ECHA
European Chemicals Agency

Committee for Risk Assessment
RAC

Annex 1
Background Document
to the Opinion proposing harmonised classification and labelling at Community level of
TDCP
(Tris[2-chloro-1-chloromethyl)ethyl] phosphate)

ECHA/RAC/CLH-0-0000000953-71-03/A1

TDCP
EC Number: 237-159-2
CAS Number: 13674-87-8

Adopted
3rd September 2010

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP)

EC Number: 237-159-2

CAS number: 13674-87-8

Registration number (s): Not applicable

Purity: 93 – 99.9% pure (w/w)

Impurities: 0.1 – 7% w/w.

There are a number of impurities which are stated as confidential by the manufacturers. This information has been presented in a confidential identity annex which has been submitted separately to ECHA.

Proposed classification based on Directive 67/548/EEC:

Carcinogen Category 3; R40

Proposed classification based on Regulation EC 1272/2008:

Category 2 Carcinogen with hazard statement H351

Proposed labelling:

Directive 67/548/EEC: Xn; R40; S(2)-36/37

Regulation EC 1272/2008: "Warning", H351 "Suspected of causing cancer".

Proposed specific concentration limits (if any):

None

Notes (if any):

None

The classification proposal is based on the properties of the substance itself. This dossier reviewed the carcinogenicity, mutagenicity and reproductive toxicity endpoints. For carcinogenicity a classification is proposed. For mutagenicity, developmental toxicity and male fertility toxicity no classification is proposed. Female fertility was not evaluated as no data were available.

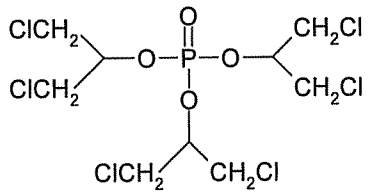
JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
EC Name:	tris[2-chloro-1-(chloromethyl)ethyl] phosphate
CAS Number:	13674-87-8
IUPAC Name:	Tris(1,3-dichloropropan-2-yl) phosphate
Synonyms	TDCP: this common acronym is used throughout this report Tris[2-chloro-1-(chloromethyl)ethyl] phosphate 2-Propanol, 1,3-dichloro-, phosphate (3:1) Tris(1,3-dichloro-2-propyl) phosphate Tris(1-chloromethyl-2-chloroethyl) phosphate 1,3-Dichloro-2-propanol phosphate (3:1) Phosphoric acid, tris(1,3-dichloro-2-propyl)ester Fyrol FR-2 Tolgard TDCP LV Tris CP

1.2 Composition of the substance

Chemical Name:	Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
EC Number:	237-159-2
CAS name:	2-Propanol, 1,3-dichloro-, phosphate (3:1)
IUPAC Name:	Tris(1,3-dichloropropan-2-yl) phosphate
Molecular formula:	$C_9H_{15}Cl_6O_4P$
Structural formula:	
Molecular weight:	430.91
Typical concentration:	93 – 99.9 % w/w

Chemical Name:	Confidential Impurities*
Typical concentration:	0.1 – 7 % w/w

* There are a number of impurities which are stated as confidential by the manufacturers. This information has been presented in a confidential identity annex which has been submitted separately to ECHA. The structures of the impurities do not suggest that they would have had a strong influence on any of the test results and will not influence the classification and labelling. No additives are used.

1.3 Physico-chemical properties

Table 2.1: Summary of physico- chemical properties

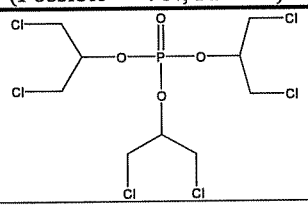
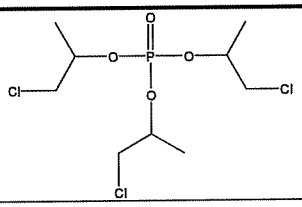
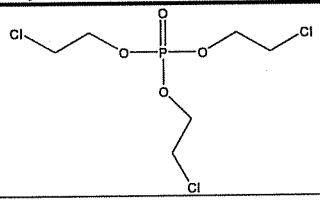
REACH ref Annex, §	Property	IUCLID section	Value	Comment/reference
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	Liquid	/
VII, 7.2	Melting/freezing point	3.2	< -20°C**	Cuthbert and Mullee, 2002a.
VII, 7.3	Boiling point	3.3	~326°C** (decomp.)	Boiled with decomposition. Cuthbert and Mullee, 2002a.
VII, 7.4	Relative density	3.4 density	1.513 at 20°C**	Cuthbert and Mullee, 2002a,
VII, 7.5	Vapour pressure	3.6	5.6×10^{-6} Pa at 25°C **	The result is consistent with the chemical structure of the main component and the other properties, in particular the boiling point. Tremain, 2002.
VII, 7.6	Surface tension	3.10		No study available, but based on the chemical structure and physico-chemical properties, TDCP not expected to exhibit surface activity.
VII, 7.7	Water solubility	3.8	18.1 mg/l at 20°C**	Cuthbert and Mullee, 2002b.
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	3.69 ± 0.36**	Cuthbert and Mullee, 2002b.
VII, 7.9	Flash point	3.11	May be above 245^0C	No closed cup result is available. Read-across from TCPP (HSA/EA, 2008b), suggests that the result is likely to be above 245^0C.
VII, 7.10	Flammability	3.13	Not expected to be flammable	Based on the chemical structure and physico-chemical properties..
VII, 7.11	Explosive properties	3.14	Not expected to be explosive	Based on the chemical structure and the known synthetic route of manufacture via an exothermic reaction.
VII, 7.12	Self-ignition temperature		513 °C	Akzo Nobel, 2000.
VII, 7.13	Oxidising properties	3.15	Not expected to be oxidising	Based on the chemical structure and analogy to similar existing chemicals.
XI, 7.17,	Viscosity	3.22	1,800 cP at 25 °C	Akzo Nobel, 2003, cited

REACH ref Annex, §	Property	IUCLID section	Value	Comment/reference
			2,200 cP at 0 °C 540 cP at 40 °C	in USEPA, undated.
	Henry's law constant		1.24 x 10^{-04} Pa.m^3/mol at 25°C	By calculation from VP and WS results.

Studies marked ** were performed with a composite sample of purity 94.2%, derived from recent representative commercial products from the main producers

TDCP is structurally similar to two other chlorinated alkyl phosphate esters, TCPP (Tris (2-chloro-1-methylethyl) phosphate) and TCEP (Tris (2-chloroethyl) phosphate). The structures, the key physical chemical properties and the classifications of each are presented in Table 2.2 below.

Table 2.2: Structures and key physico- chemical properties for TDCP, TCPP and TCEP

	Tris [2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP)	Tris (2-chloro-1-methylethyl) phosphate (TCPP)**	Tris (2-chloroethyl) phosphate (TCEP) *
CAS number	13674-87-8	13674-84-5	115-96-8
Classification in the Annex I of Directive 67/548/EEC	Proposal: Carc. Cat. 3; R40. (Possible***: N; R51-53)	not classified	Carc. Cat. 3; R40 Repr. Cat. 2; R60 Xn; R22 N; R51-53
Structure			
Molecular weight	430.91	327.57	285.49
Physical state	Liquid	Liquid	Liquid
Melting point	<-20 ^{0}C	<-20 ^{0}C	<-70 ^{0}C
Boling point	Ca. 326 0C (decomp)	Ca. 288 0C (decomp)	320 0C (decomp)
Relative density	1.513	1.288 at 20 0C	1.4193 at 25 0C
Vapour Pressure	5.6 x 10-6 Pa at 25 ^{0}C	1.4 x 10-3 Pa at 25 ^{0}C	1.14 x 10-3 Pa at 20 ^{0}C (extrapol.)
Water solubility	18.1 mg/l	1080 mg/l at 20 ^{0}C	7820 mg/l at 20 ^{0}C
Log Kow	3.69 ± 0.36	2.68 ± 0.36	1.78

* taken from BAUA, 2006
** taken from HSA/EA 2008b
*** taken from HSA/EA 2008a

Although the structures and physiochemical properties of the three substances may be seen as sufficiently comparable to suggest a read- across approach, some differences in the target organs and critical effects for the three substances do not support a full direct read-across from data on either TCEP or TCPP.

2 MANUFACTURE AND USES

TDCP is an additive flame retardant, i.e. it is physically combined with the material being treated rather than chemically combined.

3 CLASSIFICATION AND LABELLING

The substance is not currently classified in Annex I of Directive 67/548/EEC or Annex VI of Regulation No. 1272/2008.

4 ENVIRONMENTAL FATE PROPERTIES

TDCP meets the screening criteria for P or vP of the PBT criteria (HSA/EA, 2008a).

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The following information on toxicokinetics is included as supporting information only. Further information can be found in the IUCLID file for TDCP.

ABSORPTION

Following oral administration of radiolabelled TDCP to rats, absorption from the GI tract was found to be > 90 %, and therefore 100% oral absorption is assumed. No data are available for the inhalation route and in accordance with the default values given in the TGD[1], 100 % absorption via the inhalation route is assumed. An in vitro percutaneous absorption study using human skin membranes was conducted to determine the absorption following topical application of [^{14}C]-TDCP. The skin membranes were exposed to TDCP for 8 hours, mimicking a normal working day. The mean total absorption was 15.4 %, 10.69 % and 6.0 %, for doses 0.003, 0.01 and 0.12 mg/cm^2, respectively (HSA/EA, 2008a).

DISTRIBUTION

There was no apparent effect of the route of administration on tissue distribution following oral and i.v. administration, with tissue/blood ratios for the total radioactivity similar for all tissues. Highest levels of radioactivity were found in the liver, kidney and lung following oral, dermal and i.v. administration. Tissue concentrations of either the parent compound or metabolites were low due to

[1] Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

a rather fast elimination: the decrease in the radioactivity of most tissues (except skin) became apparent by 7 hours post exposure, and a marked decrease was obvious in all tissues by 24 hours after TDCP administration, and by day 10 the remaining radioactivity was only 1-5 % of that observed 15 minutes post-exposure (HSA/EA, 2008a).

METABOLISM

In vitro, mixed function oxidases (MFO) in microsomes of rat liver homogenate appear to play an important role in the metabolism of TDCP. The metabolite bis(1,3-dichloroisopropyl)hydrogen phosphate accounted for 75% of the MFO-metabolised TDCP. TDCP was also shown to be metabolised by glutathione-S-transferase present in the soluble fraction of rat liver, and it appears that TDCP is directly conjugated with glutathione. In a separate in vitro study, the metabolism of TDCP in the soluble fraction resulted in almost exclusively in one metabolite, which is possibly a γ-glutamylcysteinyl conjugation product of the parent TDCP. The following metabolites were also generated by the microsomal fraction of liver homogenate: bis(1,3-dichloro-2-propyl) phosphate (64 % of total metabolites), 1,3-dichloro-2-propanediol (20%), 1,3-dichloro-2-propanol (5.7 %) and an unknown metabolite (11 %).

Following i.v. administration of TDCP to rats, the metabolites isolated from rat urine were bis(1,3-dichloro-2-propyl) phosphate (67.2 % of total urine radioactivity), an unidentified polar metabolite (32 %), 1,3-dichloro-2-propyl phosphate (0.29 %) and un-metabolised TDCP (0.45 %) (HSA/EA, 2008a).

EXCRETION

Elimination of TDCP was rapid. Following oral administration, recovery of radioactivity after 168 hours was urine (43.2 %), faeces (39.2 %), expired air (16.24 %) and carcass (2.51 %). The decrease in radioactivity in all tissues was biphasic. The longest t½ was recorded in adipose tissue in both phases of elimination (17.8 and 92.4 hours, respectively).

Following i.v. administration, approximately 34 %, 20 % and 20 % of total radioactivity was excreted in the urine, faeces and expired air, respectively. The half-life of TDCP clearance in tissues was between 1.5 and 5.4 hours (HSA/EA, 2008a).

5.2 Acute toxicity

TDCP has a low acute toxicity, with an oral LD_{50} (rat) greater than 2000 mg/kg bw. The dermal LD_{50} (rat) following occluded contact for 24 hours, is greater than 2000 mg/kg bw. For inhalational exposure, the 4 hour LC_{50} (rat) is greater than 5.22 mg/l. (HSA/EA., 2008a).

No classification for acute toxicity is proposed and the above information is included as supporting information only. Further information on this endpoint can be found in the IUCLID file for TDCP.

5.3 Irritation

Skin and eye irritation have not been evaluated as part this dossier. Information on this endpoint can be found in the IUCLID file for TDCP.

5.4 Corrosivity

Corrosivity has not been evaluated as part this dossier. Information on this endpoint can be found in the IUCLID file for TDCP.

5.5 Sensitisation

Skin and respiratory sensitisation have not been evaluated as part of this dossier. Information on these endpoints can be found in the IUCLID file for TDCP.

5.6 Repeated dose toxicity

There is neither 28 day nor 90 day repeat dose toxicity study available for TDCP. However some information can be extracted from the rat carcinogenicity 2-year study (Stauffer Chemical Company, 1981a; Freudenthal, R.I. and Henrich, R.T., 2000).

In the 2-year carcinogenicity study described in details in section 5.8.1, groups of 60 male and 60 female rats were fed diets containing TDCP to achieve dose levels of 0, 5, 20 and 80 mg/kg/day. The study was conducted for 24 months. Ten animals of each sex were selected for interim sacrifice at 12 months, however not all endpoints were evaluated at this time point for each dose group.

Significantly greater mortality was recorded for high dose males. There was a clear adverse effect on body weight in the high-dose male and female groups throughout the study, with body weights at termination more than 20 % lower than controls.

A significant reduction in red blood cell parameters was also noted for high-dose animals. Some individual animals in mid and high dose groups exhibited marked elevation in blood urea nitrogen values at 18 and 24 months which is consistent with renal pathology in these animals.

Absolute and relative liver weights were increased at both 12 and 24 months in high dose animals. There was an increase in foci of hepatocellular alteration and sinusoidal dilation in both males and females at the highest dose.

Kidney weights were increased in mid- and high-dose animals. Kidney enlargement was observed in mid and high dose males and high dose females. Cysts were evident in males at all doses and in mid and high dose females. Microscopically, there was an increase in the incidence of hyperplasia of the convoluted tubule epithelium in females at the high dose and in males in all treatment groups when compared to control animals at 24 months. There was also an increase in chronic nephropathy in males at the mid and high doses and in females at the high dose at 24 months.

Thyroid weights were also increased in mid- and high-dose animals. In addition to these findings, erythroid/myloid hyperplasia of the rib marrow, erythroid/myloid metaplasia of the spleen and hyperplasia of the parathyroid glands were also increased in high-dose animals.

Gross observations in the male reproductive tract noted in the mid and high dose animals included various discolorations, masses/nodules, enlargement and flaccidity in the testes as well as small seminal vesicles. These observations were made in animals which were killed at 24 months and which died or were killed when moribund after the 12 month interim sacrifice, although no information is available as to the exact time of death. The corresponding testes weights were not

significantly higher than control males. Details of the histological observations on the male reproductive organs are discussed in section 6.9.

A **LOAEL of 5 mg/kg/day** (based on the hyperplasia, considered a pre-neoplastic lesion, observed in the kidneys in all treated groups and the testicular effects observed at this dose) can be derived from this study.

Elsewhere:

In a 90-day study to investigate the possible neurotoxicity of TDCP in hens, doses of 0, 4, 20 and 100 mg/kg/day TDCP were administered to hens. There were no mortalities in TDCP-treated birds. Under the conditions of the test, there was no evidence of TDCP induced delayed neurotoxicity (Stauffer Chemical Company, 1979b).

In an epidemiology study carried out on 289 male workers in a TDCP manufacturing plant as an adjunct to a mortality study, no adverse health effects linked to TDCP exposure were determined, but it should be underlined that all air samples were under the limit of detection of TDCP (Stauffer Chemical Co., 1983b.

No data are available on inhalation and dermal repeated dose toxicity (HSA/EA, 2008a).

Repeated dose toxicity has not been evaluated as part of this dossier and the above information is included as supporting information only. Further information on this endpoint can be found in the IUCLID file for TDCP.

5.7 Mutagenicity

5.7.1 In vitro data

The available in vitro mutagenicity data for TDCP is summarised in Table 6.1, below.

Table 6.1 Summary of in vitro mutagenicity data for TDCP

Test	Endpoint	Result	Comments	Ref.
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Non-mutagenic	Test substance: TDCP: LV. Purity not stated	SafePharm Labs (1984 & 1985b)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Non-mutagenic	Studies did not meet current regulatory stds Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1976 & 1977a)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Significant positive response at 500 µg/plate +S9 (TA 100)	Test substance: Fyrol FR-2. Purity 95.7%	Stauffer Chem. Co. (1983a)
Ames modified quantitative suspension assay	Gene mutation	Mutagenic only at toxic doses (>1000µg/plate (+&-S9)	Not a true positive response Test substance: Fyrol FR-2. Purity 95.7%	Stauffer Chem. Co. (1983a)

Test	Endpoint	Result	Comments	Ref.
Ames assays	Gene mutation	Positive response +S9 in strains TA 100 & 1535 from 333 µg/plate.	Dose-related response (Interlaboratory comparison) Test substance: Tris(1,3-dichloro-2-propyl)phosphate. Purity 94.4%	Mortelmans et al. (1986)
Ames assays	Gene mutation	Weakly mutagenic +S9 with TA 100. Positive in 6 independent expts + PB-induced S9. Positive in 2 expts + PCB-induced S9 and in 3 expts +PB-induced S9. Confirmatory results with PCB-induced mouse & guinea pig liver S9.	Dose dependency observed in multiple assays Test substance: Fyrol FR-2. Purity not stated	Gold et al. (1978)
Ames (Pour plate assay)	Gene mutation	Weakly mutagenic + S9 with TA 100.	Test substance: TDCP. Purity not stated	Lynn et al. (1981)
Ames assay	Gene mutation	Positive at 0.5mg/ml +S9.	Test substance: Tris-dichloropropylphosphate. Purity not stated	Ishidate (1983)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Positive mutagenic response +S9 with TA 100 at 500 µg/plate	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro plate mutagenicity assay, fungi (	Gene mutation	Non-mutagenic in Sacc. cereviseriae	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1976 & 1977a)
In vitro mouse lymphoma assay with L5178Y cells	Gene mutation	Positive +S9 at >80µg/ml. Non-mutagenic -S9.	Clear dose-related increase Test substance: TDCP LV. Purity not stated	Inveresk (1985)
In vitro chromosome aberration assay	Chromosome aberration	Negative with or without S9	Test substance: Fyrol FR-2. Purity not stated	Covance (2004)
In vitro mouse lymphoma assay	Gene mutation	Negative with or without S9	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Sister chromatid exchange assay (L5178Y $TK^{+/-}$ cells)	SCE	Negative	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Chromosome aberration assay (L5178Y $TK^{+/-}$ cells)	Chromosome aberration	Increase at highest dose analysed (118 µg/ml) +S9.	Considered equivocal. Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Chromosomal aberration assay	Chromosome aberration	Positive +S9 at 0.5 mg/ml	Test substance: Tris-dichloropropylphosphate. Purity not stated	Ishidate (1983)
Sister chromatid exchange (CECT assay)	SCE	Negative	Test substance: Fyrol FR-2. Purity not stated	Bloom (1982 & 1984)
In vitro transformed foci in BALB/3T3 cells	Cell transformation	Negative	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem Co. (1978b)
In vitro point mutation assay in V79 cells	Gene mutation	Negative	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)

Test	Endpoint	Result	Comments	Ref.
In vitro UDS assay	DNA damage & repair	Minimal response at 0.1mM	Not possible to quantify response Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro transformation assay in Syrian hamster embryo cells	Cell transformation	Positive at 20 & 30µM	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro Salm. typhimurium mutagenicity assay with hepatocyte activation	Gene mutation	Small increase in revertants at 0.05 mM (non-induced rat livers). No increase using PB-induced hepatocytes	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)

5.7.2 In vivo data

The available in vivo mutagenicity data for TDCP is summarised in Table 6.2 below.

Table 6.2 Summary of in vivo mutagenicity data for TDCP

Test	Endpoint	Result	Comments	Ref.
In vivo Mouse micronucleus assay	Clastogenicity	Non-clastogenic	Test substance: Tolgard TDCP LV. Purity not stated.	SafePharm Labs Ltd. (1985c)
In vivo Mouse bone marrow cytogenetic assay	Chromosome aberration	Non-clastogenic	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978c)
In vivo/in vitro urine mutagenicity assay	Mutation	Negative	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978d)
In vivo/in vitro unscheduled DNA synthesis assay	DNA damage & repair	Negative	Test substance: TDCP. Purity >99% w/w	Covance Laboratories Inc. (2005)
Recessive lethal mutation assay in Drosophila	Chromosomal mutation	Negative	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978e)

5.7.3 Human data

No data available for this dossier.

5.7.4 Other relevant information

No data available for this dosser.

5.7.5 Summary and discussion of mutagenicity

No data from humans are available on the mutagenicity of TDCP.

There is evidence to suggest that TDCP is mutagenic *in vitro*. Among the 22 reported in vitro assays, the Ames assays and mammalian cells (mouse lymphoma L5178Y), both in presence of metabolic activation (S9), are positive. The in vitro transformation assay in Syrian hamster embryo (SHE) cells is also positive; it should be noted that this assay points out earliest identifiable stage in

carcinogenicity (morphological cell transformation) and thus in contrast to other short-term in vitro assays SHE detects both genotoxic and epigenetic carcinogens. TDCP also caused an increase in the occurrence of chromosome aberrations in mouse lymphoma cells in the presence of metabolic activation. In contrast, a chromosome aberration study in Chinese hamster ovary (CHO) cells did not induce any increase in chromosome aberrations or polyploidy.

However no confirmation was found in the 5 in vivo studies: TDCP was not clastogenic in a mouse micronucleus assay conducted according to OECD Guideline 474 and did not induce unscheduled DNA synthesis in liver cells in an in vivo/ in vitro UDS assay conducted according to OECD Guideline 486. Elsewhere, negative results were also obtained in a mouse bone marrow cytogenetic assay (MN) which is considered as an indicator of chromosomal aberrations induction, as also in an in vivo/in vitro urine mutagenicity assay that can point mutagenic effects of very end-metabolic products.

Regarding notably the five negative in vivo assays, it is considered that TDCP is not genotoxic in vivo and thus no classification for mutagenicity is proposed.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

There is only one oral 2-year carcinogenicity study in rats available, which has limitations regarding guideline conformance, reporting and statistical analysis (Stauffer Chemical Company, 1981a; Freudenthal, R.I. and Henrich, R.T., 2000). The study was not conducted according to an EC/40/2008 guideline or equivalent frame (e.g. guidance document OECD 453 concerning combined chronic toxicity/carcinogenicity studies) or even under Good Laboratory Practices scheme. Nevertheless it follows relatively correctly the expected protocol. The reporting is sometimes limited, for instance the detailed histological description is missing whereas this would have allowed a more accurate evaluation of the importance of the observed tumours (notably a better separation of the different stages would have been useful to further discuss about development from benign to malign tumours). The highest doses are very probably above the maximum tolerated dose (MTD) as body weight in both sexes is lower especially after the 58th week and becomes more than 20% lower than control at the end of the study; however lower and middle doses also induce carcinogenesis effects that are statistically significant for several organs and this without important body weight changes.

The statistic tests were not named or described, and were not compared to historical control. However on this point Ireland provided later on the reviewed statistics of 24 studies initiated between 1991 and 1997 (Giknis and Clifford, 2001) on Crl:CD(SD)BR Crl:CD(SD)BR IGS (International Genetic Standard) rat colonies from Charles River Laboratories at six different industrial or contract testing facilities in the United States, Canada and Japan. This review shows that interpretation of the results for all testes or kidney endpoints doesn't change significantly under the light of these historical controls. For example, after 24 months TDCP oral exposure hyperplasia of the convoluted tubular epithelium occurs in 58% (28/48) male rats, this rate is significantly higher than study control 4% (2/45) but also than the 104-weeks historical control (6 studies reported this endpoint, control range was 1.43-4%). It should be noted that the basic limitation of this comparison is that the 2-year study on Charles River Sprague-Dawley rats was conducted by Stauffer in 1981, this means around 10-15 years earlier.

Groups of 60 male and 60 female Sprague Dawley rats were fed diets containing TDCP (Fyrol FR-2, purity 95% w/w) to achieve dose levels of 0, 5, 20 and 80 mg/kg/day of TDCP for 24 months. Ten animals of each sex per group were selected for interim sacrifice at 12 months. Animals were routinely observed for morbidity, mortality and clinical signs of toxicity. Body weights and food consumption were measured and blood and urine samples taken periodically from selected animals for haematology, clinical chemistry and urinalysis. Full necropsy was carried out on all animals. Tissues from control and high dose animals were examined microscopically, as were gross lesions, tissue masses, liver, kidney and testes of low and mid dose animals.

Mortality rates in all groups were low during the first 12 months and low in most groups from 12 through to 17 months, with the exception of the high dose males where there was a slight increase in the number of deaths. After month 17, the mortality rate increased in all groups and remained high until the end of the study (this can be expected in ageing animals). Total mortality in low- and mid-dose males and in all TDCP-treated females was considered comparable to that of the controls. Significantly greater mortality ($p<0.05$) was recorded for high dose males, (38/60 and 26/60 animals died in the high and control groups, respectively).

There was a clear adverse effect on body weight at 80 mg/kg/day, throughout the study, with body weights at termination >20 % lower than control animals. Slight decreases (most differences did not exceed 5 %) in male body weights in the 20 mg/kg/day at some intervals of the study may also have been related to treatment. Food consumption for controls and high dose animals was generally comparable except for slight increases in values for the high dose groups during the last few months of the study.

Table 6.3: Tumour incidence in Sprague Dawley rats fed TDCP in a 2 year assay

			Dose group (mg/kg/day)			
Organ	**Tumour identification**	**Sex**	**0**	**5**	**20**	**80**
Kidney	Renal cortical adenoma					
	12 months[a]:	M	0/15	0/12	0/13	0/13
		F	0/11	0/13	0/9	0/10
	24 months only:	M	1/45	3/49	9/48*	32/46*
		F	0/49	1/48	8/48*	29/50*
Testes	Interstitial cell tumour					
	12 months[a]:	M	0/14	0/12	3/13	3/11
	24 months:	M	7/43	8/48	23/47*	36/45*
Liver	Hepatocellular adenomas					
	12months[a]:	M	0/15	0/12	0/13	3/14
		F	0/11	0/13	0/9	1/10
	24 months:	M	2/45	7/48	1/48	13/46*
		F	1/49	1/47	4/46	8/50*
	Hepatocellular carcinomas					
	12months[a]:	M	0/15	0/12	0/13	0/14
		F	0/11	0/13	0/9	0/10
	24 months only:	M	1/45	2/48	3/48	7/46
		F	0/49	2/47	2/46	4/50
Adrenal	Cortical adenomas					
	12 months[a]:	M	0/15	#	#	2/13
		F	5/11	#	#	1/10

	24 months:	M F	5/44 8/48	3/14 5/27	5/16 2/33	3/44 19/49*

* Statistical significance ($p<0.05$)

[a] Scheduled deaths at 12 months and unscheduled deaths prior to 12 months. The report states the numbers of animals "suitable for evaluation" for each tissue listed, although in some cases the results of actual incidence in these tissues is not reported. It has therefore been assumed, that where tissues were suitable for evaluation but no results for 12 month assessment for that lesion are reported, that no lesion was present.

Animals not evaluated at 12 months

Examination of the tissues from the 12-month interim group and those animals found dead prior to 12 months found an increased incidence of neoplastic nodules in the livers of rats in the 80 mg/kg/day group, which were identified as hepatocellular adenomas. There was also an increase in interstitial (Leydig) cell tumours in the testes of males at 20 and 80 mg/kg/day. The incidence of neoplasm in all other tissues was similar in control and treated animals at this time.

At 24 months, the incidence of renal cortical adenomas in males was 1/45 (2 %), 3/49 (6 %), 9/48 (19 %) and 32/46 (70 %) at 0, 5, 20 and 80 mg/kg/day, respectively (reaching statistical significance from 20 mg/kg/day). In females, the corresponding incidences were 0/49 (0 %), 1/48 (2 %), 8/48 (17 %) and 29/50 (58 %), respectively, with statistical significance again from 20 mg/kg/day. There was no reported incidence at 12 months. In addition to the tumours, there was an increase in the incidence of hyperplasia of the convoluted tubule epithelium at 24 months in females at 80 mg/kg/day and in males in all treatment groups when compared to control animals.

In the livers of male animals at 24 months, the incidence of hepatocellular adenomas was 2/45 (4 %), 7/48 (14.5 %), 1/48 (2 %) and 13/46 (28 %) at 0, 5, 20 and 80 mg/kg/day, respectively, with statistical significance reached at 80 mg/kg/day. In females, the corresponding incidences were 1/49 (2 %), 1/47 (2 %), 4/46 (9 %) and 8/50 (16 %), respectively, with statistical significance again at 80 mg/kg/day. At the 12 month interim sacrifice, the incidence of hepatocellular adenomas was 3/14 and 1/10 for males and females respectively at 80 mg/kg/day compared to none in control animals.

At 24 months, the incidence of hepatocellular carcinoma was also increased in males and females, with the incidence in males being 1/45 (2 %), 2/48 (4 %), 3/48 (6 %) and 7/46 (15 %) at 0, 5, 20 and 80 mg/kg/day, respectively, although this did not reach statistical significance. The corresponding values in females were 0/49, 2/47 (4 %), 2/46 (4 %) and 4/50 (8 %). There was no reported incidence at 12 months.

At 24 months the incidence of Leydig cell tumours of the testes (benign tumours) was 7/43 (16 %), 8/48 (17 %), 23/47 (49 %) and 36/45 (80 %), at 0, 5, 20 and 80 mg/kg/day, respectively. The effects were statistically significant at 20 and 80 mg/kg/day. At 12 months, 3/13 (23%) mid dose animals and 3/11 (27%) high dose animals were observed to have Leydig cell tumours whereas no tumours were observed in control animals.

The incidence of adrenal cortical adenomas in high dose females at 24 months (19/49, 38%) could be seen to a certain extent as increased compared to control group 8/48 (17 %), however historical control reviewing 24 studies for this endpoint show that the usual incidence range is 1.43-34.00 %.

5.8.2 Carcinogenicity: inhalation

No studies are available.

5.8.3 Carcinogenicity: dermal

No studies are available.

5.8.4 Carcinogenicity: human data

The mortality of workers employed at a TDCP manufacturing plant was investigated in a retrospective cohort study of male workers who were employed for a minimum of 3 months during the 1956-77 study period and were followed through to 1980 (Stauffer Chemical Co., 1983b). Of the 289 workers eligible for the study, 50% had worked at the plant for < 5 years while 42 workers had been employed for ≥ 15 years. Ten workers died during the study period. The report indicates that all workers were exposed to 'extremely low levels of TDCP in the work environment'. Breathing zone sampling was performed between 1978 and 1981; TDCP levels were always below the limit of detection (8 ppb).

The overall mortality of the study population was 75 % of that expected in a comparable population of US males. For the category 'all causes', the SMR (observed deaths/expected deaths x 100) was 75 (no confidence interval reported). Mortality due to 'all malignant neoplasm' was slightly higher than expected with an SMR of 131. Three cases of lung cancer were observed (vs. 0.8 expected). However, the numbers were too small to calculate a p-value. One case had worked as a janitor in the plane office and was considered non-exposed. The second case had only worked at the plant 2 years prior to onset of disease and the third case had worked for 19 years, as a production operator and a mechanic. All three decedents were moderate to heavy cigarette smokers. Overall, it was concluded that there was no evidence linking these lung cancers with TDCP exposure.

As all air samples were always below the limit of detection and as it was a very small study, one cannot place much reliance on the negative result.

5.8.5 Other relevant information

No data available.

5.8.6 Summary and discussion of carcinogenicity

A retrospective cohort study is available from a TDCP manufacturing plant (Stauffer Chemical Co., 1983b). The study included 289 workers, who were employed at the plant for a minimum of three months during the study period of 21 years. No evidence of an increased cancer risk among the workforce was found, but as all air samples were always below the limit of detection and as it was a very small study, one cannot place much reliance on the negative result.

A rat 2-year carcinogenicity study (Stauffer Chemical Company, 1981a; Freudenthal, R.I. and Henrich, R.T., 2000) although not conducted according to an EC/40/2008 guideline or equivalent frame provides sufficiently clear observations to support a Carcinogen diagnostic which may request classification (the most important arguments are presented first):

- TDCP seems in a dose-dependant manner to induce neoplastic development, notably renal cortical adenomas (neoplasm of epithelium) occur with rates of 1/45 (2%), 3/49 (6%), 9/48 (19%)*, 32/46 (70%)* incidence for respectively the 0, 5, 20 and 80 mg TDCP/kg/day (the symbol "*" means "statistically significant"). A LOAEL of 5 mg/kg/day, based on increased incidence of hyperplasia of the convoluted tubule epithelium in treated males was derived. The incidence of benign testicular Leydig cell tumours was also increased possibly even in a time-dependant manner: 0/14 (0%), 0/12 (0%), 3/13 (23%), 3/11 (27%) at 12 months and

7/43 (16%), 8/43 (18%), 23/47 (49%)*, 36/45 (80%)* at 24 months at 0, 5, 20 and 80 mg/kg/day respectively.

- Tumours occur in multiple sites. Liver or adrenal cortical observations if compared to the historical control may be considered as on the border line; all the more as it's known that chronic administration of an MFO inducer can induce in rat liver hyperplasic response similar to adenomas (an increase in liver weight and, upon histological examination, centrilobular hypertrophy characterized by an increase in cytoplasmic volume, resulting from smooth endoplasmic reticulum, hypertrophy, and increasing nuclear polymorphism). However, some other organs clearly appear as carcinogen targets of TDCP, at least the kidneys and testis, but the small increase in gliomas of the brain - which is mentioned in the original study report - may also be questioned.

- Tumours occur in both sexes. Increasing doses of TDCP in diet have effects in the same organs of both sexes; however incidence responses appear most of time slightly greater in males and no ovary tumour was observed.

- Several stages of the multistage process of carcinogenesis can be identified: Hyperplasia is often considered as a pre-neoplastic lesion, which can lead to tumour formation. The study report does not provide enough detailed information to conclude whether the hyperplasia observed following treatment with TDCP would progress to cancer or whether the tumours observed with TDCP arise through a different mechanism. However, it is not unreasonable to assume that the tumours have developed through hyperplasic changes. It can be said even without some missing histopathological observations that the benign to malign development of at least cortical renal tumours is very probable. This can also be supported by two facts: 1) the ratio tumours / hyperplasia is equal for lower and middle doses at 24 months (0.3) and 2) a logical time sequence for the middle dose may be seen from the rate 1/13 hyperplasia incidence at 12 months (with no nephropathy) towards 28/48 hyperplasia incidence plus 9/48 cortical tumours at 24 months (and 36/48 chronic nephropathy).

- Routes of exposure can be multiple: the unique carcinogenicity study is oral and elsewhere an oral absorption rate higher than 90% was measured. However, the dermal route can not be excluded as 8-hours exposure of human skin membranes revealed potential dermal absorption rates in the range of 6.0-15.4%. The inhalation route may be concluded as not relevant regarding TDCP low vapour pressure, but this assumption should be moderated as TDCP could also absorb on dust (log Koc = 3.25). In the TDCP manufacturing plant retrospective cohort study of 289 male workers employed for a minimum of 3 months during the 1956-77 (Stauffer Chemical Co., 1983b), it wasn't observed any cancer (among the 3) liable to TDCP. However it wasn't measured (air monitoring only between 1978 and 1981) any levels higher than the limit of detection (8 ppb); in addition, it's unknown if dust was included in air samples or if workers were protected.

- Comparison of absorption, distribution, metabolism and excretion between test animals and humans: The use of MFO inhibitors showed that mixed function oxidases (MFOs) in microsomes play an important role in the metabolism of TDCP. The metabolites generated by the microsomal fraction were bis(1,3-dichloro-2-propyl) phosphate (BDCP 64 % of total metabolites), 1,3-dichloro-2-propanediol (20 %), 1,3-dichloro-2-propanol (5.7 %) and an unknown metabolite (11 %). TDCP was also shown to be mainly metabolised by glutathione-S-transferase present in the soluble fraction of rat liver, TDCP by the soluble fraction resulted almost exclusively in the conjugation of TDCP in one γ-glutamylcysteinyl conjugation product. Metabolism was through dealkylation of the phosphate group. The resulting halogenated alkyl group was metabolised to CO2 that was expired or incorporated

into endogenous molecules. Normal human liver contained rather slightly less NADPH-cytochrome c reductase and cytochrome P-450 than is found in the adult rat, even wide variations can be observed, this metabolic pattern and also the half-life of TDCP clearance in tissues (no bioaccumulation) can be estimated between 1.5 and 5.4 hours should all the more be taken into account as indicators of better behaviours than what could occur in human.

- Mechanism of action is unknown. As discussed in section 5.7 above, TDCP may be assumed to be a non-genotoxic carcinogen and thus rather act via a threshold mechanism. In a study investigating the in vivo binding of TDCP to macromolecules of mouse liver, kidney and muscle (Morales & Matthews, 1980), TDCP was greatest in the liver (51 ± 4 pmoles/mg) followed by kidney (31 ± 8) pmoles/mg) and muscle (5.2 ± 0.8 pmoles/mg). The highest concentration of bound radioactivity in the three tissues was to low molecular weight RNA (67 and 93 pmoles/mg for liver and kidney, respectively) followed by protein (57, 43 and 7.2 pmoles/mg, respectively), rRNA (28, 13 and 5.6 pmoles/mg, respectively) and DNA (8.3 and <1.0 pmoles/mg for liver and kidney, respectively). More than 95 % of the radioactivity associated with these macromolecules was covalently bound (Morales & Matthews, 1980). However, although there is some evidence that TDCP is mutagenic in vitro, the five negative in vivo mutagenic studies (see mutagenic section) covering notably gene mutation (UDS test) and clastogenicity (micronucleus test) bring to consider that TDCP may not be a genotoxic. As genetic events are central in the overall process of cancer, other mechanistic hypotheses are welcome to support the interpretation of the carcinogen potential of TDCP, for the main targets two were drawn: In testis, it may be possible that an alteration in the Hypothalamus-Pituitary-Testis axis induces through a luteinising hormone chronic stimulation Leydig cells tumours, however no arguments as modifications in steroid hormone levels were provided in support of the hypothesis. And in kidney a glutathione metabolite of TDCP could be cleaved by β-lyase to form reactive thioaldehydes, resulting in cytotoxicity and hyperplasia, leading to tumour formation; In support of this hypothesis, In vitro metabolism studies with TDCP identified a glutathione metabolite of TDCP that was not present in vivo (HSA/EA, 2008a).

Overall, based on the results from one carcinogenicity study in rat, in which oral exposure to TDCP increase incidence of tumours in the kidney, liver, testes and adrenal glands, together with evidence that TDCP is not genotoxic in vivo, lead to the conclusion that TDCP can be considered only as "suspected human carcinogen", which means a proposal for classification as Carc. Cat. 2 H351[2] (Carc. Cat. 3; R40[3]).

This proposal is in line with a previous provisional agreement at the TC C&L Meeting to classify TDCP as Carc. Cat 3; R40[4].

Higher classification, that means Carc. 1B - H350 [2] (Carc. Cat 2 / R45 [3]), is not appropriate for TDCP as evidence is only available from a single animal carcinogenicity study, as the dose response relationship concerns only benign tumours (even potentially able to develop in malignant

[2] Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures

[3] Dangerous Substances Directive (67/548/EEC)

[4] Commission Working Group on the Classification and Labelling of Dangerous Substances Meeting on the Health Effects of Pesticides, Existing Chemicals & New Chemicals November 14-18, 2005

forms) and as TDCP is not geno-toxic *in vivo* and therefore may be assumed to be a non-genotoxic carcinogen.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

A fertility study in male rabbits was carried out using 40 male and 80 female Dutch belted rabbits (Stauffer Chemical Company, 1982b). Ten male rabbits were assigned to each of four dose groups and treated with 2, 20, or 200 mg/kg/day TDCP (Fyrol FR-2, purity 96% w/w) in Mazola oil for twelve weeks by oral gavage. Animals were examined throughout the treatment period for signs of treatment-related toxicity. During the last week of treatment, each male was mated with one female and then with the second three days later. The females were returned to their cages and sacrificed mid-gestation. The reproductive tract was removed and examined to determine the number of corpora lutea in each ovary, the number of implantation sites and viable foetuses. Males were sacrificed at the end of the mating period and the reproductive tract (testes, epididymides, spermatic cord with blood and lymphatic vessels and ductus deferens, ampullary gland, vesicular gland, seminal vesicle, prostate gland, paraprostatic gland, urinary bladder, urethra, and bulbo-urethral glands) was removed for histological examination. Sperm were taken from one epididymus and analysed for sperm concentration, motility and morphology. Viability was not measured due to the subjectivity in sample readings.

Two animals in each of the 0, 2 and 20 mg/kg/day groups and one in the 200 mg/kg/day died prior to scheduled sacrifice. These deaths were not considered treatment-related. There were no clinical signs of toxicity.

Mating, fertility and pregnancy parameters were unaffected by treatment. There were no treatment-related effects on numbers of corpora lutea, implantations, viable foetuses or resorptions. Sperm analysis was not affected by treatment. There were no histopathological changes detected in the male reproductive tract.

There was a treatment-related increase in absolute and relative kidney (14 % and 19 %, respectively) and liver weights (18 and 23 %, respectively), in the 200 mg/kg/day males. **Overall, it is considered that there is no concern for male fertility in the rabbit.**

In the 2-year carcinogenicity study (Stauffer Chemical Company, 1981a; and also reported in Freudenthal, R.I. and Henrich, R.T., 2000) detailed in section 6.8.1 some effects were observed on the reproductive system of male rats. As discussed earlier, not all information from this study was available to the submitting Member State, but all available information is presented here. Ten animals of each sex per group were selected for interim sacrifice at 12 months. For some effects at this time, only control and high dose animals were evaluated. All animals in the control and all treatment groups were evaluated at 24 months.

Mortality rates were low until 17 months, with the exception of the high dose males where there was a slight increase in the number of deaths in the 12 to 17 month period. After 17 months, the mortality rate increased in all groups and remained high until the end of the study. Significantly greater mortality ($p < 0.05$) was recorded for high dose males when compared with the controls (38/60 versus 26/60, respectively).

There was a clear adverse effect on body weight at 80 mg/kg/day through the study, with body weights at termination greater than 20% lower than control animals.

In animals which were killed at 24 months and which died or were killed when moribund after the 12 month interim sacrifice (which are counted in the 24 month results), gross observations noted in the male reproductive tract of animals treated at 20 and 80 mg/kg/day included various discolorations, masses/nodules, enlargement and flaccidity in the testes as well as smaller seminal vesicles (when compared with control animals). The corresponding testes weights were not significantly higher than control males. Histological changes were also noted in the testes, the epididymides and the seminal vesicles both in control animals and all treatment groups.

A summary of the neoplastic and non-neoplastic observations in the male reproductive organs is presented in table 6.3 below.

Table 6.3 Summary of observations on male reproductive organs of Sprague Dawley rats in the 2-year carcinogenicity study with TDCP (mg/Kg/day). Statistical analysis was not performed by authors of this study (Stauffer Chemical Company, 1981a).

	Finding	**Control 0**	**Low 5**	**Mid 20**	**High 80**
12 Months	**Testes**				
	Interstitial cell tumour	0/14 (0%)	0/12 (0%)	3/13 (23%)	3/11 (27%)
	Seminiferous tubules: germinal epithelial atrophy with associated oligospermia	5/14 (36%)	2/12 (17%)	3/13 (23%)	7/11 (64%)
	Epididymides				
	Oligospermia	0/14 (0%)	NM	NM	1/11 (9%)
	Seminal Vesicles				
	Decreased secretory product	0/15 (0%)	NM	NM	1/10 (10%)
24 Months	**Testes**				
	Interstitial cell tumour	7/43 (16%)	8/48 (17%)	23/47 (49%)	36/45 (80%)
	Seminiferous tubules: germinal epithelial atrophy with associated oligospermia	30/43 (70%)	29/48 (60%)	42/47 (89%)	44/45 (98%)
	Eosinophilic material in tubular lumen	2/43 (5%)	4/48 (8%)	12/47 (26%)	11/45 (24%)
	Sperm stasis	5/43 (12%)	5/48 (10%)	11/47 (23%)	14/45 (31%)
	Periarteritis nodosa	5/43 (12%)	10/48 (21%)	19/47 (40%)	16/45 (36%)
	Epididymides				
	Oligospermia	11/41 (27%)	9/32 (28%)	7/13* (54%)	35/44 (80%)
	Degenerated seminal product	8/41 (20%)	7/32 (22%)	3/13 (23%)	22/44 (50%)
	Seminal Vesicles				
	Decreased secretory product	1/41 (2%)	11/13[a] (85%)	17/19[a] (89%)	22/42 (52%)
	Atrophy	0/41	4/13[a]	6/19[a]	10/42

		(0%)	(31%)	(32%)	(24%)

NM: Not measured

[a] Not all animals evaluated for this effect

In the testes, the incidence of germinal epithelial atrophy with associated oligospermia was increased above control values in the high dose group (statistical analysis was not performed on this data) at 12 months and in the mid and high dose animals at 24 months; it should be noted that control values were relatively high, 36% at 12 months, 70% at 24 months. The incidence of sperm stasis was increased above control values (12 %) at the mid and high doses (23 % and 31 % respectively, statistical analysis not performed) at 24 months. There was an increase in the incidence of amorphous eosinophilic material in the tubular lumens, as also periarteritis nodosa incidence in all treated groups at 24 months. The report indicated that the testes were "suitable for evaluation" at 12 months, although no result was presented in the report for this time point. So, it is assumed that the testes were evaluated for these effects at 12 months but that no effects were observed.

In epididymides oligospermia was noted in one high dose animal as soon as after 12 months. There was none noted in any control animals. Epididymides from the low and mid dose animals were not evaluated, apart from one unscheduled mid dose animal. At 24 months, 27 % of the control group showed oligospermia, whereas 28 %, 54 % and 80 % displayed this symptom at the low, mid and high doses respectively. Degenerated seminal product was observed in both control and treated animals at 24 months (this was not examined in the low and mid doses at 12 months; it can only be presumed that it was examined at the high dose at 12 months, and did not occur), with a great increase only in the high-dose group (20 %, 22 %, 23 % and 50 % incidence in the control-, low, mid- and high- groups respectively).

In the seminal vesicles, secretory product was decreased in one high dose animal at 12 months (but not in any control animals and the effect was not examined in the low and mid doses at 12 months). At 24 months, 2 % of control animals displayed decreased secretory product compared with 84 %, 89 % and 52 % incidence in the low, mid and high dose animals respectively; it should be noted that not all animals in the low and mid dose groups were evaluated. At 24 months atrophy of the seminal vesicles was also observed in all treated animals at 24 months (30 %, 31 % and 23 % of the low, mid and high dose animals respectively), but not in any control animals; again not all animals in the low and mid dose groups were evaluated for this effect. Only the control and high dose 12 month animals were examined for atrophy of the seminal vesicles; no indication was given on an effect observed in the high dose animals.

As discussed in section 6.8.1, there was an increase in Leydig cell tumours of the testes in mid and high dose animals at both 12 and 24 months. Atrophy in seminiferous tubules is often observed adjacent to large tumours, especially Leydig cell tumours. Also, atrophy in seminal vesicles is commonly observed in association with testicular atrophy. It could be so interpreted as that the effects observed on the male reproductive organs are secondary to the Leydig cell tumours. However, the temporal sequence may introduce some uncertainty about this interpretation as it is likely testicular toxicity precedes observable neoplasia. Indeed, at 24 months some effects on the testes (periarteritis nodosa) and seminal vesicles (decreased secretory product and atrophy) were clearly observed from the low dose without any tumour incidence increase observed in Leydig cell; e.g. seminal decreased secretory product incidence was 85% (11/13) in the low dose group whereas only 2% (1/41) in the control group and at the same moment interstitial cell tumour incidence is 17% (8/48) versus 16% (7/43) in controls. At 24 months, 49% (23/47) Leydig-cell-tumours were observed in the middle dose whereas reduced secretory product was observed with incidence of 89%. The LOAEL of 5 mg/kg/day derived from the testicular toxicity (repeated dose toxicity part of the 2-year carcinogenicity study) is thus lower than the one for which occur the Leydig-cell-

tumours (20 mg/kg/day). Even effects noted in the male reproductive system are mainly observed in animals at 24 months and some of them are comparable to control, it cannot be exclusively attributed to natural ageing process of rats. It's to note that no observation was made for epididyme oligospermia and decreased seminal secretory products low and mid doses at 12 months. At the end it can be made all the same the hypothesis that early carcinogenic stages infer effects, via notably testosterone levels; decrease of seminal weights are notably an observation in favour of this hypotheses.

No evaluation of the female reproductive system was included in the 2-year carcinogenicity study with TDCP.

Based on negative results in the rabbit fertility study and because the observations made in the rat 2-year carcinogenicity study couldn't be linked to an early fertility function impairment, conclusion is that there is insufficient evidence for classification of TDCP as a male reproductive toxicant.

5.9.2 Developmental toxicity

Two developmental toxicity studies in rats are available for TDCP. Both raise the conclusion that no developmental toxicity occurs without maternal toxicity.

In the first study, TDCP (Fyrol FR-2, assumed purity of 100 % w/w) was administered daily to 20 mated Sprague Dawley female rats/dose group by oral gavage from days 6-15 of gestation at 0, 25, 100 and 400 mg/kg/day (Stauffer Chemical Company, 1978f). General observations were made daily, body weights measured on days 0, 6, 11, 15 and 19 of gestation. All surviving females were sacrificed on day 19 and the dams and foetuses examined grossly. Numbers of corpora lutea, implantations, resorptions, live foetuses and dead foetuses were noted. One third of the foetuses were examined by serial whole body sectioning using Wilson's technique. The remaining foetuses were eviscerated, fixed and examined for skeletal abnormalities using alizarin red staining.

There were three mortalities at 400 mg/kg/day which may have been caused by intubation errors, as findings at necropsy were not considered indicative of treatment-related effects. Clinical signs of toxicity were marked in most animals at the high dose and consisted of urine stains, hunched appearance, salivation, alopecia, rough coat, bloody crust around the nose, thinness and depression. Some clinical signs were also noted in the mid dose group and these may have been treatment-related (alopecia, hunched appearance, rough hair coat and urine stains). There was a significant body weight loss in mid and high dose animals from days 6-11 of treatment. These treated animals lost 15.6 g and 28.9 g, respectively, when compared to untreated animals who gained 22.1 g during this period. From days 11-15, mean weight gain of mid and low dose groups was not different from control, while mean weight gains were reduced in the 400 mg/kg/day group (50% of control). The overall mean weight gain from days 0-19 was significantly reduced ($p<0.05$) at 400 mg/kg/day (56% of controls). Mean food consumption was significantly reduced to 84.8% at 100 mg/kg/day (days 7-11) and at 400 mg/kg/day to an average of 45% throughout treatment. There were no specific findings at necropsy, which were indicative of a treatment-related effect. A NOAEL for systemic maternal effects of 100 mg/kg/day can be derived from this study.

Pregnancy rates were unaffected by treatment. The mean number of corpora lutea and implantation sites and the implantation efficiencies of the treated animals surviving to day 19 of gestation were similar to or exceeded control values. At 400 mg/kg/day, the rate of resorptions was statistically significantly increased when compared to controls (14.4 % compared to 6.7 %). The foetal viability index for this dose group was statistically significantly lower than control. No increase was seen at the low or mid doses.

There was a slightly lower mean foetal weight (2.21g) and crown-rump length (3.18 cm) for the 400 mg/kg/day litters when compared to controls (2.42g and 3.35 cm, respectively) although these did not reach statistical significance (Data for mean weight and crown-rump length from two of the 100 mg/kg/day litters were removed as they appeared to be of an older gestation age). The finding of increased incidence of dilated lateral ventricles of the brain was slight and within the historical control range. There was considerable evidence of retarded skeletal development in the high dose group; incomplete ossification of intraparietal and supraoccipital, nonossified hyoid and nonossified centres in the sternebrae, nonossified centre of the sacral and caudal portions of the vertebrae, nonossified arches of the sacral vertebrae and incomplete ossification of the pubis, and nonossified centres in the metacarpals and metatarsals. Such findings are consistent with the reduced foetal weight, length and viability at this dose level and indicate developmental retardation which may be related to the maternal toxicity seen at 400 mg/kg/day. The finding of increased incidence of foetuses with angulated ribs at 400 mg/kg/day may have been related to treatment but is of unknown biological significance (no historical control data for this effect was included in the report). A NOAEL of 100 mg/kg/day can be derived for developmental toxicity, based on the statistically significant increased resorptions and the decreased foetal viability index at 400 mg/kg/day.

In a second study, (Tanaka et al., 1981), in which only the abstract of the study is in English, groups of 15-24 female Wistar rats were dosed orally with 0, 25, 50, 100, 200 and 400 mg/kg/day TDCP in olive oil during days 7 through 15 of gestation. At the highest dose level, 11 out of 15 dams died and toxic symptoms included piloerection, salivation and haematuria. At this dose level, maternal body weight gain and food consumption were significantly reduced when compared to control values. Maternal kidney weight was significantly increased in the mid and high dose groups when compared to controls (absolute kidney weights were increased by 8.7 % and 35.5% in the mid and high dose groups and the relative weights were increased by 12.2 % and 65.3 %, respectively).

At 400 mg/kg/day, a significant increase in foetal death occurred. As indicated above, 11 out of the 15 dams dosed at this level died. One of the remaining dams had total dead implants. The remaining 3 dams had live foetuses. The number of live foetuses from this treatment group was 22 compared to a total of 194 in the control group (all other treatment groups were comparable to the controls). The number of dead foetuses in the high dose group was 26 compared to 6 in the control group. The number of dead foetuses in the other treatment groups was comparable to controls. There was no evidence of an adverse effect of TDCP on skeletal development of the foetuses at any dose level. In postnatal examination performed at dose levels of 200 mg/kg/day and below, there was no change in the performance of the offspring in functional tests such as open field, water maze, rota rod, inclined screen, pain reflex and preyer's reflex examinations. From this study, a NOAEL of 200 mg/kg/day can be derived for both maternal and developmental toxicity based on effects observed at 400 mg/kg/day.

5.9.3 Human data

No data available for this dossier.

5.9.4 Other relevant information

TC C&L discussion

The classification and labelling of TDCP was discussed at TC C&L Meeting[5], where it was provisionally agreed to classify TDCP as Carc. Cat 3 R40. At this meeting, it was also provisionally agreed to classify TDCP as Repr. Cat. 3; R62. During the follow-up period to this meeting, Ireland revised the classification proposal to no classification for fertility and it was agreed that this revised proposal would be discussed at the next meeting TC C&L Meeting. However, the due to other priorities on the agenda, TDCP was never discussed.

Comparison with other flame retardants

The effects on male fertility have been investigated for the two structurally related substances, TCPP (tris(2-chloro-1-methylethyl) phosphate) and TCEP (tris(2-chloroethyl) phosphate).

In a two-generation reproductive toxicity study with TCPP, no effects were observed on the male reproductive system (reported in HSA/EA, 2008b). For TCEP, an effect on male reproductive organ weight was noted in mice and effects on sperm parameters were observed in mice and rats (reported in BAUA, 2006). TCEP is classified as Repr. Cat. 2; R60.

The effects on female fertility have been investigated for both TCPP and TCEP. In a two-generation reproductive toxicity study with TCPP, an increase in oestrus cycle length and a decrease in uterus weight were observed in treated females (reported in HSA/EA, 2008b).

In a continuous breeding study in mice with TCEP an impairment of fertility, seen as a decrease in the number of litters produced, was observed. However, in a cross-over mating trial, pregnancy and fertility indices were lower in treated male / control females only, indicating male mice are more sensitive to TCEP treatment than female mice (reported in BAUA, 2006).

In a separate study investigating vaginal cytology in mice and rats following treatment with TCEP for 18 weeks, no effect on oestrus cyclicity was observed in mice. In rats, an increase in cycle length and variations in relative frequencies of oestrus stages were observed in the low and mid dose but not the high dose, and therefore the biological significance of the effect is questionable (reported in BAUA, 2006).

The fact that the effects on female fertility are not observed for both substances indicates that a read-across from female fertility data on either substance to TDCP should be used carefully and can be made best use only as a secondary argument

5.9.5 Summary and discussion of reproductive toxicity

No data from humans are available on the reproductive toxicity of TDCP.

Developmental toxicity:

In two developmental toxicity studies in rats, there was no evidence of embryotoxicity in the absence of maternal toxicity. Therefore, no classification for developmental toxicity is proposed.

Fertility toxicity:

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[5] Commission Working Group on the Classification and Labelling of Dangerous Substances Meeting on the Health Effects of Pesticides, Existing Chemicals & New Chemicals, November 14-18, 2005.

In a fertility study in male rabbits, no treatment related effects on mating, fertility or pregnancy parameters were observed. Sperm analysis was not affected and there were no histopathological changes detected in the male reproductive tract.

In a 2-year carcinogenicity study in rats, for the majority of observations, only control and high dose animals were evaluated at 12 months and no significant differences were noted at this time point. However, some effects were noted in the testes, epididymes and seminal vesicles in all animals, including control animals at 24 months but with a trend for higher incidence in the treated groups. The increase in Leydig cell tumours was only observed in the mid and high dose males at both 12 and 24 months. These non-neoplastic effects on the male reproductive organs may be secondary to not observable Leydig cell tumoral changes. However, as no clear-cut evidence of fertility toxicity was found after 12 months and the findings made at the age of 24 months were of unclear relevance (notably 70% control animals spontaneously developed testis atrophy) it can not be argued adverse effects on sexual function and fertility (CLP guidance 3.7.2.3.1).

Finally, considering the negative male rabbit fertility study on one side and the insufficient evidence from the rat carcinogenic 2-year study, TDCP cannot be classified as toxic for male fertility. In addition, fertility effects in females cannot be assessed as no data were available.

5.10 Other effects

Not relevant.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not relevant.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Environmental hazard assessment has not been evaluated as part of this dossier. Information on these endpoints can be found in the IUCLID file for TDCP. It should be outlined that it was concluded in HSA/EA 2008a: "Data presented in this report are consistent with the classification N R51-53 (toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment). This is based on the lowest acute E(L)C50 of 1.1 mg/l (fish) and the lack of ready biodegradability".

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

As, harmonised classification for carcinogens is considered a Community-wide action no additional justification is needed to propose to classify TDCP as Carc. Cat 3; R40[6] / Carc. 2 H351[7].

OTHER INFORMATION

TDCP was on the 4th Priority list adopted under Council Regulation (EEC) 793/93. A risk assessment report, addressing human health and the environment was prepared by the Rapporteur, Ireland, and agreed at Technical Committee for New and Existing Substances (TC NES). For further information please refer to the risk assessment report (HSA/EA, 2008).

[6] Directive 67/548/EEC

[7] Regulation (EC) No. 1272/2008

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