD TG407 studies include:

- The 90 d study was conducted in Sprague Dawley rats while the OECD TG414, OECD TG416 and OECD TG407 studies were conducted with Wistar rats. The rationale for use of SD rats in the 90 d study is unclear. Published data suggests that epididymal sperm concentration is more variable in Sprague-Dawley strain than in Wistar strain (Marty et al., 2009).
- The dose volume used in the 90 d study was 10 ml/kg vs 5 ml/kg in the OECD TG414, OECD TG416 and OECD TG407 studies. Since a dose volume of 5 ml/kg was used in the 28 d study that was used for dose selection in the 90 d study, the rationale for use of a different dose volume is unclear. However, for the same mg/kg dose, the aqueous dosing solutions used in this 90 d study were half as concentrated as those used in the OECD TG407, OECD TG414, and OECD TG416 studies. It is unclear if this may have had an impact on the study results.
- The test article in the 90 d study was the free amine, as it was for the 28 d study. Although this was dictated by the Chinese Authorities, with hindsight inclusion of one or more dose groups with the XTJ 568 dihydrochloride salt would have allowed for bridging to the previously-conducted OECD TG416 and OECD TG414 studies.

3.3 Developmental Toxicity Study

Method: OECD TG 414

Test substance: Dihydrochloride Salt

Study reference: Notox Project 489241

Date: 20 May 2010

As noted in an email dated 07 April 2016 (B. Bossuyt to L. Segal), this study was conducted in 2009 as part of the former EU Notification of New Substances (NONS) requirements. Due to tonnage increases from 100 to 1000 tonnes per year in 2006-2007, the Belgian Authorities requested Huntsman perform prenatal developmental and 2-generation reproductive toxicity

studies. At the time they were conducted these studies were part of the Dangerous Substances Directive 6th Amendment notification procedure.

The following are PC comments and conclusions regarding this study:

1. The dose range finding (DRF) study was performed with 6 presumed pregnant female Han Wistar rats per group, randomly allocated to 4 groups (control plus 3 doses), dosed daily post coitum from day 6-19. The doses selected for this DRF were 150, 450 and 1000 mg/kg/d. For each dose level, animals were dosed for 10 days before the next dose level was started. The starting dose level for this DRF was based upon the results of a previous 28-day oral gavage study (Notox 375694) with XTJ 568 free amine in m/f Wistar rats, in which 150 mg/kg/d was the LOAEL and 50 mg/kg/d was the NOAEL. In the previous 28-day study with XTJ 568 free amine, all animals dosed at 1000 mg/kg/d were sacrificed or found dead by day 19, with markedly reduced weight gain and food consumption. In contrast, no mortality or significant clinical signs were seen in the DRF in pregnant females with XTJ 568 dihydrochloride at up to 1000 mg/kg/d during 14 days of daily gavage exposure (gestation day 6-19). Lower body weight and body weight gains were noted at 1000 mg/kg/d, and during the first 5 days of treatment reduced absolute and relative (to bodyweight) food consumption was seen at 1000 and 450 mg/kg/d, with partial recovery thereafter. Litter sizes, m/f ratios, foetal body weights, and external morphology were unaffected at any dose level. Consequently, based on the results of the DRF the doses selected for the definitive study were 150, 450, and 1000 mg/kg/d.
2. In the definitive OECD TG414 study, 4 groups of 24 pregnant Wistar rats each were dosed by gavage from gestation day 6-19 with 0 (elix water), 150, 450 or 1000 mg/kg/d of XTJ 568 dihydrochloride. According to the analytical data (appendix 4), each of the dosing formulations had mean accuracies between 90-110% of the target concentrations and formulation homogeneity met the acceptance criteria (< 10% variation).
3. There were no treatment-related clinical signs. At 1000 mg/kg/d, bodyweight, bodyweight gain was lower than control starting on the 2nd day of dosing. Food consumption at 1000 mg/kg/d was lower than control during the whole treatment period. At 450 mg/kg/d, a slight transient reduction in bodyweight gain was also evident but this was judged to be not toxicologically significant. Food consumption at 450 mg/kg/d was lower than control during the first week of dosing and reached statistical significance from gestation day 6-9, but recovered during the 2nd week of dosing. This was judged to be not toxicologically significant. No effects on bodyweight, bodyweight gain or food consumption occurred at 150 mg/kg/d.
4. There were 21, 20, 24 and 20 pregnant dams in the 0, 150, 450 and 1000 mg/kg/d groups, respectively. There were no differences between control and treated groups in corpora lutea, implantations, viable/dead foetuses, early/late resorptions, or pre-/post-implantation loss. Mean litter sizes were comparable across all dose groups and sex ratio was unaffected by treatment. Foetal bodyweight, and the incidence of external, visceral and skeletal malformations was also unaffected by exposure to XTJ 568 dihydrochloride.
5. The NOAEL for maternal toxicity was considered to be 450 mg/kg/d based on reduced bodyweight and bodyweight gain in dams at 1000 mg/kg/d. The NOAEL for developmental toxicity was considered to be 1000 mg/kg/d.

Overall Comments:

PC considers this rat oral (gavage) developmental toxicity study with XTJ 568 dihydrochloride to be well conducted and reported, and the results appear to be reliable. The analytical data in this study report supports the accuracy and homogeneity of the dosing formulations used in the study.

It should be noted that the lack of effects in pregnant Wistar rats dosed at 450 and 1000 mg/kg/d in this OECD TG 414 study with XTJ 568 dihydrochloride is in marked contrast to the results of the 28-day study with XJT 568 free amine in young adult m/f rats of the same strain completed in 2003, in which all animals dosed at 1000 mg/kg/d were sacrificed by day 19, with markedly reduced weight gain and food consumption and severe irritation of the stomach.

The Belgian Authority's CLH report for XTJ 568 does not dispute the design or conclusions of the rat oral (gavage) developmental toxicity study with XTJ 568 dihydrochloride. The results of the study do not trigger a Reproductive Category 2 classification according to CLP guidance, since the NOAEL for developmental effects (1000 mg/kg/d) exceeds the NOAEL for maternal toxicity (450 mg/kg/d). In addition, no evidence of test-item related developmental effects were observed at 1000 mg/kg/d.

3.4 2 Generation Reproductive Toxicity Study

Method: OECD TG 416

Test substance: Dihydrochloride Salt

Study reference: Notox Project 489273

Date: 15 July 2010

As noted in an email dated 07 April 2016 (B. Bossuyt to L. Segal), this study was conducted in 2009 as part of the former EU new substance notification requirements (ELINCS). Due to tonnage increases (100 - 1000 tpa) in 2006-2007, the Belgian Authorities requested Huntsman to perform prenatal developmental and 2-generation reproductive toxicity studies. At the time they were conducted these studies were still part of the ELINCS notification procedure.

The following are PC comments and conclusions regarding this study

1. The doses for this 2-generation reproductive toxicity (OECD TG 416) study were selected based upon the DRF study in the rat oral (gavage) developmental toxicity study (Notox Project 489241) with XTJ 568 dihydrochloride. The DRF study was performed with 6 presumed pregnant female Han Wistar rats per group, randomly allocated to 4 groups (control plus 3 doses), dosed daily post coitum from day 6-19. The doses selected for this DRF were 150, 450 and 1000 mg/kg/d. For each dose level, animals were dosed for 10 days before the next dose level was started. The starting dose level for this DRF was based upon the results of a previous 28-day oral gavage study (Notox 375694) with XTJ 568 free amine in m/f Wistar rats. No mortality or significant clinical signs were seen in the DRF in pregnant females with XTJ 568 dihydrochloride at up to 1000 mg/kg/d during 14 days of daily gavage exposure (gestation day 6-19). Lower body weight and body weight gains were noted at 1000 mg/kg/d, and during the first 5 days of treatment, reduced absolute and relative (to bodyweight) food consumption was seen at 1000 and 450 mg/kg/d, with partial recovery thereafter. Litter sizes, m/f ratios, foetal body weights, and external morphology were unaffected at any dose level. Consequently, based on the results of the DRF in the OECD TG 414 study, the doses selected for the OECD TG 416 study were 150, 450, and 1000 mg/kg/d.

2. Four groups of Wistar rats were dosed by gavage with 0 (elix water), 150, 450 or 1000 mg/kg/d of XTJ 568 dihydrochloride in a dose volume of 5 ml/kg, adjusted to latest bodyweight. The F0 rats were dosed for a minimum of 70 days prior to mating and continued until sacrifice. Initially a 1-generation study was planned with XTJ 568, however, based on possible treatment-related findings, it was decided to extend the study into a 2-generation study. The F1 rats were exposed in utero and during lactation, until weaning and then directly, by gavage, following weaning for a minimum of 70 days prior to mating and up to sacrifice. F2 rats were exposed to XTJ 568 in utero and during lactation. Each study group in the F0 and F1 generations was composed of 24 rats/sex.

3. According to Section 6.1.2 of the study report, three different test item batches were used in this study:

   a. Batch 8666-080-C from study start until 12 March 2009

   b. Batch 8470-004-A form 13 March 2009 until 02 June 2009

   c. Batch 8470-054-C from 03 June 2009 to end of study

   The salt of the 3rd batch was made from a different diamine precursor than the previous batches, however, the difference in the amine content (approx. 1.4%) fell within the normal variation in the amination process for XTJ 568 and therefore the 3 batches were considered comparable.

4. The analytical data in appendix 5 indicates that each of the dosing formulations had mean accuracies between 80-120% of the target concentrations and formulation homogeneity met the acceptance criteria (< 10% variation). The dosing formulations were stable for 6 hrs at room temp and for 8 days when stored in a fridge. Dosing formulations were prepared daily 6 hr or less prior to dosing from study start until January 11 2009, and weekly from January 12 2009 to end of the study (approx. 6 wks after start of dosing the F0 generation and a month prior to mating F0).

5. After consulting the Sponsor half, the F0 males were sacrificed one week earlier than originally planned due to a shortage of the test article. The earlier than planned sacrifice of half of the F0 males was not considered to influence the integrity of the study.

6. Necropsy of the F1 males was delayed 1-3 weeks because of the need to consult the sponsor re: the possible need for a second mating of the F1 males. The second mating of F1 males (as recommended in OECD TG 416) was considered because the litter size in the F1 high dose group was statistically significantly lower than F1 controls. However, after consultation with the Sponsor, it was decided not to re-mate the F1 males to produce a second litter, as this was not seen as adding value to the study.

7. Evaluations of sperm concentration and morphology were performed in a blinded manner. F1 sperm motility was also performed in a blinded manner, but F0 sperm motility was not. Although sperm production rate was calculated and statistical analysis performed, it was not reported in the study report as it was not included in the protocol.

8. As noted on page 33 (deviations), the methodology used for sperm motility assessments in F0 males did not follow the protocol and was not consistent with the method used for F1 males.

9. One high dose F0 female was sacrificed for humane reasons due to severe wounds in the abdominal region. This was considered unrelated to treatment.

10. Slight to severe salivation was noted in all male and female rats in the two highest dose levels during 1 or more days during the study. This was considered to be related to the taste of the formulations rather than toxicity.

11. In the F0 generation:

    a. 150 mg/kg/d: no test-item related findings on parental, reproductive or developmental parameters were observed.

    b. 450 mg/kg/d: significant decrease in sperm concentration and motility was observed along with an increased incidence of abnormal sperm morphology (separated head); one female showed an acyclic estrous cycle.

    c. 1000 mg/kg/d: decreased bodyweight and bodyweight gain was observed for males and females; lower food consumption in females during post coitum and lactation periods; treatment-related changes to testes (minimal to slight multifocal degeneration of seminiferous tubules); uterus (minimal to moderate attenuated myometrium) and vagina (slight to moderate attenuated epithelium); significant decrease in sperm concentration and motility was observed along with an increased incidence of abnormal sperm morphology (separated head); significant reduction in pup bodyweight during lactation period.

12. In the F1 generation:

    a. 150 mg/kg/d: no test-item related findings on parental, reproductive or developmental parameters were observed.

    b. 450 mg/kg/d: lower bodyweight in males during the whole observation period due to a developmental delay which occurred during lactation; increased incidence of abnormal sperm morphology (separated head).

    c. 1000 mg/kg/d: lower bodyweight in males during the whole observation period due to a developmental delay which occurred during lactation; lower bodyweight in females during almost the whole observation period; lower food consumption in females during lactation; reduced size of testes, epididymides and/or seminal vesicles in 3/24 males; treatment-related changes to testes (minimal to slight multifocal degeneration of seminiferous tubules), epididymides (cell debris) and vagina (epithelial mucification); 5/24 females showed an irregular estrous cycle; significant decrease in testes and epididymides weight; significant decrease in sperm concentration and motility was observed along with an increased incidence of abnormal sperm morphology (separated head); significantly lower number of implantation sites vs control; litter size significantly lower than control (7.9 vs 11.1); mean bodyweight significantly lower than control during lactation phase; delayed balanopreputial separation and vaginal opening (considered secondary to reduced bodyweight).

13. NOAEL for parental toxicity was considered to be 450 mg/kg/d based on reduced bodyweight and bodyweight gain in F0 and F1 males and females at 1000 mg/kg/d.

14. NOAEL for reproductive toxicity was considered to be 150 mg/kg/d based on:

    a. effects on sperm parameters in males at 450 and 1000 mg/kg/d in F0 and F1;

b. effects on estrous cycle in females at 450 and 1000 mg/kg/d in F1

15. The NOAEL for developmental toxicity was considered to be 450 mg/kg/d based on reduced F1 litter size and mean number of pups, and reduced F0 and F1 pup bodyweight.

Overall Comments:

PC considers this rat oral (gavage) 2-generation reproductive toxicity study with XTJ 568 dihydrochloride to have been adequately conducted and reported, and in general the results appear to be reliable. However, one area of concern is that the methodology used for sperm motility assessments in F0 males did not follow the protocol and was not consistent with the method used for F1 males. In addition, F0 sperm motility assessments were not performed in a blinded manner. However, the majority of the sperm assessments (number, morphology, motility) were conducted in a blinded manner and in accordance with the protocol, so this is not seen as a significant flaw in the study.

The analytical data in this study supports the accuracy and homogeneity of the dosing formulations used in the study. However, it should be noted that three different test item batches were used in this study as described above.

It should also be noted that the effects seen at 450 and 1000 mg/kg/d in young adult male and female Wistar rats and pregnant females in this OECD TG 416 study with XTJ 568 dihydrochloride is in marked contrast to the results of the 28 day study with XTJ 568 free amine performed in 2003 with m/f rats of the same strain, in which all animals dosed at 1000 mg/kg/d were sacrificed or found dead by day 19, with markedly reduced weight gain and food consumption and severe irritation of the stomach.

The EU CLP criteria for reproductive toxicity classification (ECHA 2015) indicates 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. In PC's view, the results of the rat oral (gavage) 2-generation reproductive toxicity study with XTJ 568 dihydrochloride satisfy the condition specified in the guidance, as discussed below:

1. The NOAEL for reproductive effects is 150 mg/kg/d, based mainly on sperm parameters in males and estrous cycle effects in females seen at 450 and 1000 mg/kg/d. Given that the method used for sperm motility assessments in F0 males did not follow the protocol and was not consistent with the method used for F1 males, and the lack of blinding in the assessment of sperm motility in F0, we would discount those results. Nonetheless, there remains the observation of a significant decrease in sperm concentration and an increased incidence of abnormal sperm morphology (separated head) at 450 and 1000 mg/kg/d. In addition, one F1 female at 450 mg/kg/d showed acyclic estrous cycle, which in conjunction with the estrous effects seen at 1000 mg/kg/d seems most likely treatment-related.

2. The NOAEL for parental toxicity is 450 mg/kg/d, based on the reduced bodyweight and bodyweight gain in F0 and F1 males and females at 1000 mg/kg/d.

3. A wide spectrum of reproductive effects were seen at 1000 mg/kg/d, a dose which also induced systemic toxicity in the F0 and F1 parental rats. These effects included:

   a. reduced size of testes, epididymides and/or seminal vesicles in 3/24 males (F1);

b. treatment-related changes to testes (minimal to slight multifocal degeneration of seminiferous tubules), epididymides (cell debris) and vagina (epithelial mucification) (F0 & F1);

c. irregular estrous cycle in 5/24 females (F1);

d. significant decrease in testes and epididymides weight (F1);

e. significant decrease in sperm concentration and motility along with an increased incidence of abnormal sperm morphology (separated head) (F0 & F1);

f. significantly lower number of implantation sites vs control (F1);

g. significantly lower litter size than control (F1)

Although some of these effects may have been secondary to parental toxicity, it seems unlikely that all of them are.

Consequently, in PC's view, 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 the 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.

4 Overall Assessment Re: Reproductive Toxicity Classification

The following is PC's overall assessment of the OECD TG 407 (28-day), OECD TG 408 (90-day), OECD TG 414 (developmental toxicity), and OECD TG 416 (2 generation reproductive toxicity) studies with XTJ 568 free diamine and XTJ 568 dihydrochloride salt, and the applicability of each to the CLP for Rep Cat 2.

4.1 28 d (OECD TG 407) study with XTJ 568 free diamine

1. Dosing solutions were not quantitatively analysed and dose solution accuracy was evaluated after study completion with separately prepared test item samples; high concentration variability; raises concerns about the accuracy of the dosing formulations used in the study.
2. LOAEL of 150 mg/kg/d based on slight body weight. effect and clinical signs is inconsistent with results from the 90 d study with XTJ 568 free amine, in which 150 mg/kg/d was the NOAEL.
3. Data are considered of limited value for assessment of CLP Reproductive Toxicity Classification.

4.2 90 d (OECD TG 408) study with XTJ 568 free diamine

1. Study was adequately conducted and reported; results appear to be reliable.
2. NOAEL of 150 mg/kg/d appears to be correct, but is inconsistent with the 28 d study with XTJ 568 free diamine, in which 150 mg/kg/d was a LOAEL.
3. Dose selection for the 90 d study may be considered deficient with respect to para 13 of OECD TG408, because the highest dose administered (150 mg/kg/d) failed to "*induce toxicity but not death or severe suffering*".
4. 90 d study was conducted with the Sprague Dawley strain, which is inconsistent with the Wistar strain used for the OECD TG414, OECD TG416 and OECD TG407 studies.
    i. Rationale for use of SD rats in the 90 d study is unclear.
    ii. Published data suggests that epididymal sperm concentration is more variable in Sprague-Dawley strain than in Wistar strain (Marty et al. (2009)
5. Dose volume used in the 90 d study was 10 ml/kg vs 5 ml/kg in the OECD TG414, OECD TG416 and OECD TG407 studies. The rationale for use of a different dose volume is unclear.
6. Reductions (not statistically significant) in % motile and progressively motile sperm were noted at 50 and 150 mg/kg/d, largely due to low values in one animal in each group; reason for the low values in single males in the 50 and 150 mg/kg/d groups not established, but not considered related to XTJ 568 exposure.
7. Sperm evaluations demonstrate that 150 mg/kg/d was a NOAEL for XTJ 568 free diamine in the SD rat, which is consistent with the NOAEL for reproductive effects (150 mg/kg/d) in the OECD TG416 study with Wistar rats.
8. Data are considered of limited value for assessment of CLP Reproductive Toxicity Classification since highest dose used was the NOAEL.

4.3 Developmental toxicity study with XTJ 568 dihydrochloride salt

1. Study was adequately conducted and reported, and in general the results appear to be reliable; analytical data supports the accuracy and homogeneity of the dosing formulations used in the study.
2. No evidence of test item related developmental effects were observed at 1000 mg/kg/d; NOAEL for developmental effects 1000 mg/kg/d
3. NOAEL for maternal toxicity (450 mg/kg/d), based on reduced bodyweight, bodyweight gain and food consumption at the high dose.
4. Data are considered useful for assessment of CLP reproductive toxicity classification, however, it should be noted that test item was dihydrochloride salt of XTJ 568 diamine; no OECD TG 414 available for XTJ 568 free diamine, therefore assessment is based on read-across from dihydrochloride salt of XTJ 568 to the XTJ 568 free diamine.
5. The results of this study do not trigger a reproductive toxicity classification for developmental toxicity, according to CLP guidance.

4.4 Two-generation reproductive toxicity study with XTJ 568 dihydrochloride salt

1. Study was adequately conducted and reported, and in general the results appear to be reliable; analytical data supports the accuracy and homogeneity of the dosing formulations used in the study.
2. One point of concern is that the methodology used for F0 sperm motility assessments:
    a. did not follow the protocol and not consistent with method used for F1
    b. not performed in a blinded manner
3. Other F0 and F1 sperm assessments (number, morphology, motility) were conducted in a blinded manner and in accordance with the protocol.
4. NOAEL for parental toxicity is 450 mg/kg/d:
    a. based on the reduced bodyweight, bodyweight gain in F0 and F1 males and females at 1000 mg/kg/d
    b. consistent with maternal toxicity observed in OECD TG 414 study.
5. NOAEL for reproductive effects is 150 mg/kg/d (F0 & F1):
    a. based on sperm parameters in males and estrous cycle effects in females seen at 450 and/or 1000 mg/kg/d:
        i. significant decrease in sperm concentration and an increased incidence of abnormal sperm morphology at 450 and 1000 mg/kg/d
        ii. In F0, 1 female at 1000 mg/kg/d with acyclic estrous cycle
        iii. In F1, 1 female at 450 mg/kg/d and 3 at 1000 mg/kg/d with acyclic estrous cycle
6. Wide spectrum of reproductive effects seen at 1000 mg/kg/d with concomitant systemic toxicity in the F0 and F1 parental rats:
    a. reduced size of testes, epididymides and/or seminal vesicles in 3/24 males (F1);

b. treatment-related changes to testes (minimal to slight multifocal degeneration of seminiferous tubules), epididymides (cell debris) and vagina (epithelial mucification) (F0 & F1);

c. irregular estrous cycle in 5/24 females (F1);

d. significant decrease in testes and epididymides weight (F1);

e. significant decrease in sperm concentration and motility along with an increased incidence of abnormal sperm morphology (separated head) (F0 & F1);

f. significantly lower number of implantation sites vs control (F1);

g. significantly lower litter size than control (F1)

7. Data are considered useful for assessment of Reproductive Category 2, however, it should be noted that test item was dihydrochloride salt of XTJ 568 diamine; no OECD TG 416 study available for XTJ 568 free diamine, therefore assessment is based on read-across from dihydrochloride salt of XTJ 568 to the XTJ 568 free diamine.

4.5 Discussion

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 PC's 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 TG 416 study. PC does 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 opening in F1 pups at 1000 mg/kg/d. However, this was 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.

4.6 Conclusion

In PC's judgement, 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 the 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 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 also on *developmental* effects in the OECD TG 416 study, for the reasons outlined in section 4.5.

References

European Commission (2008) REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

ECHA (2015) Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1 June 2015

FBS Public Health, Food Chain Safety and Environment, Proposed 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-2ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine EC 447-920-2
26th November 2016

Marty M. *et al*., (2009) Inter-Laboratory Control Data for Reproductive Endpoints Required in the OPPTS 870.3800/OECD 416 Reproduction and Fertility Test. Birth Defects Research (Part B) 86: 470–489.