ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Substance name: Trixylyl Phosphate
CAS number: 25155-23-1
EC number: 246-677-8

General comments

Date	Submitted by Person/Organisation/MSCA	Comment	Response	Rapporteur's comments
2009/07/16	Hungary / National Institute of Chemical Safety	In view of the experimental data and the precautionary principle the proposed classification and labelling can be supported but in our opinion there is a need for further information and/or testing to confirm the proposed classification and classification as Repr. Cat. 3; R62 should be considered.	This screening study provided clear results showing absence of gravid dams at the highest dose and a strong reduction in gravid dams at the mid dose. A confirmation of these clear results in an additional test seems therefore not needed. Classification with Repr. Cat. 3; R62 was considered but not proposed because there was a clear effect on fertility supported by effects on the reproductive organs that were not considered secondary to the systemic toxicity. Therefore, classification with Repr. Cat. 2; R60 is proposed.	We agree that no further information is needed. Data from the screening test are sufficient for Reprotox Cat. 2 classification due to effects on fertility (absence/reduction of number of pregnant females in combination with histopatological changes in male reproductive organs). In our opinion further testing would be necessary to strengthen the data if a classification for developmental toxicity was proposed.
2009/07/27	Ireland / Health & Safety	(1) The Irish CA is in agreement with the proposal of The Netherlands to classify	(1) Thank you for your support.	We agree in the classification as Repr. Cat 2 R60 under DSD

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	Authority	trixylyl phosphate as Repr. Cat 2 R60 (Repr. 1B H360). (2) We note that in the Annex XV report, under the heading "Proposal for Harmonised Classification and Labelling", proposed precautionary statements in accordance with the CLP Regulation have been stated. The inclusion of the precautionary statements in the Annex XV report may not be required. Art 37(1) of the CLP Regulation states that "*the proposal shall follow the format set out in part 2 of Annex VI and contain the relevant information provided for in Part 1 of Annex VI*". Part 1 of Annex VI does not include reference to precautionary statements and we also note that current entries in Table 3.1 of Annex VI do not list precautionary statements. Precautionary statements are selected taking the intended use of the substance into consideration and therefore these may not require harmonisation as part of this proposal.	(2)We agree that precautionary statements are not required in an Annex XV report and will remove them.	(Repr 1B H360F under CLP Regulation). (2) Agree that P statements are not required. Precautionary statements are labelling elements that are not a part of the harmonized classification and labelling in the CLP and therefore they should not be proposed here.

Toxicity to reproduction

Date	Submitted by Person/Organisation/MSCA	Comment	Response	Rapporteur's comments
2009/07/10	Sweden / Swedish Chemicals Agency	(1) The OECD TG422 is a screening test and is therefore less sensitive to detect adverse effects because lower number of animals tested, shorted dosing duration 2 w compared to 10 w in a 2-generation study and also fewer effects examined e.g. no sperm parameters tested. The trixylyl phosphate would then be a potent reproductive toxicant since it has been revealed in this study. Therefore we agree with the proposed classification for fertility as Repr. Cat. 2; R60.	(1) Thank you for your support.	(1) Agree
		(2) The classification is strengthened by structure activity relationship with other tri-substituted phosphates like Tris(2-chloroethyl)phosphate (TECP), also classified for fertility as Repr. Cat. 2; R60.	(2) We agree that several tri-substituted phosphates 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	(2) We agree that although the data with other aryl phosphates may strengthen the classification, using read-across from the general group of tri-substituted phosphates to support the classification is not justified.

			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 is therefore not justified. A chapter on read-across will be included in chapter 5.9.4.	
		(3) The suggested mechanism that trixylyl phosphate interferes with the steroid production seems plausible. With this limited information of the mechanism, however, it must be regarded as essentially unknown and therefore relevant to humans.	(3) We agree that there is only limited information on the mechanism in our original proposal. However, some additional data on the mechanism of effects on fertility of possibly structurally related substances was provided by Germany which add to the evidence that an effect on steroid production could be part of the mechanism. The German data will be included in the background document. We agree that the effects should be regarded as relevant to humans.	(3) We agree that the information provided by Germany support about the possible mechanism of action of TXP, i.e. interfenece in steroid production, based on the comparison of the effects between TXP and TCP (an analogue to TXP). This mechanism cannot be disregarded as not-relevant for humans.
		(4) Developmental toxicity It is mentioned that the number of implantations were strongly reduced or	(4) The uterus of the dams was examined for visible implants and stained with ammonium sulphide for implantation sites. At the high dose	More data are needed to conclude on this particular end-point about possible developmental effect.

		there were no implants. Had not the implantation occurred or was the cause implantation losses? If there are post-implantation losses a classification for developmental toxicity could be argued as well.	2 animals showed implantation sites which did not result in parturition. At the mid dose, implantation sites were only observed in two animals with successful parturition. Post-implantation loss was therefore only observed in two dams at the high dose. We regard this as an indication for an effect on development but not sufficient to propose a classification for this endpoint. This will be included in the background document.	
2009/07/24	Frauke Schröder/ Germany / Baua	(1) Based on the availability of data on the test results of a study according to OECD testguideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) trixylyl phosphate has been identified to possess a toxic potential adverse to fertility, for the reason of which classification and labelling of trixylyl phosphate according to Directive 67/548/EEC as Repr. Cat 2; R60 and according to Regulation EC 1272/2008 as Repr. 1B with hazard statement H360 is proposed. Justification provided by the submitter of this proposal has been considered and is found to be plausible and appropriate. Thus, the German CA	(1) Thank you for your support. There seems to be a difference in opinion on the required hazard statement. We prefer to use H360 instead of H360F because here is no data (no developmental studies) showing the absence of an effect on development and even some indications of an effect on development (post implantation loss in two animals at the highest dose).	The comment is taken into account (H360F is proposed).

		supports the classification of the substance based on regulation (EC) No 1272/2008 in category 1B as a presumed human reproductive toxicant with the hazard statement H360F. (2) The existing data are from an animal study and already show histological changes in the reproductive organs of the male rats at the lowest concentration tested (25 mg/kg). The histological alterations occur in the absence of other toxic effects and are accompanied by a clearly reduced fertility in the next dose tested. Exposure of the animals to 200 mg/kg results in only 18 % pregnant female rats after successful mating (2/11 females underwent parturition). Therefore a clear impairment of fertility is given in the dose group of 200 mg/kg. Dosing the animals with 1000 mg/kg leads to complete infertility as none of the successfully mated females underwent parturition. As staining of the uterus reveals only two gravid animals in the highest dose group and no additional gravid animal in the mid dose group, the reduced pregnancy rate is not the result of post-implantation loss.	(2) Thank you for these additional studies on TXP and analogues of TXP. The study with TXP will be included in the summaries of the background document and the studies with the analogues in a separate chapter on read-across. Much more data on the effects of TCP on fertility are available for example in the evaluation of the IPCS (Environmental Health Criteria 110, 1990). A full search for analogues of Trixylyl Phosphate was not considered necessary given the availability of data with the substance. Further, read-across is difficult to justify between UVCBs.	(2) We agree to include the data for read across Since no analysis of whether TCP is enough closely related to TXP has been proposed it is difficult to judge whether the data on TCP may be used to characterise TXP. However, the observed adverse effects of TCP are very similar those caused by TXP. Since the mechanism of action of TCP is known (intereference in stereoid production) the similarity of adverse effects observed in both substances and their structural similarity make the comparison of the mode of actions reasonable (i.e. it is likely that TXP exhibit the same mode of action as TCP). However, for

		Because the histological changes in the male reproductive organs at the lowest dose tested and the obvious impairment of fertility in the mid dose group fulfil the assumption for classification of "…alterations to the female and male reproductive system and/or adverse effects on …, fertility, parturition, …" (3.7.1.3 CLP regulation) the substance has to be classified. The mentioned data from a combined repeated dose toxicity study with the reproduction / developmental toxicity screening test (OECD guideline 422) suggests the classification in category 1B. Further evidence for gonadal and resulting reproductive toxicity of trixylyl phosphate is provided from the findings of an additional 90 day inhalation study on rats (Wall et al., 1990) with trixylenyl phosphate based hydraulic fluids, which has been reported to induce testicular degeneration and cytoplasmatic vacuolization in adrenocortical and ovarian interstitial cells. The study of Wall et al., 1990 is reported in (1). Further support for the classification		classification purposes the available data on TXP are sufficient and data on TXP analogues may be used as supportive information.

		proposal is provided from test results and available data of structurally closely related triaryl phosphate compounds, e.g. of tricresyl phosphate (TCP) from reproductive toxicity testing in mice and rats. In a feeding study on Swiss CD-1 mice (2) 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 on F344 rats (3) 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 (1), (4) 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 are very similar to those observed in rats after exposure to tricresyl phosphate and suggest interference with steroidogenic tissues and with cholesterol storage as a mechanism of action also for trixylyl phosphate. (1) Pathologic Effects of Butylated Triphenyl Phosphate –base Hydraulic Fluid and Tricresyl Phosphate on the Adrenal Gland, Ovary, and Testis in the Fischer-344 Rat. Latendresse JR et al., (1994) Toxicologic Pathology Volume 22, Number 4, 341-352.		

		(2) Reproductive Toxicity of Tricresyl Phosphate in a Continuous Breeding Protocol in Swiss (CD-1) Mice. Chapin RE et al., (1988) Fundamental and Applied Toxicology 10, 344-354. (3) Reproductive toxicity of Butylated Triphenyl Phospahte and Tricresyl Phosphate Fluids in F344 Rats. Latendresse JR., et al (1994) Fundamental and Applied Toxicology 22, 392-399. (4) Pathogenesis of Cholesteryl Lipidosis of Adrenocortical and Ovarian Interstitial Cells in F344 Rats Caused by Tricresyl Phosphate and Butylated Triphenyl Phosphate. Latendresse JR., et al (1993) Toxicology and Applied Pharmacology 122, 281-289.		
		(3) Remarks on test descriptions: Page 12/Table 5.7 Summary of Reproductive Performance It would be appreciated, if the data from the recovery group cross-over and within-group mating could be included in the table. Page 13/14 During the text it is repeatedly stated, that	(3) The requested data were included in the background document but were not always available. Included Data on the number of dams with	(3) OK (OK) OK

		no implants or reductions in implantations had been observed during the study. It would be appreciated, if data on the numbers of implantations, resorptions and on corpora lutea (if available) could be included in Table 5.7.	implantations was included.	
		(4) Page 14 “Based on reproductive outcome, a NOAEL of 25 mg/kg bw/day could be established. However, since histological changes in reproductive organs were already observed at the lowest dose level (25 mg/kg bw/day), for effects on reproductive organs, only a LOAEL could be established (25 mg/kg bw/day).” Derivation of a NOAEL for fertility of 25 mg/kg bw/day (as also indicated as NOAEL in the IUCLID5) from the submitted study is very formal and not sustainable. Based on the effects on the gonads and taking into consideration the limitations of the submitted test format, which is an in vivo screening test, with in particular a short premating treatment period of two weeks only) we would prefer to set the dose level of 25 mg/kg bw/day as the LOAEL for fertility. This is	(4) We agree with the proposed LOAEL for fertility and adapted the text in the background document accordingly.	(4) We agree with LOAEL

		of relevance for the further human health risk assessment, also because any more testing will not be allowed in case of success of the submitted classification proposal.		

Other hazards and endpoints

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2009/07/24	Frauke Schröder / Germany / Baua	German CA comment on physicochemical characteristics: We do not have access to robust study summaries relating to physicochemical characteristics, either. However, from the structural formula it can be excluded that the substance is pyrophoric or evolves any flammable gases in contact with water or humid air. Furthermore it has no potential for an explosion hazard or oxidizing properties. It needs to be mentioned that the determination of the flash point was in an open cup.	We also do not expect the stated physicochemical properties. However, as this proposal focuses on the reproductive effects, we have not been actively collecting information on physical-chemical endpoints. The text on the determination of the flash point will be adapted.	OK