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:

2,4,6-tri-tert-butylphenol

EC Number: **211-989-5**

CAS Number: **732-26-3**

Index Number: **/**

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SUPPORT ON HOW TO COMPILE ANNEX I TO THE CLH REPORT

1 PHYSICAL HAZARDS

Not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Acute oral toxicity study (Anonymous 4, 1992)

Study reference:

Anonymous 4, 1992

Detailed study summary and results:

Test type

OECD TG 401

Test substance

- 2,4,6-TTBP

Test animals

- *Species/strain/sex :* rat / SD / both sexes
- *No. of animals per sex per dose :* 5/sex/group

Administration/exposure

- *Mode of administration :* gavage
- *Duration of test/exposure period :* single exposure
- *Doses/concentration levels :* 200 and 2000 mg/kg bw
- *Post exposure observation period :* 14d
- *Vehicle:* arachis oil

Results and reliability

- *LD50 value :* > 200 and < 2000 mg/kg bw
- *Number of deaths at each dose level :* at the high dose level, 2 females were found dead 1d after exposure. Moreover, 1 male was killed in extremis 4d after treatment and 3 females were killed in extremis 1d after treatment.

- *Clinical signs:* At 2000 mg/kg bw, clinical signs were noted such as ataxia, hunched posture, lethargy, decreased respiratory rate and laboured respiration, ptosis and loss of righting reflex. Surviving animals recovered 3 or 10 days after treatment.

  No clinical signs were observed at the low dose.
- *Necropsy findings :* animals which died or which were killed in extremis during the study exhibited haemorrhagic lungs, dark or pale liver, patchy pallor of the liver or red-coloured possible necrosis of the liver and haemorrhagic or pale gastric mucosa.

3.1.2 Human data

No human data available

3.1.3 Other data

No other data available

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 Acute dermal toxicity study (Anonymous 5, 2015)

Study reference:

Anonymous 5, 2015

Detailed study summary and results:

Test type

OECD TG 402

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 99.88%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes

Administration/exposure

- *Mode of administration :* dermal exposure, occlusive
- *Duration of test/exposure period :* 24h
- *Doses/concentration levels :* 2000 mg/kg bw
- *Post exposure observation period :* 2w
- *Control group :* no
- *Vehicle:* corn oil
- *Area covered (e.g. x% of body surface) :* approx. 10% of the tot. body surface

- *Total volume applied :* 10mL/kg

Results and reliability

- *LD50 value :* > 2000 mg/kg bw
- *Number of deaths at each dose level :* no mortality observed

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* 1 female exhibited a general erythema 2d after exposure
- *Necropsy findings :* no effects observed

3.2.2 Human data

No human data available

3.2.3 Other data

No other data available

3.3 Acute toxicity - inhalation route

Not evaluated in this CLH dossier

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 Skin irritation study (Anonymous 6, 1992)

Study reference:

Anonymous 6, 1992

Detailed study summary and results:

Test type

OECD TG 404

Test substance

- 2,4,6-TTBP
- *Degree of purity :* no information available

Test animals

- *Species/strain/sex :* rabbit / NZW / male
- *Number of animals :* 3 males

Administration/exposure

- *Duration of test/exposure period :* 4h
- Semi-occlusive

- *Total dose:* 0.5g
- *Vehicle:* 0.5ml of distilled water
- *Time points at which grading/scoring took place :* 1, 24, 48 and 72h

Results and discussion

- *numerical skin grades at 1, 4, 24, 48 and 72 hours*

  Table 1 : irritation score

	animals	Observation time (h)			
		1	24	48	72
erythema	1	1	1	1	0
	2	1	0	0	0
	3	1	0	0	0
edema	1	1	0	0	0
	2	0	0	0	0
	3	0	0	0	0

- *Mean erythema score (mean of the 24, 48 and 72h examinations) :* 0.22/4
- *Mean edema score (mean of the 24, 48 and 72h examinations) :* 0/4

3.4.2 Human data

No human data available

3.4.3 Other data

No other data available

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 Eye irritation study (Anonymous 7, 1992)

Study reference:

Anonymous 7, 1992

Detailed study summary and results:

Test type

OECD TG 405

Test substance

- *2,4,6-TTBP*
- *Degree of purity :* no information available
- *Is the substance skin corrosive or skin irritant?* no

Test animals

- *Species/strain/sex :* rabbit / NZW / both sexes
- *No. of animals per sex per dose :* 2 males and 1 female

Administration/exposure

- *Total dose:* 0.1 ml (approx.. 62mg)
- Single exposure
- *Post exposure observation period :* 72h
- *Control group :* the left eye remained untreated and was used as control
- *Vehicle:* no vehicle
- *Removal of test substance (e.g. water or solvent) :* no

Results and discussion

- *Irritant/corrosive response data :*

  Table 2 : irritation score

Rabbit		Male 1				Male 2				Female 1			
Observation time		1	24	48	72	1	24	48	72	1	24	48	72
cornea	Degree of opacity	0	0	0	0	0	0	0	0	0	0	0	0
	Area of opacity	0	0	0	0	0	0	0	0	0	0	0	0
iris		1	0	0	0	1	0	0	0	0	0	0	0
Conjunctivae	Redness	1	1	0	0	1	0	0	0	1	1	0	0
	Chemosis	1	0	0	0	1	0	0	0	0	0	0	0
	Discharge	1	1	0	0	1	0	0	0	1	0	0	0
Mean score for ocular irritation		11	4	0	0	11	0	0	0	4	2	0	0

- *Mean score of the 24, 48 and 72h examinations :*
  - Corneal opacity score : 0/4
  - Iris score : 0/2
  - Conjunctivae (redness) : 0.22/3
  - Chemosis score : 0/4
- *Overall irritation score :* 8.7, 2.0, 0.0 and 0.0 respectively after 1, 24, 48 and 72h of exposure

3.5.2 Human data

No human data available

3.5.3 Other data

No other data available

3.6 Respiratory sensitisation

Not evaluated in this CLH dossier

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Local Lymph Node Assay (Anonymous 8, 2015)

Study reference:

Anonymous 8, 2015

Detailed study summary and results:

OECD TG 429

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 99.88%

Test animals

- *Species/strain/sex :* mouse / CBA/J / female
- *No. of animals per sex per dose :* 5 females/group

Administration/exposure

- *Induction*
  - *concentration(s) and volume of test substance :* 0, 10, 25 and 50%
  - *induction vehicle :* N,N-dimethyl formamide
  - dorsal surface of both ears was topically treated (25µL/ear)
  - *the spacing between doses :* day 1,2 and 3
  - At d6, an injection of PBS containing ^{3}H-methyl thymidine was performed and after 5h, animals were killed

Results and discussion

- *skin reactions / irritation :* no irritation of the ears
- *systemic toxicity :* no mortality and no clinical signs were observed
- *body weight :* no modification observed
- *macroscopy of the auricular lymph nodes :* the auricular lymph nodes of 2 females exposed to 25% and of all animals exposed to 50% appeared larger in size.
- *stimulation index :* 1.7, 3.3 and 4.6 respectively at 10, 25 and 50%
- *EC3 :* 22.2%

3.7.2 Human data

No human data available

3.7.3 Other data

No other data available

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Bacterial reverse mutation assay (Anonymous 9, 2015)

Study reference:

Anonymous , 2015

Detailed study summary and results:

Test type

OECD TG 471

AMES test

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 99.88%

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable : S. Typh.* TA98, TA10, TA1535, TA1537 and *E Coli* WP2uvrA
- *Test concentrations :*
    - First experiment : *S. Typh.* TA100 and *E. Coli* WP2uvrA with concentrations of 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate in the absence and presence of rat liver S9-mix. Based on the results, the doses of 17, 52, 164, 512 and 1600 µ/plate were used fir the assay with the tester strains *S. Typh.* TA1535, TA1537 and TA98 in the absence and presence of rat liver S9-mix.
    - Second experiment : up to the dose level of 1600 µg/plate in the tester strains *S. Typh.* TA1535, TA1537, TA98, TA100 and *E. Coli* WP2uvrA.
- *Vehicle:* DMSO

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation :* only in tester strains *S. Typh.* TA1535 and TA1537 without S9-mix
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation :* negative

Table 3 : Mean number of revertant colonies/3replicate plates for the first experiment

Dose level (µg/plate)	*S. Typh.*				*E. Coli*
	TA98	TA100	TA1535	TA1537	WP2uvrA
Without S9-mix					
Positive control	13040±64	776±46	760±64	339±27	1326±78
Negative control	17±6	103±19	12±10	5±4	28±3
1.7	NT	113±18	NT	NT	26±11
5.4	NT	108±17	NT	NT	27±6
17	19±2	114±27	6±1	6±2	27±8

52	10±4	92±8	13±2	7±2	29±13
164	14±11	96±8	11±1	11±3	30±1
512	15±4	104±24	12±4	10±2	30±8
1600	7±1	85±4	6±2	4±2	24±2
5000	NT	99±10	NT	NT	23±5
With S9-mix					
Positive control	908±359	1389±88	275±64	324±28	218±13
Negative control	24±2	108±10	10±5	8±3	33±3
1.7	NT	106±17	NT	NT	36±6
5.4	NT	108±17	NT	NT	52±27
17	25±2	116±24	8±4	11±1	37±5
52	18±2	97±6	11±6	6±4	34±7
164	24±5	98±13	8±4	10±2	35±2
512	9±2	105±11	10±4	4±2	35±6
1600	13±2	89±13	8±2	6±2	31±2
5000	NT	111±9	NT	NT	32±7

Table 4 : Mean number of revertant colonies/3 replicate for the second experiment

Dose level (µg/plate)	*S. Typh*				*E. Coli*
	TA98	TA100	TA1535	TA1537	WP2uvrA
Without S9-mix					
Positive control	1964±166	699±73	72±24	62±4	153±32
Negative control	10±3	86±12	9±2	4±4	24±7
17	11±6	91±16	6±6	5±5	21±2
52	11±3	79±16	5±3	5±5	26±7
164	12±6	90±2	9±3	4±1	30±10
512	11±4	89±8	17±4	7±4	23±11
1600	8±1	81±3	4±1	2±2	28±4
With S9-mix					
Positive control	627±35	875±128	775±73	147±17	341±19
Negative control	15±4	62±4	7±4	6±1	51±4
17	17±6	73±3	4±1	6±2	50±10
52	12±3	64±4	11±3	7±3	47±6
164	11±4	68±5	8±3	8±6	61±7
512	19±8	65±6	6±2	3±2	44±5
1600	15±7	86±25	8±2	5±0	58±8

3.8.1.2 *In vitro* mammalian cell gene mutation test (Anonymous 10, 2015)

Study reference:

Anonymous 10, 2015

Detailed study summary and results:

Test type

OECD TG 476

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 99.88%

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable :* Mouse lymphoma L5178Y cells
- *Type and composition of metabolic activation system:* rat liver microsomal enzymes (S9-mix)
- *Test concentrations :* First experiment : 3h treatment : 0.1 to 45µg/ml (without S9-mix) and 0.1 to 100µg/ml (with S9-mix)

  Second experiment : 3h treatment : 0.01 to 25µg/ml (without S9-mix) and 0.01 to 60µg/ml (with S9-mix)

  Third experiment : 24h treatment : 0.1 to 30 µg/ml (without S9-mix)
- *Vehicle:* DMSO

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation :* In absence or presence of metabolic activation, 2,4,6-TTBP did not induce a significant increase in the mutation frequency in the two experiment

Table 5 : first experiment : cytotoxicity and mutagenic response after 3h treatment

Dose level (µg/ml)	RSG (%)	CE day 2 (%)	RS day 2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					
Negative control 1	100	50	100	100	159
Negative control 2	100	65	100	100	126
0.1	116	57	98	114	99
1	103	111	193	199	49
5	101	50	86	87	128
10	127	70	122	155	100
20	47	55	95	44	120
25	21	102	177	37	123
35	7	81	141	10	108
45	12	57	98	11	114
Positive control	68	39	67	46	671
With S9-mix					
Negative control 1	100	56	100	100	135
Negative control 2	100	60	100	100	83
0.1	104	57	98	101	133
1	91	61	106	96	105
10	101	68	118	118	77
20	82	67	116	95	130
50	67	84	145	97	106

70	22	49	84	18	106
80	14	47	81	11	189
100	11	72	125	13	124
Positive control	40	23	40	16	1676

Table 6 : second experiment : cytotoxicity and mutagenic response after 3h treatment

Dose level (µg/ml)	RSG (%)	CE day 2 (%)	RS day 2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					
Negative control 1	100	120	100	100	66
Negative control 2	100	110	100	100	86
0.01	94	101	88	83	87
0.1	108	120	104	113	56
0.5	112	110	96	107	75
1	96	129	112	107	76
5	113	111	97	110	82
10	105	101	88	92	92
20	60	127	111	66	75
25	26	111	97	25	89
Positive control	87	59	52	45	994
With S9-mix					
Negative control 1	100	91	100	100	103
Negative control 2	100	75	100	100	127
0.01	108	94	113	122	85
0.1	95	89	107	102	101
1	105	81	98	103	82
5	90	102	123	111	75
10	93	111	134	125	58
35	47	118	142	67	74
50	23	98	118	28	90
60	11	93	112	13	102
Positive control	52	42	51	27	1752

Table 7 : third experiment : cytotoxicity and mutagenic response after 24h treatment

Dose level (µg/ml)	RSG (%)	CE day 2 (%)	RS day 2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					
Negative control 1	100	90	100	100	92
Negative control 2	100	98	100	100	92
0.1	93	94	100	93	101

1	102	104	110	112	109
5	95	120	127	121	101
10	83	108	115	95	73
15	80	63	67	54	48
20	51	137	145	73	69
25	33	108	115	38	95
30	20	107	114	23	106
Positive control	84	81	87	72	798

3.8.1.3 *In vitro* mammalian chromosome aberration test (Anonymous 11, 1998)

Study reference:

Anonymous 11, 1998

Detailed study summary and results:

Test type

Japanese guideline

Test substance

- 2,4,6-TTBP
- *Degree of purity :* unknown

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable :* Chinese hamster ovary (CHO)
- *Type and composition of metabolic activation system:* S9-mix
- *Test concentrations :*
    - without S9-mix : 0.015, 0.022, 0.024 and 0.026mg/ml for 6h treatment ; 0.0098, 0.013, 0.017 and 0.022mg/ml for 24h treatment ; 0.010, 0.020, 0.025 and 0.030mg/ml for 48h treatment
    - With S9-mix : 0.026, 0.035, 0.047, 0.062, 0.083, 0.11 and 0.15mg/ml for 6h treatment
- *Vehicle:* DMSO

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation*

Table 8 : chromosome aberration

Treatment time (h)	Met. act.	Dose level (mg/ml)	Mitotic index	Nb. of cells scored	Mean aberrations per cell	Cells with aberrations (%)	
						Numerical	Structural
6	-	Neg. control	8.0	200	0.010	2.5	1.0
		Neg. control	8.3	200	0.015	1.0	1.5
		0.015	9.7	200	0.025	3.0	2.0
		0.022	7.0	200	0.010	2.0	1.0
		0.024	6.4	200	0.025	2.5	2.5
		0.026	4.6	200	0.090	4.0	3.5

		Pos. control	9.1	200	0.335	3.5	24.5**
24	-	Neg. control	4.0	200	0.025	1.5	2.5
		Neg. control	3.6	200	0.115	1.0	5.5
		0.0098	6.2	200	0.035	2.0	3.5
		0.013	4.2	200	0.050	3.5	4.5
		0.017	5.5	200	0.030	0.0	3.0
		0.022	4.0	200	0.050	2.5	4.5
		Pos. control	5.5	200	0.630	3.5	39.0**
48	-	Neg. control	5.0	200	0.015	4.5	1.5
		Neg. control	4.7	200	0.005	3.0	0.5
		0.010	5.1	200	0.005	3.5	0.5
		0.020	4.5	200	0.015	4.5	1.5
		0.025	5.1	200	0.005	3.0	0.5
		0.030	0.8	200	0.010	5.5	1.0
		Pos. control	5.2	200	0.940	8.0	37.5**
6	+	Neg. control	7.6	200	0.045	2.5	4.5
		Neg. control	8.0	200	0.045	4.0	4.0
		0.026	10.1	200	0.050	6.0	3.5
		0.035	8.1	200	0.065	2.5	5.5
		0.047	7.7	200	0.025	3.5	2.5
		0.062	5.5	200	0.045	5.5	4.5
		Pos. control	7.2	200	3.030	3.0	74.0**

** : $p<0.01$

2,4,6-TTBP did not induced structural and numerical chromosome aberrations.

3.8.2 Animal data

No study available

3.8.3 Human data

No human data available

3.8.4 Other data

No other data available

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Chronic toxicity study (Matsumoto K. *et al.*, 1991)

Study reference:

Matsumoto K *et al.*, 1991, Chronic toxicity of 2,4,6-tri-tert-butylphenol in rats, The J. of Toxicological Sciences, 16, p.167-179.

Detailed study summary and results:

Test type

No guideline followed

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 97%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 40/sex/group

Administration/exposure

- *Route of administration :* feed
- *duration of test/exposure period :* 24 months
- *doses/concentration levels :* 0, 30, 100, 300 or 1000 ppm (equivalent to approx.. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d)
- *vehicle:* no information available

Results and discussion

- *mortality and time to death (indicate number died per sex per dose and time to death) :* no effects
- *clinical signs :* no effects
- *body weight gain :* significantly lower in females at the highest dose level after 12m of exposure and thereafter

Table 9 :BW data (in g)

	Males					Females				
Dose level (in ppm)	0	30	100	300	1000	0	30	100	300	1000
BW (after 6m of exposure)	362	365	372	406*	402*	188	192	198	185	181
BW after (12m of exposure)	419	431	431	449*	444	240	274**	253	237	198**
BW after (18m of exposure)	458	448	462	482	451	278	287	285	250*	217**
BW after (24m of exposure)	411	443	393	429	379	296	297	270	262	192**

* : $p<0.05$; ** : $p<0.01$

- *food/water consumption :* no effects
- *clinical chemistry and haematology :* few significant changes were observed (see table 10)

Table 10 : clinical chemistry and hematology data

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of exposure	Hb (g/dl)	16.6	16.3	16.4	15.9*	15.7**	16.2	15.8	15.7	15.1**	14.9**
	MCV (fl)	48.3	48.1	47.9	47.2**	46.6**	52.3	51.3**	50.6**	49.9**	47.1**
	Plt ($x10^3$/µl)	741	733	723	784	916**	715	762	801	831*	891**
	BUN (mg/d)	15.8	15.7	16.8	15.4	15.9	18.2	16.1**	17.1	16.5	16.6*
	GOT	87	83	74	79	73	111	83*	76**	77**	72**

	(mU/ml)										
	PL (mg/dl)	167	172	186	184*	206**	204	232**	255**	256**	304**
	T-chol (mg/dl)	82	86	90	102**	157**	137	184**	199**	220**	427**
	Gamma-GTP (mU/ml)	0.8	1.4	1.3	0.8	0.9	0.5	1.4	0.7	1.2	4.3**
After 12m of exposure	Hb (g/dl)	15.5	15.7	15.7	15.4	14.3**	15.3	15.2	15.1	14.8*	14.2**
	MCV (fl)	49.1	47.7*	48.6	47.1**	45.5**	52.9	52.8	52.3**	50.6**	47.3**
	Plt ($x10^3$/µl)	745	770	762	818*	925**	683	804	794*	815*	908**
	BUN (mg/d)	13.4	13.6	14.5	16.1**	17.3**	16.0	16.4	16.8	16.2	17.5
	GOT (mU/ml)	90	89	84	77	69	141	57**	59**	59**	58**
	PL (mg/dl)	174	172	157	181	213**	219	225	252**	261**	325**
	T-chol (mg/dl)	122	119	103	141	210**	167	196*	232**	284**	518**
	Gamma-GTP (mU/ml)	2.1	1.5*	1.2**	1.6	2.3	1.8	1.6	1.5*	1.7	3.4
After 18m of exposure	Hb (g/dl)	16.0	15.8	15.6	15.9	15.1	15.7	15.1*	14.9	14.5**	13.1**
	MCV (fl)	50.5	50.5	51.0	49.8	46.7**	54.5	53.1**	52.5**	51.6**	48.2**
	Plt ($x10^3$/µl)	778	786	834	862	897	682	705	795	833*	929**
	BUN (mg/d)	13.5	15.0	18.0**	16.7**	17.7**	13.9	14.0	15.8	17.1	17.3**
	GOT (mU/ml)	63	65	122	62	57	86	62*	82	60**	130
	PL (mg/dl)	218	227	214	219	267	196	246**	261**	273**	330**
	T-chol (mg/dl)	245	277	183	206	344	163	247**	253**	304**	497**
	Gamma-GTP (mU/ml)	1.6	1.8	2.4	2.6	4.4**	1.7	0.7**	3.2	1.0	4.5
After 24m of exposure	Hb (g/dl)	14.9	13.0	11.9	14.9	12.8	14.7	14.6	14.0	14.2	13.0
	MCV (fl)	53.5	54.7	56.3	47.9	45.1*	53.4	53.3	51.4	49.9**	48.2*
	Plt ($x10^3$/µl)	726	951	1032	912	1311**	684	792	830	825	1252**
	BUN (mg/d)	22.8	17.1	34.7	25.7	37.0	14.0	14.2	14.1	19.1	37.5
	GOT (mU/ml)	64	57	100	77	51	83	66	58	75	63
	PL (mg/dl)	201	209	257**	254*	315**	214	244**	255**	275**	285**
	T-chol (mg/dl)	167	194	285**	336*	595**	187	266**	362*	443**	516**
	Gamma-	6.0	3.9	5.0	5.7	5.6	2.2	1.5	0.5*	3.7	2.4

	GTP (mU/ml)										

* : p<0.05; ** : p<0.01

- *organ weights :* significant changes observed (see table 11). Only liver, kidneys and adrenals were examined.

Table 11: relative organ weights

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of expo	liver w (g%)	2.4	2.29	2.39	2.56*	3.14**	2.42	2.72**	2.90**	3.36**	5.02**
	kidney w (g%)	0.49	0.50	0.48	0.48	0.52	0.63	0.61	0.59	0.63	0.65
	adrenal w (mg%)	11	12	12	11	14*	25	26	25	24	26
After 12m of expo	liver w (g%)	2.30	2.39	2.32	2.76**	3.40**	2.19	2.19	2.53**	3.02**	5.39**
	kidney w (g%)	0.49	0.51	0.50	0.51	0.56**	0.55	0.52*	0.56	0.57	0.76**
	adrenal w (mg%)	11	11	11	12	13*	18	17	17	18	22**
After 18m of expo	liver w (g%)	2.47	2.57	2.63	2.85**	3.86**	2.09	2.44**	2.61**	3.22**	5.26**
	kidney w (g%)	0.53	0.54	0.51	0.52	0.58	0.56	0.57	0.57	0.65	0.78**
	adrenal w (mg%)	11	12	11	12*	15	17	18	16	19	20
After 24m of expo	liver w (g%)	2.76	2.72	3.46*	3.62**	5.58**	2.44	2.80**	3.40**	4.65**	6.61**
	kidney w (g%)	0.61	0.59	0.86	0.65	0.89*	0.59	0.65	0.73**	0.89**	1.12**
	adrenal w (mg%)	16	14	21	15	18	19	17	20	22	26*

* : p<0.05; ** : p<0.01

- *histopathological findings:* at the 2 highest dose levels, swelling, focal necrosis and vacuolisation of hepatocytes were observed after 6months of exposure and thereafter. Only liver, kidneys and adrenals were examined. no more information available.
- *tumour incidence data by sex, dose and tumour type :* no neoplastic lesions observed. No more information available.

3.9.2 Human data

No information available

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No information available

3.9.4 Other data (e.g. studies on mechanism of action)

No information available

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 12, 2015)

Study reference:

Anonymous 12, 2015

Detailed study summary and results:

Test type

OECD TG 407, 421 and 422

Test substance

- 2,4,6-TTBP
- *Degree of purity* : 99.88%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 10/sex/group

Administration/exposure

- *Route of administration :* gavage
- *duration and frequency of test/exposure period :*
    - males : 29D (beginning 2w prior mating)
    - females : 2w prior mating and until at least D4 of lactation
- *doses/concentration levels :* 0, 3, 10 and 30 mg/kg bw/d
- *vehicle:* corn oil

Description of test design:

Following a minimum premating period of 14 days for males and females, one female was cohabited with one male of the same treated dose level, avoiding sibling mating. Detection of mating was confirmed by evidence of sperm in the vaginal lavage or by the appearance of an intravaginal copulatory plug. This day was designated as D0 post-coitum. Once mating occurred, the males and females were separated.

Results and discussion

For P and F1 adults (per dose):

- *mortality :* no effects
- *clinical observations:* no effects observed
- *body weight data for P animals :* lower bw data was observed in females at the 2 highest dose level (not at all observation time)

Table 12 : bw data (in g) (10animals examined by group)

		Males				Females			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
Premating	D1	317	315	317	314	215	216	215	220
	D8	340	341	347	345	226	230	2280	232
Mating	D1	359	357	363	362	227	233	235*	235*
	D15	370	382	391*	384	251 (n=1)	288 (n=1)	/	/
Post-coitum	D0					231 (n=9)	234 (n=9)	238	240*
	D14					278 (n=9)	288	287	284
	D20					345 (n=9)	358	353	347
Lactation	D1					267 (n=9)	275	276	277
	D4					270	280	271	273

* : p<0.05

- *haematological and clinical biochemistry findings :* significant changes were observed (see table 14 and table 15)

Table 13 : significant haematology changes (5 animals examined by group)

	Males				Females			
Dose level (in mg/kg bw/d)	0	3	10	30	0	3	10	30
Neutrophils (% WBC)	15.2	17.9	16.0	19.4	31.9	31.3	28.7	20.2*
Lymphocytes (% WBC)	81.9	79.1	81.7	78.1	65.5	66.5	68.4	77.1*
RBC (10^{12}/L)	8.39	8.51	8.37	8.54	6.8	6.64	7.22	7.41*
Reticulocytes (% RBC)	2.2	2.2	2.1	2.2	6.8	5.4	4.0	3.9*
MCV (fL)	53.3	52.6	53.0	52.1	56.8	55.7	53.2*	51.1**
MCH (fmol)	1.13	1.11	1.14	1.11	1.19	1.18	1.12**	1.07**
PT (s)	16.4	16.1	15.5	15.9	15.8	15.4	15.5	14.3**

* : p<0.05 ; ** : p<0.01

Table 14 : clinical biochemistry data (5 animals examined per group)

	Males				Females			
Dose level (in mg/kg bw/d)	0	3	10	30	0	3	10	30
ASAT (U/L)	81.0	76.3	77.0	82.3	71.5	77.8	87.6**	74.8
Tot. protein (g/L)	58.6	58.9	57.6	59.0	63.1	62.5	64.3	70.3**
Albumin (g/L)	31.6	31.7	30.6	30.8	31.7	31.5	32.8	36.8**
Tot. bilirubin (µmol/L)	2.4	2.1	2.0**	1.7**	2.3	2.1	1.9**	1.5**
Glucose (mmol/L)	8.41	7.70	9.24	9.17	5.79	6.10	6.81	7.39*
Colesterol (mmol/L)	1.62	1.94	1.99	2.23	1.52	2.17	2.82**	4.57**
Potassium (mmol/L)	3.87	4.16	4.16	4.20*	3.46	3.63	3.72	3.98**
Calcium (mmol/L)	2.50	2.51	2.53	2.49	2.62	2.65	2.61	2.80*

* : p<0.05 ; ** : p<0.01

- *toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation :* no changes

  Table 15 : reproduction data

Dose level (in mg/kg bw/d)	0	3	10	30
Females paired	10	10	10	10
Mating index (%)	100	100	100	100
Fertility index (%)	90	100	100	100
Conception index (%)	90	100	100	100
Gestation index (%)	100	100	100	100

- *precoital interval (number of days until mating and number of estrous periods until mating) :* mean precoital time : 3.6, 3.0, 3.3 and 3.3 respectively at 0, 3, 10 and 30 mg/kg bw/d
- *number of implantations, corpora lutea :*
  - corpora lutea : 12.9, 13.8, 15.2, 13.7 respectively at 0, 3, 10 and 30 mg/kg bw/d
  - Implantations : 12.7, 13.3, 12.9 and 11.4 respectively at 0, 3, 10 and 30 mg/kg bw/d (lower number of implatations at the highest dose, due to one female)
- *duration of gestation (calculated from day 0 of pregnancy) :* 21.2, 21.0, 21.0 and 21.4d respectively at 0, 3, 10 and 30 mg/kg bw/d
- *necropsy findings :* 3 males and 1 females of the high dose level exhibited enlargement of the liver.
- *organ weight changes :* a higher liver weight was observed in females at 10 mg/kg bw/d and in both sexes at 30 mg/kg bw/d.

Table 16 : organ weight data (in g or %)

		Males				Females			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
FBW		351 (n=10)	363 (n=10)	371* (n=10)	367 (n=10)	235	249	246	246
Brain weight	Abs (g)	1.97	2.01	2.08*	1.99	1.91	1.87	1.89	1.93
Epididymides	Abs (g)	1.091	1.134	1.156	1.122				
	Rela (%)	0.310	0.314	0.311	0.307				
Liver weight	Abs (g)	8.07	8.68	9.24	11.38**	7.09	7.98	8.95**	12.08**
	Rela (%)	2.25	2.40	2.52	3.13**	3.01	3.20	3.64**	4.91**
Prostate	Abs (g)	0.542	0.570	0.663	0.623				
	Rela (%)	0.151	0.158	0.181	0.171				
Seminal vesicles	Abs (g)	1.485	1.472	1.420	1.644				
	Rela (%)	0.413	0.410	0.388	0.450				
Spleen weight	Abs (g)	0.531	0.563	0.667*	0.586	0.587	0.567	0.518	0.511

	Rela (%)	0.148	0.156	0.182*	0.160	0.251	0.227	0.211	0.208
Testes/ovaries weight	Abs (g)	3.31	3.55	3.66	3.41	0.125	0.140	0.190	0.135
Thyroid weight	Abs (g)	0.016	0.018	0.013	0.017	0.012	0.014	0.013	0.017
Uterus	Abs (g)					0.721	0.687	0.658	0.655
	Rela (%)					0.307	0.276	0.268	0.268

* : p<0.05 ; ** : p<0.01

- *histopathological findings:*
  - liver : slight to moderate hepatocellular hypertrophy was noted in both sexes at 10 and 30 mg/kg bw/d. Furthermore, hepatocellular necrosis was observed in 1 male and 1 female of the highest dose.
  - Cecum : mucosal hypertrophy was present in males at the mid and high dose levels.
  - Spleen : decreased hematopoiesis was observed in females of the mid and high dose levels.

Table 17 : microscopic data

			Males				Females			
Dose level (in mg/kg bw/d)			0	3	10	30	0	3	10	30
Cecum	Hypertrophy, mucosa	Grade 1	0	0	1	1	0	0	0	0
		Grade 2	0	0	0	2	0	0	0	0
Liver	Hypertrophy hepatocellular	Grade 1	0	0	2	1	0	0	0	0
		Grade 2	0	0	0	4	0	0	5	0
		Grade 3	0	0	0	0	0	0	0	5
	Necrosis hepatocellular	Grade 1	0	0	0	1	0	0	0	1
Spleen	Hematopoiesis	Grade 1	2	/	1 (n=1)	2	0	0	3	2
		Grade 2	0	/	0	0	1	2	1	3
		Grade 3	0	/	0	0	4	3	1	0

Grade 1 : minimal/very few/very small ; Grade 2 : slight/few/small ; Grade 3 : moderate/moderate number/moderate size

For F1 and F2 pups/litters (per dose):

- *number of live births :*
  - Number of dead pups at first litter check : 0, 0, 1 and 0 respectively at 0, 3, 10 and 30 mg/kg bw/d
  - Number of living pups at first litter check : 101, 125, 121 and 94 respectively at 0, 3, 10 and 30 mg/kg bw/d
  - % Postnatal loss : 0.0, 0.0, 6.6 and 12.8 % respectively at 0, 3, 10 and 30 mg/kg bw/d (8** pups and 12** pups died respectively at 10 and 30 mg/kg bw/d)
- *sex ratio :* % of males/females living pups at first litter check : 50/50, 50/50, 50/50 and 55/45 respectively at 0, 3, 10 and 30 mg/kg bw/d
- *viability index :* Viability index : 100.0, 100.0, 93.4** and 87.2** respectively at 0, 3, 10 and 30 mg/kg bw/d
- *mean litter or pup weight by sex and with sexes combined :*

Table 18 : body weight of pups (in g)

Dose level (in mg/kg bw/d)		0	3	10	30
Day	Sex				
1	M	6.3	5.9	5.7**	5.7**
	F	6.1	5.7	5.4**	5.4*
	M+F	6.2	5.8	5.5**	5.6*
D4	M	9.6	8.8*	8.2**	7.7**
	F	9.4	8.3	7.7**	7.5**
	M+F	9.5	8.5	8.0**	7.6**

* : p<0.05 ; ** : p<0.01

- *external, soft tissue and skeletal malformations and other relevant alterations :* Only incidental macroscopic findings among surviving pups was noted (missing tail or tail point or dehydrated appearance), the incidence was also within the range considered bormal for pups of this age (no more information available).
    - 0 mg/kg bw/d : 2 pups exhibited blue spot snout and 2 blue spot head
    - 3 mg/kg bw/d : 3 pups exhibited blue spot back, 3 scabs and 1 blue snout
    - 10 mg/kg bw/d : 1 pups exhibited blue spot neck, 3 pallor
    - 30 mg/kg bw/d : 1 pups exhibited pallot, 1 absence of milk in stomach, 1 missing tail, 2 tail point and 2 dehydrated appearance

3.10.1.2 Range finding study of the combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 12, 2015)

Study reference:

Anonymous 12, 2015

Detailed study summary and results:

Test type

Range finding study

No guideline followed

Test substance

- 2,4,6-TTBP
- *Degree of purity* : 99.88%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 3 females/group

Administration/exposure

- *Route of administration :* oral
- *duration and frequency of test/exposure period :* 10d
- *doses/concentration levels, rationale for dose level selection :* 50, 100 and 250 mg/kg bw/d

- *vehicle: corn oil*

For P

- *time of death during the study and whether animals survived to termination :* at the highest dose, 2 animals were sacrificed in extremis on day 10 and one animal was found dead on d9
- *clinical observations: description, severity, time of onset and duration :*
  - ≥ 50 mg/kg bw/d : hunched posture was observed.
  - ≥ 100 mg/kg bw/d : lethargy, piloerecction and uncoordinated movements were noted.
  - 250 mg/kg bw/d : abnormal gait, labored respiration, ventro-lateral recumbency, deep respiration.
- *body weight data :* slight reduce was observed at the 2 highest dose levels
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals* : terminal body weight, kidney and liver weight were determined. Liver weight was higher at 50 and 100 mg/kg bw/d (no more information available)
  - 50 mg/kg bw/d : higher liver weight in all animals
  - 100 mg/kg bw/d : notably increased liver weight (abs and rela) in all animals
  - 250 mg/kg bw/d : not determined (all animals sacrificed/found dead before scheduled necropsy
- *haematological and clinical biochemistry findings if available :* no information available
- *necropsy findings :* enlarged liver with yellowish foci was noted in all animals exposed at the 2 highest dose levels. At 250 mg/kg bw, hardened liver (2 animals), irregular surface of the forestomach (1 animal), black brown foci on the adrenal glands (1animal), reddish foci on the mesenteric lymph nodes (2 animals) were observed.
- *histopathological findings: nature and severity :* not examined

3.10.2 Human data

No information available

3.10.3 Other data (e.g. studies on mechanism of action)

No information available

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

See chapter 3.1 and chapter 3.2

3.11.2 Human data

No information available

3.11.3 Other data

No information available

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Combined 28-day repeated dose toxicity study with the reproduction/ developmental toxicity screening test (Anonymous 12, 2015)

See chapter 3.10.1.1

3.12.1.2 Chronic toxicity study (Matsumoto K. et al, 1991)

Study reference:

Matsumoto K. et al., 1991

Detailed study summary and results:

Test type

OECD TG 452

Test substance

- 2,4,6-TTBP
- *Degree of purity :* 97%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 40/sex/dose

Administration/exposure

- *route of administration* : oral (feed)
- *duration and frequency of test/exposure period :* 24 months
- *doses/concentration levels :* 0, 30, 100, 300 and 1000 ppm
- *post exposure observation period*
- *vehicle:* no information available

Results and discussion

- *body weight and body weight changes :* bwg significantly reduced in females exposed to 1000 ppm after 12m of exposure

Table ?? : body weight data (in g)

	Males					Females				
Dose level (in mg/kg bw/d)	0	30	100	300	1000	0	30	100	300	1000
After 6m of exposure	362	365	372	406*	402*	188	192	198	185	181
After 12m of exposure	419	431	431	449*	444	240	274**	253	237	198**
After 18m of exposure	458	448	462	482	451	278	287	285	250*	217**
After 24m of exposure	411	443	393	429	379	296	297	270	262	192**

* : p<0.05 ; ** : p<0.01

- *description, severity, time of onset and duration of clinical signs :* no effects
- *haematological and clinical biochemistry findings:* significant modifications were observed

Table ?? : clinical chemistry and hematology data

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of exposure	Hb (g/dl)	16.6	16.3	16.4	15.9*	15.7**	16.2	15.8	15.7	15.1**	14.9**
	MCV (fl)	48.3	48.1	47.9	47.2**	46.6**	52.3	51.3**	50.6**	49.9**	47.1**
	Plt ($x10^3$/µl)	741	733	723	784	916**	715	762	801	831*	891**
	BUN (mg/d)	15.8	15.7	16.8	15.4	15.9	18.2	16.1**	17.1	16.5	16.6*
	GOT (mU/ml)	87	83	74	79	73	111	83*	76**	77**	72**
	PL (mg/dl)	167	172	186	184*	206**	204	232**	255**	256**	304**
	T-chol (mg/dl)	82	86	90	102**	157**	137	184**	199**	220**	427**
	Gamma-GTP (mU/ml)	0.8	1.4	1.3	0.8	0.9	0.5	1.4	0.7	1.2	4.3**
After 12m of exposure	Hb (g/dl)	15.5	15.7	15.7	15.4	14.3**	15.3	15.2	15.1	14.8*	14.2**
	MCV (fl)	49.1	47.7*	48.6	47.1**	45.5**	52.9	52.8	52.3**	50.6**	47.3**
	Plt ($x10^3$/µl)	745	770	762	818*	925**	683	804	794*	815*	908**
	BUN (mg/d)	13.4	13.6	14.5	16.1**	17.3**	16.0	16.4	16.8	16.2	17.5
	GOT (mU/ml)	90	89	84	77	69	141	57**	59**	59**	58**

	PL (mg/dl)	174	172	157	181	213**	219	225	252**	261**	325**
	T-chol (mg/dl)	122	119	103	141	210**	167	196*	232**	284**	518**
	Gamma-GTP (mU/ml)	2.1	1.5*	1.2**	1.6	2.3	1.8	1.6	1.5*	1.7	3.4
After 18m of exposure	Hb (g/dl)	16.0	15.8	15.6	15.9	15.1	15.7	15.1*	14.9	14.5**	13.1**
	MCV (fl)	50.5	50.5	51.0	49.8	46.7**	54.5	53.1**	52.5**	51.6**	48.2**
	Plt ($x10^3$/µl)	778	786	834	862	897	682	705	795	833*	929**
	BUN (mg/d)	13.5	15.0	18.0**	16.7**	17.7**	13.9	14.0	15.8	17.1	17.3**
	GOT (mU/ml)	63	65	122	62	57	86	62*	82	60**	130
	PL (mg/dl)	218	227	214	219	267	196	246**	261**	273**	330**
	T-chol (mg/dl)	245	277	183	206	344	163	247**	253**	304**	497**
	Gamma-GTP (mU/ml)	1.6	1.8	2.4	2.6	4.4**	1.7	0.7**	3.2	1.0	4.5
After 24m of exposure	Hb (g/dl)	14.9	13.0	11.9	14.9	12.8	14.7	14.6	14.0	14.2	13.0
	MCV (fl)	53.5	54.7	56.3	47.9	45.1*	53.4	53.3	51.4	49.9**	48.2*
	Plt ($x10^3$/µl)	726	951	1032	912	1311**	684	792	830	825	1252**
	BUN (mg/d)	22.8	17.1	34.7	25.7	37.0	14.0	14.2	14.1	19.1	37.5
	GOT (mU/ml)	64	57	100	77	51	83	66	58	75	63
	PL (mg/dl)	201	209	257**	254*	315**	214	244**	255**	275**	285**
	T-chol (mg/dl)	167	194	285**	336*	595**	187	266**	362*	443**	516**
	Gamma-GTP	6.0	3.9	5.0	5.7	5.6	2.2	1.5	0.5*	3.7	2.4

	(mU/ml)										

* : p<0.05; ** : p<0.01

- *organ weight :*

table ?? : relative organ weight value

		Males					Females				
Dose level (in mg/kg bw/d)		0	30	100	300	1000	0	30	100	300	1000
After 6m of exposure	Liver w. (g%)	2.40	2.29	2.39	2.56*	3.14**	2.42	2.72**	2.90	3.36**	5.02**
	Kidney w. (g%)	0.49	0.50	0.48	0.48	0.52	0.63	0.61	0.59	0.63	0.65
	Adrenal w. (mg%)	11	12	12	11	14*	25	26	25	24	26
After 12m of exposure	Liver w. (g%)	2.30	2.39	2.32	2.76**	3.40**	2.19	2.19	2.53**	3.02**	5.39**
	Kidney w. (g%)	0.49	0.51	0.50	0.51	0.56**	0.55	0.52*	0.56	0.57	0.76**
	Adrenal w. (mg%)	11	11	11	12	13*	18	17	17	18	22**
After 18m of exposure	Liver w. (g%)	2.47	2.57	2.63	2.85**	3.86**	2.09	2.44**	2.61**	3.22**	5.26**
	Kidney w. (g%)	0.53	0.54	0.51	0.52	0.58	0.56	0.57	0.57	0.65	0.78**
	Adrenal w. (mg%)	11	12	12	12*	15	117	18	16	19	20
After 24m of exposure	Liver weight (g%)	2.76	2.72	3.46**	3.62**	5.58**	2.44	2.80**	3.40**	4.65**	6.61**
	Kidney weight (g%)	0.61	0.59	0.86	0.65	0.89*	0.59	0.65	0.73**	0.89**	1.12**
	Adrenal weight (mg%)	16	14	21	15	18	19	17	20	22	26*

* : p<0.05; ** : p<0.01

- *gross pathology findings:* swelling, focal necrosis and vacuolisation of hepatocytes were noted after 6months of exposure and thereafter. No neoplastic lesions were observed. Only liver, kidneys and adrenals were examined. No more information available).

3.12.1.3 Subacute toxicity study (Takahashi O. and Hiraga K., 1987)

Study reference:

Takahashi O. and Hiraga K., 1978

Detailed study summary and results:

Test type

No guideline followed

GLP compliance : unknown

Investigation of the relationship between haemorrhage induced by butylated hydroxytoluene and its antioxidant properties or structural characteristics

Test substance

- 2,4,6-TTBP

- *Degree of purity :* >99%

Test animals

- *Species/strain/sex :* rat / SD/ male
- *No. of animals per sex per dose :* 10 males

Administration/exposure

- *route of administration* : oral (feed)
- *duration and frequency of test/exposure period :* 3w (continuous in the diet)
- *doses/concentration levels :* 1.98 mmol/kg/d
- *vehicle:* unchanged

Results and discussion

- *mortality and time to death :* all animals died during the exposure period. LT50 was of 7.4D

  Table ?? : lethal times

Day of exposure	5D	6D	8D	9D	10D	11D
Number of animals died	1	1	2	3	2	1

- *body weight and body weight changes :* no information available
- *food/water consumption :* no information available
- *description, severity, time of onset and duration of clinical signs :* no information available
- *haematological findings:* no information available
- *clinical biochemistry findings:* no information available
- *gross pathology findings:* Necropsy was performed in all dead animals and showed haemothorax, haematocoelia, intracranial haematoma, intranasal haemorrhage, intramuscular haematoma, intratesticular haematoma and intraepididymis haemorrhage.

3.12.2 Human data

No information available

3.12.3 Other data

No information available

3.13 Aspiration hazard

Not evaluated in this dossier

4 ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.