COMMITTEE FOR RISK ASSESSMENT

BACKGROUND DOCUMENT TO THE OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING OF

TRIXYLYL PHOSPHATE
EC number: 246-677-8
CAS number: 25155-23-1

Final

27 January 2010

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Trixylyl Phosphate

EC Number: 246-677-8

CAS number: 25155-23-1

Registration number (s): -

Purity: > 99% w/w

Impurities: -

Proposed classification based on Directive 67/548/EEC:

Repr. Cat.2; R60

Proposed classification based on Regulation EC 1272/2008:

Repr. 1B with hazard statement H360F[1]

Proposed labelling:

Directive 67/548/EEC: T; R60; S: 53-45

Regulation EC 1272/2008: GHS08, Danger, H360F

Proposed specific concentration limits (if any):

None

Proposed notes (if any):

None.

This proposal is based on the oral combined repeated dose and reproductive/developmental study submitted by the company Supresta Netherlands BV in accordance with the requirements of 67/548/EEC. This dossier reviewed the reprotoxicity endpoints only. Classification for carcinogenicity, mutagenicity or respiratory sensitisation was not considered. The classification is based on the properties of the substance itself.

[1] It is the view of RAC that hazard statement H360F is the most appropriate, given the available toxicological profile of trixylyl phosphate, but RAC recognised that H360 could be applied if the available criteria are applied strictly.

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	**Trixylyl Phosphate**
EC Name:	**Trixylyl Phosphate**
CAS Number:	**25155-23-1**
CAS Name	**phenol, dimethyl-, 1, 1', 1''-phosphate**
IUPAC Name:	**tri-x,y-xylyl orthophosphate, where x and y denotes o, m, p or mixture**

1.2 Composition of the substance

This substance is an UVCB containing over 50 different constituents and no additives. Trixylyl Phosphate is produced through the reaction of phosphorus oxytrochloride and xylenols. The xylenols are present in a distillation fraction of naturally occurring coal tar derivatives which also contains different ethyl phenyls. Reaction of the different xylenols and ethyl phenyls results in alkylated triphenyl phosphates with a high amount of possible isomers that cannot be easily analysed. Therefore, the exact composition is unknown. Some other constituents are provided in the confidential sections of the IUCLID file. However, the substance is tested as such and the individual constituents are not the basis for the classification.

A more precise chemical naming could be: Reaction product of phosphorous oxytrichloric acid and a mixture of xylenols containing > 95% tri (dimethylphenyl and ethylphenyl) phosphates

Chemical Name:	**Trixylyl Phosphate**
EC Number:	**246-677-8**
CAS Number:	**25155-23-1**
IUPAC Name:	**tri-x,y-xylyl orthophosphate, where x and y denotes o, m, p or mixture**
Molecular Formula:	**$C_{24}H_{27}O_4P$**
Structural Formula:	**not applicable as this constituent contains several constituents**
Molecular Weight:	**410.45**
Typical concentration (% w/w):	
Concentration range (% w/w):	**95-98%**

1.3 Physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	liquid	IUCLID (2001)
VII, 7.2	Melting/freezing point	3.2		
VII, 7.3	Boiling point	3.3	> 300 ° C	Supresta, 2008
VII, 7.4	Relative density	3.4 density	1.13-1.15 g/ml at 20° C	MSDS Chemtura
VII, 7.5	Vapour pressure	3.6	8.5x10-5 Pa at 20°C	Supresta, 2008
VII, 7.6	Surface tension	3.10		
VII, 7.7	Water solubility	3.8	18.6 ug/ll at 25°C	HPV, 2004
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	5.63 at 25° C	IUCLID (2001)
VII, 7.9	Flash point (open cup)	3.11	246.1° C	IUCLID (2001)
VII, 7.10	Flammability	3.13		
VII, 7.11	Explosive properties	3.14	not explosive	IUCLID (2001)
VII, 7.12	Self-ignition temperature		575° C	MSDS Chemtura
VII, 7.13	Oxidising properties	3.15		
VII, 7.14	Granulometry	3.5		
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17		
XI, 7.16	Dissociation constant	3.21		
XI, 7.17	Viscosity	3.22	108-143 cps at 25° C	MSDS Chemtura
	Auto flammability	3.12	565.6° C	IUCLID (2001)
	Reactivity towards container material	3.18		
	Thermal stability	3.19		

Table 1: Summary of physico- chemical properties

The provided information on physical-chemical properties is based on available summaries such as the MSDS and IUCLID. As this proposal is made for a substance that is not yet registered, we do not have access to robust study summaries and do not have knowledge on the exact composition of the tested substance. Therefore, the summary above should be seen as an indication of the properties. However, as no classification is considered for physico-chemical endpoints, this will not affect this proposal.

2 MANUFACTURE AND USES

Not relevant for this dossier.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

The substance is not currently classified in Annex I of Directive 67/548/EEC.

3.2 Self classification(s)

The origin of the observed pregnancy defect was found to be prior to implantation, therefore, the findings are considered by Supresta as an effect of fertility, not on development (in accordance with Directive 93/21/EEC). Since the underlying mechanism is not clear, the relevance for humans is not evident according to Supresta. Therefore, Supresta has proposed to classify Trixylyl Phosphate in category 3 for fertility (substances which cause concern for human fertility) and to label Trixylyl Phosphate with Xn; R62.

4 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this type of dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

The provided combined oral repeated dose and reproductive/developmental toxicity study was performed with Phosflex TXP with a purity of 99% Phosflex TXP according to the available analysis certificate. Phosflex TXP is one of the commercial names of the substance Trixylyl Phosphate put on the market by the notifier of this substance. Trixylyl Phosphate and therefore also Phosflex TXP is a reaction product of phosphorous oxytrichloric acid and a mixture of xylenols containing > 95% dimethylphenyl and ethylphenyl phosphates.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No data available.

5.2 Acute toxicity

Data not reviewed, not relevant for this dossier.

5.3 Irritation

Data not reviewed, not relevant for this dossier.

5.4 Corrosivity

Data not reviewed, not relevant for this dossier.

5.5 Sensitisation

Sensitisation has not been considered as part of this dossier.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

A combined oral repeated dose and reproductive/developmental toxicity study has been carried out with Sprague-Dawley rats (Experimur, 2004) using Trixylyl Phosphate (Phosflex TXP Lot # 02223D0200 T#127, purity 99%) administered in a vehicle of corn oil (Sigma Lot# 062K0006). The study was conducted under GLP and according to OECD guideline 422, using doses of 0, 25, 200 and 1,000 mg/kg bw/day, administered by oral gavage. Groups of eleven rats/sex were exposed at each treatment level, from 2 weeks prior to mating throughout gestation and lactation. Males were dosed for 33 days in total, females for 48 days. The control and high-dose groups included five additional animals/sex, which were used for recovery experiments (3 weeks for females and 4 weeks for males).

Data were analyzed for homogeneity of variance using Levene's test. When variances were homogeneous ($p>0.001$), the data were further analyzed by one-way analysis of variance

(ANOVA). When main effect differences were found, all post-hoc comparisons between treated groups and controls were conducted using Dunnett's test. Chi-Square analysis was used for the reproductive performance parameters. Motor activity was analyzed using repeated measures analysis of variance. Statistical significance was established at $p \leq 0.05$.

Statistical significant changes are summarized in Tables 5.1 (males), 5.2 (females) and 5.3 (recovery groups). No relevant effects were observed on food intake, body weight, clinical observations or functional performance and motor activity. Serum chemistry analysis showed a significant decrease in alkaline phosphatase at all dose levels in females and in the 200 and 1000 mg/kg bw/day treated males. In addition, blood urea nitrogen and calcium were significantly incresed in mid- and high-dosed males, and alanine aminotransferase was increased in mid- and high-dosed males and mid-dosed females. Lactate dehydrogenase was decreased only in high-dosed males, A/G ratios were decreased only in high-dosed females and cholesterol and phosphate levels were increased in females treated with the highest dose of Trixylyl Phosphate. In recovery males of the high dose group, only a small increase in calcium and phosphate levels was observed. At the highest dose level (1000 mg/kg bw/day) there was a significant decrease in hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin in females, however, it was stated that these hematological changes were still within historical ranges. Males of the mid- and high dose group had a decreased relative eosinophil reticulocyte count, whereas females of the highest dose group had an increase in absolute neutrophil reticulocytes. Plasma cholinesterase was reduced in males and females of the 200 and 1000 mg/kg bw/day core groups, but was normal in recovery animals.
At 200 and/or 1000 mg/kg bw/day, absolute weight of adrenals, ovaries and liver was increased, whereas weight of the testes, heart and epididymides was significantly decreased. In females, absolute and relative weight of the adrenals was also increased at the lowest dose level (25 mg/kg bw/day). All effects on organ weights were dose-related. In recovery animals, only the liver weight of females was still significantly increased.

Table 5.1: Summary of significant changes observed in **male rats**.

Parameter (Units)	**Control**	**Low Dose** 25 mg/kg	**Mid Dose** 200 mg/kg	**High Dose** 1000 mg/kg
Clinical chemistry				
ALK P (U/L)	135.4 ± 16.3	123.2 ± 20.4	93.2 ± 10.1*	105.0 ± 11.3 *
BUN (mg/dL)	16.4 ± 2.4	17.2 ± 1.5	20.0 ± 1.6*	21.6 ± 2.1*
Ca (mg/dL)	9.2 ± 0.2	9.2 ± 0.3	9.6 ± 0.1*	9.6 ± 0.2*
LDH (U/L)	1907.4 ± 204.4	1844.4 ± 634.7	1503.0 ± 433.0	1032.0 ± 114.5*
ALT (U/L)	24.2 ± 2.4	26.6 ± 3.2	43.4 ± 3.4*	47.4 ± 12.8*
Differential counts				
Eos (%)	2.04 ± 0.885	1.36 ± 0.522	0.84 ± 0.279*	1.10 ± 0.15 8*
Plasma Cholinesterase Activity				
Plasma ChE	194 ± 18	190 ± 25	149 ± 33*	141 ± 22*
Organ weights				
Adrenals (g)	0.062 ± 0.014	0.075 ± 0.010	0.109 ± 0.024*	0.120 ± 0.021*
Adrenals (%)	0.0178 ± 0.0042	0.0215 ± 0.0030	0.0315 ± 0.0065*	0.0366 ± 0.0072*
Testes (g)	3.58 ± 0.17	3.52 ± 0.26	3.42 ± 0.44	2.92 ± 0.31*
Testes (%)	1.0284 ± 0.0700	1.0166 ± 0.1034	0.9876 ± 0.1159	0.8857 ± 0.0718*
Heart (g)	1.221 ± 0.099	1.235 ± 0.092	1.194 ± 0.067	1.115 ± 0 .092*
Epididymides (g)	1.18 ± 0.09	1.18 ± 0.15	1.10 ± 0.15	1.00 ± 0.07*
Liver (g)	10.37 ± 1.01	10.65 ± 0.84	11.58 ± 1.24*	12.12 ± 1.3 6*
Liver (%)	2.9665 ± 0.1373	3.0578 ± 0.0989	3.3330 ± 0.1758*	3.6703 ± 0.2108*

Mean values ± SD. * Significantly different from controls; p≤0.05

Table 5.2: Summary of significant changes observed in **female rats**.

Parameter (Units)	**Vehicle** Control	**Low Dose** 25 mg/kg	**Mid Dose** 200 mg/kg	**High Dose** 1000 mg/kg
Clinical chemistry				
A/G ratio	1.22 ± 0.11	1.16 ± 0.05	1.14 ± 0.11	1.04 ± 0.05*
ALK P (U/L)	83.8 ± 21.5	61.0 ± 7.5*	54.8 ± 10.2*	49.2 ± 9.9*
CHOL (mg/dL)	66.8 ± 9.7	79.4 ± 11.8	84.4 ± 13.7	100.2 ± 11.8*
PO4 (mg/dL)	5.6 ± 0.7	6.1 ± 0.2	6.2 ± 0.5	6.9 ± 0.9*
ALT (U/L)	24.6 ± 2.2	23.4 ± 3.5	39.0 ± 11.9*	38.2 ± 12.5
Haematology				
HGB (g/dL)	15.8 ± 0.8	15.8 ± 0.3	15.7 ± 0.4	14.9 ± 0.6*
MCV (fL)	53.6 ± 0.8	54.0 ± 1.0	52.4 ± 2.6	49.0 ± 1.9*
MCH (pg)	19.1 ± 0.4	19.1 ± 0.8	18.6 ± 1.0	17.7 ± 0.8*
Plasma Cholinesterase Activity				
Plasma ChE	237 ± 32	213 ± 24	148 ± 14*	140 ± 17*
Organ weights				
Adrenals (g)	0.073 ± 0.013	0.100 ± 0.015*	0.142 ± 0.023*	0.156 ± 0.033*
Adrenals (%)	0.029 ± 0.004	0.038 ± 0.004*	0.056 ± 0.008*	0.062 ± 0.013*
Ovaries (g)	0.126 ± 0.021	0.149 ± 0.019	0.211 ± 0.037*	0.213 ± 0.028*
Ovaries (%)	0.049 ± 0.007	0.056 ± 0.006	0.083 ± 0.012*	0.085 ± 0.013 *
Heart (g)	0.93 ± 0.10	0.91 ± 0.09	0.85 ± 0.07	0.84 ± 0.07*
Liver (g)	8.41 ± 1.10	8.50 ± 1.15	8.35 ± 0.91	9.61 ± 0.86*
Liver (%)	3.28 ± 0.28	3.21 ± 0.34	3.30 ± 0.27	3.83 ± 0.35*
Brain (%)	0.718 ± 0.044	0.699 ± 0.044	0.749 ± 0.053	0.770 ± 0 .047 *

Mean values ± SD

* Significantly different from controls; p≤0.05

Table 5.3: Summary of significant changes observed in male and female **recovery rats**.

	Males		**Females**	
Parameter	**Vehicle**	**High Dose**	**Vehicle**	**High Dose**
(Units)	Control	1000 mg/kg	Control	1000 mg/kg
Clinical chemistry				
Ca (mg/dL)	9.3 ± 0.2	9.6 ± 0.1*		
PO4 (mg/dL)	6.3 ± 0.4	7.3 ± 0.4*		
Differential counts				
LUC (%)	0.32 ± 0.130	0.86 ± 0.422*		
Bas (x10^3 cells/µL)			0.02 ± 0.005	0.01 ± 0.000*
Organ weights				
Brain (%)	0.4694 ± 0.0192	0.4402 ± 0.0165*	0.710 ± 0.041	0.626 ± 0.030*
Liver (g)			7.11 ± 0.91	9.15 ± 1.12*
Liver (%)			2.61 ± 0.27	3.05 ± 0.20*

Mean values ± SD

* Significantly different from controls; p≤0.05

In both sexes, the weight changes of the reproductive organs were combined with histological changes (see Tables 5.4, 5.5 and 5.6). In males there was evidence at all dose levels of degeneration of the germinal epithelium of the testes, combined with sloughed epithelial cells in the lumen of the epididymides. Retention of step 19 spermatids was observed only at the highest dose level. In females, mild diffuse hyperplasia of the interstitial cells of the ovaries was observed at all dose levels. Furthermore, histological analysis revealed diffuse cytoplasmic vacuolation of the adrenals in males at all dose levels and females at 200 and 1000 mg/kg bw/day, and mild fatty degeneration of hepatocytes in mid- and high-dosed females. The incidence and severity of all histological changes was decreased in the recovery groups, indicating a reversible mechanism of effects. This study provides supporting evidence for a proposed classification based on effects on fertility.

Table 5.4: Summary of histological changes observed in female rats.

Parameter	Treatment Group			
	Vehicle Control	Low Dose 25 mg/kg	Mid Dose 200 mg/kg	High Dose 1000 mg/kg
Mild diffuse hyperplasia interstitial cells ovaries	0/5	5/5	5/5	5/5
Diffuse cytoplasmic vacuolation adrenals	0/5	0/5	1/5	2/5
Mild fatty degeneration hepatocytes	0/5	0/5	4/5	5/5

Table 5.5: Summary of histological changes observed in male rats.

Parameter	Treatment Group			
	Vehicle Control	Low Dose 25 mg/kg	Mid Dose 200 mg/kg	High Dose 1000 mg/kg
Degeneration germinal epithelium testes	0/5	2/5	2/5	5/5
Sloughed epithelial cells lumen epididymis	0/5	1/5	4/5	5/5
Diffuse cytoplasmic vacuolation adrenals	0/5	4/5	5/5	5/5

Table 5.6: Summary of histological changes observed in male and female recovery rats.

Parameter	Treatment Group			
	Males		Females	
	Vehicle Control	High Dose 1000 mg/kg	Vehicle Control	High Dose 1000 mg/kg
Degeneration germinal epithelium testes	0/5	4/5		
Sloughed epithelial cells lumen epididymis	0/5	1/5		
Mild diffuse hyperplasia interstitial cells ovaries			0/5	2/5
Diffuse cytoplasmic vacuolation adrenals	1/5	5/5	0/5	1/5
Mild fatty degeneration hepatocytes			0/5	1/5

5.6.2 Repeated dose toxicity: inhalation

No data available.

5.6.3 Repeated dose toxicity: dermal

No data available.

5.6.4 Other relevant information

No data available.

5.6.5 Summary and discussion of repeated dose toxicity:

No relevant effects were observed on food intake, body weight, clinical observations or functional performance and motor activity in the combined oral repeated dose and reproductive/developmental toxicity study in rats. Significant changes in mid- and high-dose groups of both sexes were observed in clinical chemistry, including changes in cholinesterase, alkaline phosphatase and alanine aminotransferase. Except for changes in calcium and phosphatase levels, all changes were completely reversed in the recovery animals. Weight analysis identified the adrenals, testes, heart, epididymides, liver and ovaries as target organs, starting at the low dose in females and at the mid dose in males. Treatment-related histological lesions were observed in all target organs and in all

dose groups, with exception of the heart. Both the effects on organ weight and the histopathological changes are at least partly reversible, considering the reduced incidence and severity of the effects in the recovery groups.
Since the histological effects in the adrenals are already present following administration of the lowest dose (25 mg/kg bw/day), a NOAEL cannot be derived based on this study.
This study provides supporting evidence for a proposed classification based on effects on fertility.

5.7 Mutagenicity

Mutagenicity has not been considered as part of this dossier, and the available data have not been reviewed.

5.8 Carcinogenicity

Carcinogenicity has not been considered as part of this dossier, and the data have not been reviewed.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

In the combined oral repeated dose and reproductive/developmental toxicity study with Sprague-Dawley rats (Experimur, 2004), rats were exposed by oral gavage to doses of 0, 25, 200 or 1,000 mg/kg bw/day of Trixylyl Phosphate from 2 weeks prior to mating throughout gestation and lactation (for more details on the study set-up and statistical analysis used, see section 5.6.1). Results are summarized in Table 5.7. There was no effect on mating. Gravidity and successful parturition was observed in all animals from the control and low dose group (25 mg/kg bw/day), but was reduced in animals from the mid dose group (200 mg/kg bw/day), where only 2/11 dams underwent parturition. In the high dose group (1000 mg/kg bw/day), none of the ten mated females underwent parturition. Analysis of the uterus revealed only 2 gravid animals in the high dose group, and no additional gravid animals in the mid dose group (besides the 2 that underwent parturition), indicating that the reduced pregnancy rate is mainly the result of decreased fertility and not post-implantation loss. Counting of the corpora lutea was not performed.

To determine the cause for the adverse effects on pregnancy observed in the core groups, additional animals from the control and high dose group were left to recover from the Trixylyl Phosphate exposure. Male recovery rats from the high dose group were used for cross over mating with naïve control females, and recovered rats from the high dose and control groups were used for within-group mating. Following both cross over mating and within-group mating, no effects were observed on pregnancy or parturition, suggesting that the effects on fertility are reversible (Table 5.8).

Table 5.7. Summary of Reproductive Performance (core group)

	Treatment Group			
Parameter	**Vehicle** Control	**Low Dose** 25 mg/kg	**Mid Dose** 200 mg/kg	**High Dose** 1000 mg/kg
number of breeding pairs	11	11	11	11
number of sperm +	11	11	11	10
percent mated	100	100	100	91
number selected for littering	11	11	11	10
number of successful parturition[a]	11 (100%)	11 (100%)	2 (18%)*	0 (0%)*
length of gestation in days (mean ± SD)	21.9 ± 0.70	22.0 ± 0.45	22.0 ± 0.00	NSP
number of dams with implants	11	11	2	2
average litter size (mean ± SD)	10.7 ± 3.5	11.4 ± 3.7	8.0 ± 0	NSP
total number of pups born	118	125	16	NSP
number of stillborn fetuses	3	5	0	NSP
number alive on day 0	115 (97%)	120 (96%)	16 (100%)	NSP
total number of males born alive	61	64	5	NSP
total number of females born alive	54	56	11	NSP
ratio of males to females	1.1:1	1.1:1	0.5:1	NSP
number alive on day 4	115 (97%)	115 (92%)	15 (94%)	NSP

[a] Successful Mating = (# Successful Parturition ÷ # Selected for Littering) x 100
NSP=No Successful Parturition
*Significantly different from controls; p≤0.05

Table 5.8. Summary of Reproductive Performance (recovery group)

	Treatment Group		
Parameter	**Crossover Naïve Control[a]**	**Vehicle Control**	**High Dose 1000 mg/kg**
number of breeding pairs	5	5	5
number of sperm +	5	3	5
percent mated	100	60	100
number selected for littering	5	3	5
number of successful parturition (b)	5 (100%)	3 (100%)	5 (100%)
length of gestation in days mean ± sd	22.8 ± 0.45	22.3 ± 1.53	23.0 ± 0.71
average litter size mean ± sd	14.0 ± 1.0	13.4 ± 2.1	12.2 ± 0.8
total number of pups born	68	42	62
number of stillborn fetuses	1	0	1
number alive on day 0	67 (99%)	42 (100%)	61 (98%)
total number of males born alive	31	20	28
total number of females born alive	36	22	33
ratio of males to females	0.9:1	0.9:1	0.9:1
number alive on day 4	67 (99%)	38 (90%)	61 (98%)

[a] = Untreated females mated with high-dose males
[b] = Successful Mating = (# Successful Parturition ÷ # Selected for Littering) x 100

In a 90-day inhalation study with tri-xylenyl phosphate (TXP) based hydraulic fluids in rats, increased cytoplasmatic vacuolization in the steroid-synthesizing adrenocortical and ovarian interstitial cells and increased testicular degeneration were observed (Wall et al, 1990 as summarised by Latendresse et al, 1994a). The substance used in this study could potentially be the same or comparable to Trixylyl Phosphate but the available data on the composition of the test substance are unsufficient to conclude this. Also details on the test methods and results are lacking. Therefore, the results of this study cannot be used either directly or by read-across for the evaluation of the properties of Trixylyl Phosphate.

5.9.2 Developmental toxicity

In the combined oral repeated dose and reproductive/developmental toxicity study with Sprague-Dawley rats (for more details on the study set-up and statistical analysis used, see section 5.6.1) (Experimur, 2004), no effects were found on litter size, survival or body weight of the offspring at

25 and 200 mg/kg bw/day. However, a reduced male:female ratio was observed in the highest dose group that resulted in successful parturition (1.1:1, 1.1:1 and 0.5:1 in the control, low and mid dose group). Nevertheless, this is based on only 2 litters in the 200 mg dose group. The reduction in the number of litters at 200 and 1000 mg/kg bw is not considered to be an effect on development because in most cases there were no implants. Two dams in the highest dose group had implants (directly visible in one dam and only after staining in the other dam) but did not parturate indicating post-implantation loss. Except for gross abnormalities, pups were not analyzed for *e.g.* malformations or skeletal retardations.

5.9.3 Human data

No data available.

5.9.4 Other relevant information

Read-across

Tri-substituted phosphates

Some other tri-substituted phosphates like tri(2-chloroethyl)phosphate have effects on fertility and are classified as Repr. Cat.2; R60. However, not all tri-substituted phosphates show effects on fertility. For example, organophosphate esters used as insecticides are also tri-substituted phosphates. These substances have been tested for fertility but only a small portion is classified for effects on fertility. This shows that not all tri-substituted phosphates affect the fertility and that the effect on fertility depends on specific substructures. Using read-across from the general group of tri-substituted phosphates to support the classification for Trixylyl Phosphate is therefore not justified.

Tri-aryl phosphates

Several repeated dose toxicity and reproductive studies with tri-aryl phosphates like tricresyl phosphate (TCP), butylated triphenyl phosphates (BTP) are published. However, just like Trixylyl Phosphate, these substances are UVCBs with sometimes limited descriptions of the identity and content of the constituents. Some studies were performed with TCP containing xylyl substituted species. This limits the possibilities for read-across.

In a feeding study with TCP on Swiss CD-1 mice (Chapin et al, 1988) using a continuous breeding protocol impaired fertility in both sexes of mice in the parental animals and affected sperm motility at even the lowest dose in F1 males was revealed. A study with TCP on F344 rats (Latendresse et al, 1994b) with daily oral administration for up to 135 days using a modified continuous breeding protocol resulted in impaired fertility in the male sex, increases in adrenal gland, liver and ovarian weights, decreases in testicular and epididymal weights and histopathological degeneration of the seminiferous tubules.

Light microscopic, morphometric, ultrastructural and histochemical studies (Latendresse et al, 1994a and Latendresse et al, 1993) for elucidation of the mode of action of TCP revealed hypertrophy and cholesteryl lipidosis - composed of cholesteryl esters (CE) - of adrenocortical and ovarian interstitial cells in treated F344 rats that were progressive with duration of exposure and correlated with organ weight increases. Further, the activity of neutral CE hydrolase, an enzyme that converts CE to cholesterol in the uptake and storage pathways, was inhibited (97% inhibition

compared to that of controls) in the TCP-treated animals. The activity of acyl coenzyme A:cholesterol acyltransferase, an enzyme that esterifies cholesterol to make CE, was also depressed (27 % compared to that of controls).

Affected target organs (adrenals, ovaries, testes) and effects (organ weight changes, histopathological changes) identified during the Combined Repeated Dose and Toxicity Reproduction/Developmental Toxicity Screening Test with Trixylyl Phosphate are very similar to those observed in rats after exposure to TCP (Table 5.9) and suggest a common mechanism and/or the same constituents that are responsible for the effects. The results with TCP indicate interference with steroidogenic tissues, and with cholesterol (the starting substance for the formation of steroidal hormones) storage as a part of the mechanism of action also for Trixylyl Phosphate.

Table 5.9 Comparison of Trixylyl Phosphate and tri-cresyl phosphate

Effect	Trixylyl Phosphate	Reference	Tri-cresyl phosphate	Reference
Adrenals	Weight increase Mild fatty degeneration	Experimur, 2004	Macroscopically enlarged Lipidosis of adrenocortical cells	Latendresse et al, 1994a
Ovaries	Weight increase Mild diffuse hyperplasia of the interstitial cells	Experimur, 2004	No effects on ovary weight Lipidosis of ovary interstitial cells	Latendresse et al, 1994a
Testis	Weight decrease Degeneration of the germinal epithelium Retention of step 19 spermatids	Experimur, 2004	Weight decrease Altered morphology of the seminiferous tubular epithelium (exfoliation, mild degeneration and retained basally located step 19 spermatids)	Latendresse et al, 1994a

As there is direct and clear evidence showing the adverse effects of treatment with Trixylyl Phosphate on fertility, the data on analogues of Trixylyl Phosphate are only considered as additional evidence. Therefore, no full literature search for all possible analogues of Trixylyl Phosphate was performed, not all available studies were summarised and no full summaries of some of the available data on analogues were provided. Further, justification of read-across for UVCBs is difficult because it is unclear which of the constituents caused which effect. Furthermore it cannot be excluded that the same constituents are present in both UVCBs and cause the similar effects by both UVCBs. Based on the results summarised above and the difficulties in comparing the structures of UVCB it can only be concluded that the studies with TCP shows that a substance with probably some comparable or same constituent results in similar effects. This indicates that the results found with Trixylyl Phosphate are not a chance finding. So, whereas the presented data on TCP can thus be used as additional evidence, on itself they would not have been sufficient to support read-across for classifying TXP.

5.9.5 Summary and discussion of reproductive toxicity

It should be noted that OECD guideline 422 only comprises a reproduction/developmental toxicity screening test, and thus not all aspects of fertility and development are covered in this study. In particular, it offers only limited means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Therefore, negative results in this study are not conclusive for the absence of effects on development. The post-implanatation loss in the two dams of the highest dose group is considered an indication for a possible developmental effect but not sufficient for classification because of the low number of gravid dams in this group.

Fertility was adversely affected by treatment with Trixylyl Phosphate at doses of 200 mg/kg/day and higher, as shown by the reduction in implantations and a decreased number of gravid dams and successful parturitions. The results from the recovery experiment show that the effects on fertility are reversible. In this study, it could not be determined whether the effects on fertility were male- or female-related, since both the cross-over mating and the within-group mating resulted in normal numbers of pregnancy and parturition. No clear evidence was found for effects on development of the fetuses, although this was not extensively analyzed in the study, due to the effects on fertility and the fact that the study was a screening test.
In males and females, there is evidence for affected reproductive organs. Dose-related weight changes were observed in testes and epididymides (significant at the high dose) as well as in ovaries (significant from the mid dose on), and histological changes were observed in these organs at all dose levels of the core groups (degeneration of the germinal epithelium of the testes, sloughed epithelial cells in the lumen of the epididymides and mild diffuse hyperplasia of the interstitial cells of the ovaries). The treatment-related histological changes in the reproductive organs are at least partly reversible, as are the changes in organ weight, as shown by the results in recovery animals.
A LOAEL for effects on fertility could be established at 25 mg/kg bw/day based on histological changes in reproductive organs observed at the lowest dose level. The combination of partly different effects on the reproductive organs in males and females and an effect on the adrenals (increased weight and diffuse cytoplasmic vacuolation) suggests an effect on the steroid production.
Overall, the results of the available combined study on repeated dose toxicity and reproduction/developmental toxicity show a clear reduction in fertility with supporting evidence on the site of action, namely a significant and dose-related weight changes in testes, epididymides and ovaries, accompanied by histological changes in these organs. These effects are considered as effects on fertility because of the strong reduction in implantations at the middle and high dose combined with the effects on the reproductive organs. The effects on fertility and the effects on the reproductive organs were seen at dose levels also inducing limited general toxicity. These effects were not considered as severe generalised toxicity or severe inanition because the effects were limited at all dose levels and because at the lowest dose level of 25 mg/kg bw/day which still showed degeneration of the germinal epithelium of the testes only diffuse cytoplasmatic vacuolation of the adrenals was observed. Further, even if the effects on the reproductive organs would be secondary to the effect on the adrenals this would probably be a direct effect and not a non-specific effect because both organs have a function in steroid production. Therefore, classification as **Repr. Cat. 2; R60** for effects on fertility is proposed**.** Studies with the structural analogue TCP result in comparable effects on the adrenals, testis and ovaries and also in a reduced fertility in rats and mice (second species). However, as there is clear evidence for effects on fertility in the available study with Trixylyl Phosphate justifying the proposed classification, a full investigation of the analogs and the possibilities for read-across was not considered necessary. However, the available information on the analogues supports the abovementioned classification.

Classification for reproductive toxicity as **Repr. 1B H360F** is proposed according to the criteria in Regulation (EC) 1272/2008 because the clear reduction in fertility and effects on the reproductive organs are not considered to be a secondary non-specific consequence of other toxic effects.

5.10 Other effects

Not relevant for this dossier.

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

Not relevant for this dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not relevant for this dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

It is proposed that the substance is classified as Repr. Cat. 2, R60. Harmonised classification and labelling for reprotoxicants is considered a Community-wide action under Article 115 and it is recommended that the classification proposal is considered for inclusion on Annex I of Directive 67/548/EEC and Annex VI of Regulation EC 1272/2008.

OTHER INFORMATION

This substance has not yet been registered under REACH. The producer/importer company has been contacted. According to the information of the producer/importer, a pre-registration of the substance will be submitted before 1 December 2008.

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Supresta (2008)
E-mail with comments to the draft Annex XV report