Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol (TBNPA)

EC Number: **253-057-0**

CAS Number: **36483-57-5 and 1522-92-5**

Index Number: **603-RST-VW-Y**

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1 PHYSICAL HAZARDS

Not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No studies available.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated.

3.2 Acute toxicity - dermal route

Not evaluated.

3.3 Acute toxicity - inhalation route

Not evaluated.

3.4 Skin corrosion/irritation

Not evaluated.

3.5 Serious eye damage/eye irritation

Not evaluated.

3.6 Respiratory sensitisation

Not evaluated.

3.7 Skin sensitisation

Not evaluated.

3.8 Germ cell mutagenicity

The studies below are included in the REACH registration. When searching in Toxnet, two additional Ames tests from 1990 seem to have been conducted. These tests are neither published nor available from the registrant, and hence not included here.

3.8.1 In vitro data

3.8.1.1 Unnamed (2004)

Study reference:

Study report unnamed, 2004: https://echa.europa.eu/registration-dossier/-/registered-dossier/6484/7/7/2

Key study 1 for in vitro genetic toxicity in the registration.

Detailed study summary and results:

In vitro cytogenicity/chromosome aberration study in mammalian cells (lymphocytes: Peripheral human lymphocytes).

GLP and OECD Test Guideline (TG) 473 was followed (In Vitro Mammalian Chromosomal Aberration Test). The purpose of the in vitro chromosomal aberration test is to identify substances that cause structural chromosomal aberrations in cultured mammalian cells.

Reliability index made by the registrant: 1

- *number of replicates:* Main test 1: 1A+1B+1C+1D, Main test 2: 2A
- *number of doses, justification of dose selection:* Following a range-finder test with doses of 33-3250 µg/ml with and without metabolic activation with S9-mix, the doses in the main studies ranged from 100 to 2000 µg/ml with and without metabolic activation. There were 5 – 7 doses in each study, e.g. 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml in Main study 1.
- *positive and negative control groups and treatment:* Positive control was MMC (Mitomycin C, a clastogen active without metabolic activation) and cyclophosphamide (CP, a clastogen requiring metabolic activation). Negative control (solvent only) was DMSO. 3hr exposure, 24 hr fixation.
- *details on slide preparation: number of metaphases analysed:* Not given in the registration
- *justification for choice of vehicle:* According to test guideline (TG)
- *solubility and stability of the test substance in vehicle if known:* -
- *description of follow up repeat study:* -
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):* The study is GLP compliant.

Test substance

- The test material FR-513 (CAS no. 36483-57-5) used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity 97%
- Impurities: Dibromoneopentyl glycol < 0.1%, which does not affect the classification
- Batch number: Not specified

Administration/exposure

- *lymphocytes:* Peripheral human lymphocytes, with and without metabolic activation
- *Type and composition of metabolic activation system:*
  - Aroclor-1254 induced rat liver S9-mix
  - Quantity not given in the registration
- *Test concentrations, and reasoning for selection of doses if applicable:* Following a range-finder test with doses of 33-3250 µg/ml with and without metabolic activation with S9-mix, the doses in the main studies ranged from 100 to 2000 µg/ml with and without metabolic activation. There were 5 – 7 doses in each study, e.g. 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml in Main study 1:
- Range finder test: control: (DMSO), 33, 100, 333, 1000, 3250µg/ml (-S9-mix) (3hr, 24 hr, 48 hr exposure; 24hr, 24hr,48 hr fixation) ; control (DMSO), 33, 100, 333, 1000, 3250µg/ml (+S9-mix) (3hr exposure; 24hr fixation)
  Main test 1: control (DMSO), 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1A: control (DMSO), 100, 333, 666, 1000, 1050, 1100, 1150, 1200 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 100, 333, 666, 1000, 1050, 1100, 1150, 1200 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1B: control (DMSO), 333, 1000, 1010, 1020, 1030, 1040, 1050 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1C: control (DMSO), 333, 666, 1000 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 333, 666, 1000, 1020, 1050, 1100, 1200 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1D: control (DMSO), 100, 300, 600, 800, 1000, 1020, 1030, 1050, 1100 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 2: control (DMSO), 17, 66, 100, 126, 150, 200 µg/ml, MMC-C (0.2 µg/ml) (-S9-mix)(24 hr exposure, 24 hr fixation); control (DMSO), 1, 3, 10,17, 66, 100 µg/ml, MMC-C (0.1 µg/ml) (-S9-mix)(48 hr exposure, 48 hr fixation); control (DMSO), 100, 167, 666, 1000, 1250, 1500, 2000 µg/ml, CP (15 µg/ml) (+S9-mix)(3 hr exposure, 3 hr fixation)
  Main test 2: control (DMSO), 17, 66, 100, 126, 150, 200 µg/ml, MMC-C (0.2 µg/ml) (-S9-mix)(24 hr exposure, 24 hr fixation); control (DMSO), 1, 3, 10,17, 66, 100 µg/ml, MMC-C (0.1 µg/ml) (-S9-mix)(48 hr exposure, 48 hr fixation)
  Main test 2A: see in materials and methods* (no more info given in the registration)
- *Vehicle:* DMSO.
- *Statistical methods:* Not given in the registration.

Results and discussion

- FR-513 was found to be clastogenic in the presence of metabolic activation, and at the highest test substance concentration (1000 microgram/ml) in the absence of metabolic activation. FR-513 has the potential to disturb mitotic processes and cell cycle progression.
- *Cytotoxic concentrations with and without metabolic activation:* yes (cytotoxic at 1000µg/ml, cell lysis at 3250µg/ml). Cytotoxicity seen as low as at 100 µg/ml, with metabolic activation.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* See above.
- *Concurrent negative (solvent/vehicle) and positive control data*
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,:* Data was not presented in such detail in the registration. However the conclusion was that the test substance was positive clastogenic in the presence and in the absence of metabolic activation.

3.8.1.2 Unnamed (2004)

Study reference:

Study report unnamed, 2004: *In vitro* gene mutation study in mammalian cells:
https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/2/?documentUUID=1de76583-e4ca-4077-803c-7bf6dc92ad76
Key study 2 for in vitro genetic toxicity in the registration.
Reliability index made by the registrant: 1

Detailed study summary and results:

Test type

Mammalian cell gene mutation assay (gene mutation) following OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test). GLP compliant.

Mouse lymphoma L5178Y cells.

- *number of replicates:* 2 experiments, both with and without metabolic activation
- *number of doses, justification of dose selection:* Dose range finding test (without/with metabolic activation): Solvent control, 33, 100, 333, 1000, 3250 µg/ml. In the main studies, concentrations varied from 10 to 535 µg/ml, and up to 8 dose groups pluss two solvent controls, e.g. 10, 50, 100, 200, 300, 400, 500 µg/ml in experiment 1 (without metabolic activation).
- *positive and negative control groups and treatment:* Positive control methyl methane sulfonate/cyclophosphamide
- *details on slide preparation* -
- *number of metaphases analysed* -
- *justification for choice of vehicle*: According to test guideline
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):* GLP compliant

Test substance

- The test material FR-513 (CAS no. 36483-57-5) used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity 97%
- Impurities: Dibromoneopentyl glycol < 0.1%, which does not affect the classification
- Batch number: Not specified

Administration/exposure

- Mouse lymphoma L5178Y cells
- *Type and composition of metabolic activation system:*
  - with and without rat liver microsomal enzymes (S9-fraction).
  - *quantity* – not given
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations:*

  Dose range finding test (without/with metabolic activation): Solvent control, 33, 100, 333, 1000, 3250 µg/ml

Experiment 1 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 200, 300, 400, 500, positive control (MMS)
Experiment 1 (with metabolic activation 8%): first solvent control, second solvent control, 50, 100, 200, 300, 375, 450, 500, positive control (CP)
Experiment 2 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 225, 250, 300, 325, 350, positive control (MMS)
Experiment 2 (with metabolic activation 12%): first solvent control, second solvent control, 100, 200, 300, 350, 400, 470, 500, 535, positive control (CP)

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation*: The test substance was cytotoxic with and without metabolic activation from concentration of 333 µg/plate.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation: Mutant frequencies:* In the absence of S-9 mix FR-513 did not induce a significant increase in mutant frequencies in the first experiment. This result was confirmed in a repeat experiment with modifications in the duration of the time treatment from 3 to 24 hours. FR-513 was mutagenic in the test system with incubations in the presence of metabolic activation. The presence of S9-mix in both tests resulted an increase in mutation frequencies more than threefold and outside the labs historical data. The increases were considered biologically relevant and FR-513 is considered mutagenic in vitro.

3.8.1.3 Unnamed (1996)

Study reference:

Study report unnamed, 1996: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/2/?documentUUID=e115ea71-ed77-449f-9943-6bca66b27e3a

Key study 3 for in vitro genetic toxicity in the registration.

Reliability score made by the registrant: 1

Detailed study summary and results:

Bacterial reverse mutation assay: In vitro gene mutation study in bacteria (Ames test)

The study was done according to OECD TG 471, and GLP, on S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (with and without metabolic activation from rodent S-9 mix - treated with Aroclor 1254). Strains TA98 and TA1537 are capable of detecting frameshift mutagens, strains TA100 and TA1535 are capable of detecting base-pair substitution mutagens.

The study was done according to OECD TG 471.

Test substance

- The test material FR-513 used in the study is equivalent to the substance identified in the CLH dossier.
- *Degree of purity:* 98%
- *Impurities (or a note that the impurities do not affect the classification):* Not given in the registration

Administration/exposure

- S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
- *Type and composition of metabolic activation system:*
  - hamster S-9 mix (treated with Aroclor 1254)
- *Test concentrations, and reasoning for selection of doses if applicable:* The concentration was 0, 5, 50, 500, 5000 µg/plate in the preliminary toxicity determination (with and without metabolic activation), and 0, 15, 50, 150, 500, 1500 µg/plate in the main test (with and without metabolic activation).

Results and discussion

- 5000 µg/plate in the preliminary toxicity determination was toxic so the highest concentration was set to 1500 µg/plate in the main test.
- Toxicity was observed in the preliminary test at the concentration of 5000 µg/plate.
- Large, dose-related increases in revertant colony numbers were observed in both mutation tests with strains TA 1535 and TA 100, at concentrations between 15 and 500 µg/plate, but this was only observed with metabolic activation.
- DMSO used as negative control. Postive control was N-Ethyl-N-nitro-N-nitrosoguanidine, 9-Amonoacridine, 2 Nitrofluorene, 2-Aminoantracene, Congo red (CAS no. 573-58-0) which demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

3.8.2 Animal data

3.8.2.1 Study report (2007)

Study reference:

Study report unnamed, 2007: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/3 Key study 1 for *in vivo* genetic toxicity in the registration.

Detailed study summary and results:

Unscheduled DNA Synthesis (UDS) test with rat liver cells (liver hepatocytes) in vivo. GLP and OECD TG 486 was followed.

Test substance

- FR 513 equivalent to the substance identified in the CLH dossier
- *Degree of purity*: Not given in the registration.
- *Impurities (or a note that the impurities do not affect the classification):* Not given.

Test animals

- Sprague-Dawley rats (CD (Ctr;CD (SD) IGS BR) strain)
- *No. of animals per sex per dose:* range finder: 2 (1 male; 1 female) (2 males 0 females); main test 1: 4 per dose (males); main test 2: 4 per dose (males)
- *Age and weight at the study initiation:* 6-9 weeks old. Weight: male 200 gr-243 gr (start of experiment)

Administration/exposure

- *Doses/concentration levels, vehicle, rational for dose selection.* All animals were dosed once. In the range finding test the dose was 2000 mg/kg. In the main studies the dose was 670 and 2000 mg/kg bw.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Archis oil
- *Details on test system and conditions, and details on route of administration, exposure:* Oral by gavage
- *Duration of study, frequency of treatment, sampling times and number of samples:* Treatment: 16 hr (experiment 1); 2 hr (experiment 2)
- *Positive and negative (vehicle/solvent) control data:* Positive control 2- Acetamididofluorene (2AAF) at 50 mg/kg bw, and Sym-Dimethylhydrazine dihydrochloride (NDHC) at 40 mg/kg bw.
- *Methods of slide preparation:* The coded slides were scored using an automated image analysis system linked to a computer programe (Grain) which followed the UKEMS guidelines for statistical analysis.

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* Negative: The test material did not induce any marked or toxcologically significant increases in the incidence of cells undergoing DNA synthesis in isolated rat hepatocytes following in vivo exposure for 2 or 16 hr. Therefore the test material was considered to be non-genotoxic under the conditions of the study.
- *Concurrent positive control data:* Both positive controls produced marked increases in the incidence of cells in repair and the vehicle control groups gave acceptable values for net nuclear grain counts.
- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable.* Administration of the test substance in the range finding study produced toxicity in the dosed animals manifested as ataxia, lethargy, red colored urine (no deaths). Lethargy and ataxia was also seen in the main studies.

3.8.2.2 Unnamed (2007)

Study reference:

Study report unnamed, 2007: |https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/3/?documentUUID=ca22dcb7-3231-4dd3-95f8-44fad85f4c68

Key study 2 for in vivo genetic toxicity in the registration.

Detailed study summary and results:

In vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus

Reliability score made by the registrant: 1

Test type

OECD test guideline 474 is relevant, but the study was done prior to the guideline. No major deviations from the guideline

Test substance

- FR 513, equivalent to the substance identified in the CLH dossier
- *Degree of purity:* 98.1%
- *Batch number:* 39084

Test animals

- *Species/strain/sex:* NMRI mice/male and female
- *No. of animals per sex per dose:* In total 81 animals (45 males and 36 females). Ten animals (5 males, 5 females) per dose.
- *Age and weight at the study initiation:* Initial age at the start of acclimatisation: 8-9 weeks (males), 11-12 weeks (females). Initial body weight at start of treatment: Males mean value 32.9g (SD ± 1.9 g); Females mean value 31.3 g (SD ± 2.7 g)

Administration/exposure

- *Doses/concentration levels, vehicle, rational for dose selection:* Preliminary test: 2000, 1500, 1000, 500,400, 300 (mg/kg b.w) main test: 300, 150, 75 (mg/kg bw. On the day of the experiment, the test item was formulated in DMSO+corn oil (30%-70%). The vehicle was chosen to its relative non-toxicity for the animals. All animals received a single standard volume of 10 mL/kg body weight orally.
- *Vehicle*: Identification, concentration and volume used, justification of choice of vehicle (if other than water): DMSO+corn oil (30%-70%)
- *Positive and negative (vehicle/solvent) control data:* Positive control substance(s): CPA; Cyclophosphamide (>98%); Dosing: 40 mg/kg b.w ; volume administration: 10 mL/kg b.w

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* FR-513 did not induce micronuclei as determined by this micronucleus test with femur bone marrow cells of the mouse. The % micronuclei was 0.085, 0.110 and 0.125 at dose 75, 150, 300 mg/kg bw 24 hours post-treatment.

3.9 Carcinogenicity

No studies available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Unnamed (2015)

Study reference: Study unnamed, 2015: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Reliability score made by the registrant: 1

Effect on developmental toxicity: Via oral route

Detailed study summary and results:

Test type

28-day oral repeat dose toxicity study, OECD Guideline 407 compliant

Test substance

- 97% TBNPA
- The impurities do not affect the classification
- Batch number 2119-72-01

Test animals

- SD rats
- 5 males and 5 females
- *Age and weight at the study initiation:* Approximately 7-8 weeks, males 201-246 g (males), 154-188 g (females)

Administration/exposure

- *Route of administration:* oral (gavage)
- *duration and frequency of test/exposure period:* 28 days
- *doses/concentration levels, rationale for dose level selection:* 30, 150, 500 mg/kg bw/day
- *control group and treatment:* yes, concurrent vehicle
- *historical control data if available:* available
- *vehicle:* corn oil

Results and discussion

The results showed no systemic toxicity effects and the No Observed Adverse Effect level (NOAEL) was determined as >500 mg/kg/day (highest dose tested). No treatment related changes in sperm count and motility were observed. Vaginal lavages which were taken early morning during the 3 week period from all females, prior to termination of the animals showed no treatment related changes in the oestrus cycle. In addition, there were no dose related changes in organ weight of ovaries, seminal vesicles, testis, ureter, uterus, vagina in comparison to control animals.

3.10.1.2 Unnamed (2016)

Study reference: Study unnamed, 2016: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/9/3

Reliability score made by the registrant: 1

Effect on developmental toxicity: Via oral route

Detailed study summary and results:

Test type: Prenatal Developmental Toxicity Study

OECD Guideline 414 (Prenatal Developmental Toxicity Study). GLP compliant.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* FR-513, equivalent to the substance identified in the CLH dossier
- *Degree of purity:* 97.6%

Test animals

- *Species/strain/sex:* Sprague-Dawley M/F, (Crl:CD(SD) strain)
- *No. of animals per sex per dose:* 20 females per dose
- *Age and weight at the study initiation:* Approximately 70 days old, 231 to 292 g.

Administration/exposure

- *Route of administration*: oral (gavage)
- *duration and frequency of test/exposure period:* Pregnant females received daily doses via gavage from day 6 to 19 during the pregnancy.
- *doses/concentration levels, rationale for dose level selection:* Dose levels were 100 (low) and 300 (medium) mg/kg bw/day. In addition there was a dose group of 1000 mg/kg bw/day, but this had to be reduced to 500 mg/kg/d (high) shortly after commencement of the treatment due to signs of post dosing toxicity, after 2-3 doses. Two females in the top dose group were killed for animal welfare reasons. Some animals received 500 mg/kg bw/d (high) already from the start. Se dosing details below in table.
- *control group and treatment:* The control group received corn oil at the same volume and duration as the treated groups.
- *vehicle:* Corn oil
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* The homogeneity and stability of formulations during storage were determined and ensured.

Description of test design: Study was done according to OECD TG 414 and GLP.

The study consisted of one control and three treated groups identified as follows:

Group	Treatment	Dose # (mg/kg/day)	Number of animals	Animal numbers
			Female	Female
1	Control	0	20	1-20
2	FR-513	100	20	21-40
3	FR-513	300	20	41-60
4	FR-513	1000 / 500 @	20	61-80

Expressed in terms of material as supplied

@ 1000 mg/kg/day dosed 23-25 May. From 26 May 2016 the dose level of 500 mg/kg/day was used. Female numbers 61 -63 received 1000 mg/kg/day during Days 6-8 of gestation, female numbers 64 -72 received 1000 mg/kg/day during Days 6-7 of gestation, and female numbers 73-77 received 1000 mg/kg/day on Day 6 of gestation only. Female numbers 78-80 received the lowered dose of 500 mg/kg/day only from Day 6 to Day 17 of gestation.

Results and discussion:

- *time of death during the study and whether animals survived to termination:* Animals were killed on Day 20 after mating for reproductive assessment and detailed fetal examination. In two females toxicity signs were so severe as to require termination after 2 or 3 doses, on Day 7 or Day 8 of gestation
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:* At 1000 / 500 mg/kg/day mean body weight loss was observed during Days 6-7 of gestation (after the first dose). Later in the study, dams in the high dose group had lower food consumption and body weight gain compared to the controls. This was due to both lower gravid uterine weight in the dosed animals and lower body weight gain when the maternal body weight was adjusted for the weight of the utreus. No effects was seen on maternal body weight in the low and medium dose groups.
- *Food consumption:* Was slighty affected only in the top dose group on days 6-9, and afterwards similar to controls.
- *clinical observations: description, severity, time of onset and duration:* Signs of chin rubbing and salivation were observed in animals of all treated groups, however, this was considered to relate to general distaste of the formulation rather than any effect of toxicity. Signs of toxicity was observed after treatment of pregnant female Sprague Dawley rats with FR-513 at 1000 mg/kg/day from Day 6 of gestation with marked post dosing signs including underactive/unresponsive behaviour, partially closed eyelid(s), unsteady muscle reaction and prostrate posture following one to three doses. In two females these findings were so severe as to require termination after 2 or 3 doses, on Day 7 or Day 8 of gestation. Following the reduction of the high dose level to 500 mg/kg/day (after the third day of dosing) the signs of toxicity were less marked or no longer

apparent. No signs of toxicity was observed in the low and medium dose groups during dosing, and no macroscopic findings were made in the dams during necropsy in any of the dose groups.

- *haematological and clinical biochemistry findings if available:* Not available
- *necropsy findings:* No macroscopical findings in any dose group.

Pups/litters (per dose):

- *mean number of live pups (litter size)*
  Embryo-fetal survival was considered to have been unaffected by treatment at 100, 300 or 1000 / 500 mg/kg/day with mean numbers of implantations, resorptions, live young and percentages of sex ratio and pre and post-implantation loss being similar to control values across all treated groups.
- *mean litter or pup weight by sex and with sexes combined:* Mean placental, male, female and overall fetal weights / litter weight at 100, 300 or 1000 / 500 mg/kg/day were similar to controls and unaffected by treatment.
- *external, soft tissue and skeletal malformations and other relevant alterations:* No dose-related major fetal abnormalities were found. In the medium dose group, there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control (11 fetuses from 7 litters; compared to 4 fetuses from 3 litters in the Controls and 15 fetuses from 12 litters in HCD). In the high dose group there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control in 12 fetuses from 8 litters. This was also within the concurrent Historical Control Data (HCD) range and was considered unrelated to treatment. At 1000 mg/kg/day there was a slightly increased incidence of other minor abnormalities compared to Controls, but all within the historical controls.

3.10.2 Human data

3.10.2.1

No data available.

3.11 Specific target organ toxicity – single exposure

Not evaluated.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Unnamed, 2015

Study reference:

Study report unnamed, 2015 https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Detailed study summary and results:

Test type

OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents), GLP compliant.

Key study 2 in the registration. Reliability 1 given by the registrant

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-513 (TBNPA)
- Degree of purity: 97%
- Batch number 2119-72-01

Test animals

- Sprague-Dawley rats/male and female
- Number of animals 33 males and 33 females. 5 males and 5 females per dose.
- Age of the main study and recovery animals at start of treatment: Approximately 7 to 8 weeks of age.
- Weight range of the main study and recovery animals at the start of treatment Males: 201 to 246 g Females: 154 to 188 g

Administration/exposure

- Oral gavage
- Dosing once daily for 28 days
- Doses: 30, 150 or 500 mg/kg/day of FR-513. Rationale for dose level selection not found
- Post exposure observation period: Two weeks
- Vehicle: Corn oil. Volume dose 5 mL/kg body weight. control group and treatment: Vehicle at the same volume dose as the treated groups

- Homogeneity and stability of FR-513 in corn oil formulations (at nominal concentrations of 6 and 100 mg/mL) was confirmed
- Actual doses: 30, 150 or 500 mg/kg/day of FR-513 (equivalent to 6, 30 and 100 mg/mL using a dose volume of 5 mL/kg body weight)
- Statistical methods not given

Results and discussion

- body weight and body weight changes: no effects observed.
- organ weights: A test-substance related response increased liver weight was evident (predominantly at ≥150 mg/kg/day), and a correlative microscopic finding of slight minimal centrilobular hypertrophy were reported. Full or partial recovery were seen at the end of the study. Slightly higher kidney weights were observed in females in the low dose group and in males in the medium dose group. All findings showed full recovery, with the exception of kidney weights which remained slightly high at the end of the recovery period for males in the top dose group.
- food/water consumption: no effects observed
- clinical signs: In the top dose group, frequent incidences of chin rubbing and/or salivation (sometimes reported as excessive) was reported at some point from week 2 in all females and in the majority of males. In one female in the medium dose group, single incidences of chin rubbing and excessive salivation occurred on days 11 and 16, respectively. The signs were occurred following dosing and dissapeared 1-2 hours after. Females receiving 500 mg/kg/day displayed transient unsteady gait approximately 20 minutes after completion of dosing the group on Days 27 and 28 of treatment and one high dose female (No. 54) appeared to be less active than the other females within the group (on Day 28) at the same time. These were transient signs which had resolved by 1 to 2 hours after dosing.
- no effect on motor activity
- ophthalmologic findings: not available
- haematological findings: Blood was collected on day 29 for the main study animals and on day 15 for the recovery animals: No clear treatment related findings.
- clinical biochemistry findings: At the end of 4 weeks treatment, there was a transient and slightly low sodium concentration (0.98X Control) and slightly high potassium concentration (1.18X Control) in top dose males
- gross pathology findings: no effects
- histopathology findings: no effects

- no mortality observed

3.12.1.2 Unnamed, 2011

Study report unnamed, 2011 https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Key study 1 in the registration. Reliability 2 given by the registrant.

Detailed study summary and results:

Test type

No guideline applicable.

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-513 (TBNPA)
- Degree of purity: 98.4%

Test animals

- Crj: CD(SD) rats, male and female (a total of 25 male and 25 female)
- 5 male and 5 female per dose
- The rats were ordered at 29 to 35 days of age and within a weight range of 118 to 145 g for
- males and 108 to 135 g for females. The animals were allowed to acclimatise to the conditions described below for 12 days before treatment commenced. For those animals selected for this study, their age at the start of treatment was 41 to 47 days and their bodyweights were in the range of 209 to 272 g for males and 161 to 197 g for females
- All animals were subject to a necropsy.

Administration/exposure

- route of administration: daily oral gavage
- The test substance was administered daily over a period of 14 consecutive days. The necropsy procedures were completed on Day 15.

  Males receiving 1000 mg/kg/day were prematurely sacrificed on day 4 of treatment for animal welfare reasons.
- Doses were 0, 100, 300 and 1000 mg/kg/day.
- post exposure observation period: no

- vehicle: corn oil, 5 mL/kg bodyweight

Results and discussion

- body weight and body weight changes: no major changes except for changes in males receiving the top dose, where some of them lost weight.
- Liver, kidneys and spleen were examined and weighed. Organ weight findings including organ / body weight ratios: no effects observed
- food/water consumption: no effects
- haematological findings: not examined
- clinical biochemistry findings: not examined
- three out of five females receiving 1000 mg/kg/day to show urine staining during the treatment period (Day 4, 11 and 15), with this sign also observed at macroscopic examination
- gross pathology findings: Enlargement of the liver with associated dark areas was seen in one female receiving 1000 mg/kg/day
- histopathology findings: not examined
- post dose salivation and chin rubbing was observed on occasion in the majority of animals at all dose levels
- Males receiving 1000 mg/kg/day (top dose) were killed early on day 4 of treatment for animal welfare reasons. Last in-life signs included, abnormal gait, unresponsive, underactive, flat posture, prostrate posture and high levels of urine staining in all males at this dosage. Macroscopic examination revealed abnormal contents and pallor of the jejunum in three of the five animals, but there were no other consistent macroscopic observations recorded for these animals.

3.12.1.3 Unnamed, 1973

Study report unnamed, 1973

Supporting study 3 in the registration. Reliability 2 given by the registrant

Detailed study summary and results:

Test type

Non-GLP study

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-1360 (TBNPA)
- Purity 98%
- Dibromoneopentyl glycol 1.4% ; Tetrabromoneopenthane 0.6%

Test animals

- Male and female Sprague-Dawley rats
- 5 animals per sex per dose
- Age at the study initiation: 6-7 weeks

Administration/exposure

- route of administration – oral in feed
- 30 days dosing in feed
- 10, 30, 100 and 300 mg/kg bw/day nominal in diet
- vehicle: not given
- control group and treatment: there was a control group
- test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation: no details given
- actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable: not given
- statistical analysis of variance and Dunnett´s test

Results and discussion

- body weight and body weight changes: No effects observed
- No effects observed on food consumption. Water consumption not examined.
- No clinical signs were recorded.
- sensory activity, grip strength and motor activity assessments (when available): Not examined
- ophthalmologic findings: Not examined
- On day 24 hematologic evaluations and urinalysis were conducted on the male and female rats from the control groups and groups receiving 300 mg/kg/bwclinical biochemistry findings: decease in Serum Glutamic Pyruvic transaminase (SGPT) (300 and 100 mg/kg/day) and increase in Blood Urea Nitrogen in males (300 mg/kg/day)At the termination of the test, blood samples were collected from all the rats for determination of serum urea nitrogen, alkaline phosphatase and glutamic pyruvic transaminase. No hematological effects observed
- Necropsy was conducted. At necropsy, a complete gross pathological examination was conducted and the weights of heart, liver, kidney, testes and brain were recorded.

- Treatment-related effects were: increase in serum urea nitrogen content in male rats receiving 300 mg/kg/day FR-1360 in their diet, and renal tubular damage and generalized hyperplasia of the mucosal lining of urinary bladders of male rats reciving 300 and 100 mg/kg/day of FR-1360 in their diet. No changes were noted in any of the female rats in this study.
- No mortality occurred

3.12.2 Human data

No data available.

3.13 Aspiration hazard

Not evaluated.

4 ENVIRONMENTAL HAZARDS

Not evaluated.