



## **Analysis of the most appropriate risk management option (RMOA)**

**Substance Name:** Triphenyl phosphate (TPP)

**EC Number:** 204-112-2

**CAS Number:** 115-86-6

**Authority:** France

**Date:** January 2018

### **Cover Note**

Triphenyl phosphate (TPP) is a flame retardant presented by industry as a potentially viable alternative to decabromodiphenyl ether (decaBDE) in a variety of polymers and applications.

The TPP is suspected to be an endocrine disruptor (ED) substance because several data on TPP and its hydroxylated metabolites are in favor of potential of ED effects.

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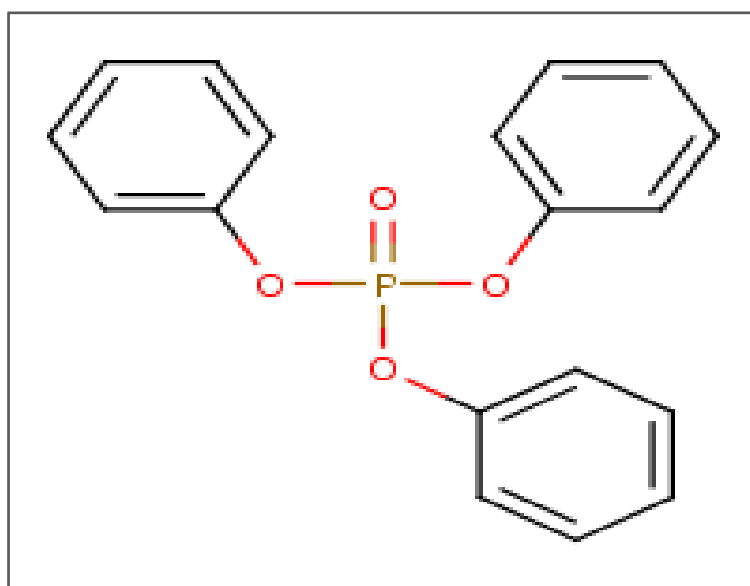
## 1 IDENTITY OF THE SUBSTANCE

**Table 1: Substance identity**

<b>EC name (public):</b>	Triphenyl phosphate (TPP)
<b>EC number:</b>	204-112-2
<b>CAS number:</b>	115-86-6
<b>CAS name</b>	Phosphoric acid, triphenyl ester
<b>IUPAC name (public):</b>	Triphenyl phosphate
<b>Index number in Annex VI of the CLP Regulation:</b>	None
<b>Physical state</b>	Solid at 20°C and 101.3 kPa; Form: pellets;
<b>Molecular formula:</b>	C <sub>18</sub> H <sub>15</sub> O <sub>4</sub> P
<b>Degree of purity:</b>	>= 99.8 % (w/w)
<b>Molecular weight or molecular weight range:</b>	326.28

**Type of substance**  Mono-constituent  Multi-constituent  UVCB

### Structural formula



## 2 OVERVIEW OF OTHER PROCESSES ON THE SUBSTANCE ITSELF/ EU LEGISLATION

**Table 2: Completed or ongoing processes**

RMOA	<input type="checkbox"/> Risk Management Option Analysis (RMOA) other than this RMOA	
REACH Processes	Evaluation	<input type="checkbox"/> Compliance check, Final decision
		<input type="checkbox"/> Testing proposal
		x CoRAP and Substance Evaluation TPP is on CoRAP list by UK in particular for potential endocrine disrupting properties concern.
	Authorisation	<input type="checkbox"/> Candidate List
		<input type="checkbox"/> Annex XIV
	Restri- -ction	<input type="checkbox"/> Annex XVII <sup>1</sup>
Harmonised C&L	<input type="checkbox"/> Annex VI (CLP) (see section 3.1)	
Processes under other EU legislation	<input type="checkbox"/> Plant Protection Products Regulation Regulation (EC) No 1107/2009	
	<input type="checkbox"/> Biocidal Product Regulation Regulation (EU) 528/2012 and amendments	
Previous legislation	<input type="checkbox"/> Dangerous substances Directive Directive 67/548/EEC (NONS)	
	<input type="checkbox"/> Existing Substances Regulation Regulation 793/93/EEC (RAR/RRS)	
(UNEP) Stockholm convention (POPs Protocol)	<input type="checkbox"/> Assessment	
	<input type="checkbox"/> In relevant Annex	

<sup>1</sup> Please specify the relevant entry.

Other processes/ EU legislation	<input type="checkbox"/> Other (provide further details below)
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### 3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

#### 3.1 Classification

##### 3.1.1 Harmonised Classification in Annex VI of the CLP

**Table 3: Harmonised classification**

Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
No current entry							

##### 3.1.2 Self classification

The following hazard classes are in addition notified among the aggregated self classifications in the C&L Inventory:

**Table 4: Self classification**

Hazard class and category code(s)	Hazard statement code(s)	Number of notifiers
Not classified	/	8
Aquatic Acute 1	H400	1036
Aquatic chronic 1	H410	1147
Aquatic chronic 2	H411	100
Aquatic chronic 4	H413	18
Eye Irrit. 2	H319	3

##### 3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

There is no current proposal for harmonised classification in Annex VI of the CLP.

### 3.1.4 CLP Notification Status

**Table 5: CLP Notifications**

	<b>CLP Notifications<sup>2</sup></b>
Number of aggregated notifications	23
Total number of notifiers	>1000

## 3.2 Additional hazard information

### 3.2.1 Health data

The data on TPP are limited, particularly for *in vivo* studies. With the exception of two experimental animal studies carried out in 2015 and 2017, the few available studies are old. However, several *in vitro* tests have been published recently.

#### 3.2.1.1 Human data

TPP concentrations in breast milk were analysed in a study on a human cohort conducted in Sweden between 1997 and 2007. Median concentration across all subjects was 8.5 ng/g of lipids (minimum and maximum values: 3.2 and 11 ng/g, respectively) (Sundkvist, Olofsson, and Haglund 2010).

Meeker and Stapleton, (2010), reported a relationship between decreased sperm counts and altered levels of thyroxine and prolactin on the one hand, and the high level of 2 organophosphate flame retardants (OPFRs) (Tris(1,3-dichloroisopropyl)phosphate (TDCPP) and TPP) in the dust of the homes of the men concerned, on the other hand (Meeker and Stapleton 2010). The authors analysed TDCPP and TPP in the dust of the houses of 50 men recruited through a U.S. infertility clinic, and assessed the relationships between the levels of these two OPFRs and reproductive and thyroid hormone levels, as well as semen quality parameters. TDCPP and TPP were detected in 96% and 98% of samples, respectively, with widely varying concentrations ranging from 0.17 and 1800 mg/g (mean value = 7.4 mg/g) for the TPP. In models adjusted for age and body mass index, each interquartile range of TPP increase in house dust samples was associated with a 19% (95% Confidence Interval (CI), -30% to -5%) decrease in sperm concentration and a 10% (95%, 2-19%) increase in prolactin levels. No causal formal link between concentration of TPP in dust and effects on fertility or thyroid function can be found in this transversal study.

Preston et al., 2010, studied the temporal variability in urinary concentrations of the TPP metabolite, diphenyl phosphate (DHP), and the relationship between DHP concentration and plasma concentration of thyroid hormones (Preston et al. 2017). Study subjects (26 male and 26 female office workers) were over the age of 18, non-smokers, and self-described as healthy. Participants were excluded if they had a current or prior diagnosis of thyroid disease, male reproductive disease, or were pregnant. Serum and urine samples were collected from adults during three sampling rounds every six months, from January 2010 to May 2011, representing winter 2010, summer 2010 and winter 2011. The authors found no significant association between DHP and free thyroxine (fT4), total triiodothyroxine (TT3), or thyroid stimulating hormone (TSH), but they found a significant positive association between exposure to DHP and total thyroxine (TT4) levels, especially in women.

<sup>2</sup> C&L Inventory database, <http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database> (accessed march 2017)

### 3.2.1.2 Toxicokinetics

Two studies are available:

One study showed that, after incubation of TPP with rat liver microsomes, the diphenyl phosphate (DPHP) is the major metabolite (Sasaki et al. 1984).

A more recent study explored the formation of TPP metabolites in primary human hepatocytes (Van den Eede et al. 2016). This study showed that DPHP and mono and di-hydroxylated TPP were the major metabolites. Quantification of biotransformation products, in hepatocytes exposed for 2 h to TPP, revealed that DPHP concentration corresponds to less than half of the depletion of TPP. According to the authors, there is a low percentage of TPP depletion which indicates that hepatic clearance would be rather slow, and in favor of high levels and persistence of TPP in the blood circulation.

### 3.2.1.3 Acute toxicity

The oral and dermal LD 50 values reported are in favor of low acute toxicity (US EPA, 2014; OECD SIDS, 2002):

- Oral route, rat and mouse: LD50 > 5000 mg/kg;
- Dermal route, rabbit: LD50 > 7900 mg/kg.

There is no available study regarding the inhalation of TPP.

### 3.2.1.4 Irritation and sensitisation

Skin irritation tests (occlusive or semi-occlusive) on rabbits reported no or moderate effects after 4, 24, and 72 h of exposure, and an experimental study in guinea pigs is in favour of no skin sensitisation (US EPA, 2014; OECD SIDS, 2002). No data on respiratory sensitisation are available.

### 3.2.1.5 Genotoxicity and mutagenicity

Although the Danish (Q)SAR database predicted that TPP may express some genotoxicity potential *in vitro* (micronucleus test in mouse erythrocytes) and *in vivo* (dominant lethal mutations in rodents and comet assay in mouse) while comparing with trisphenylphosphite, several experimental mutagenicity and genotoxicity tests show that TPP is not mutagenic on *in vitro* bacterial cells or mammalian cells and did not elicit DNA damage in Hamster fibroblasts cells (OECD SIDS, 2002, ATSDR, 2012, ECHA, 2012). As the 3 *in vitro* tests are negative, there is no need for further *in vivo* data.

### 3.2.1.6 Immunotoxicity

A 120-day feeding study was carried out on rats (5 groups of 10 males and 10 females) (Hinton et al. 1987). The animals were fed diets containing 0, 0.25, 0.5, 0.75 and 1% of TPP, corresponding to 0, 161, 345, 517, and 711 mg/kg bw/day of TPP in the individuals. They were observed for clinical symptoms and body weights (unspecified frequency), and food consumption was recorded weekly. Blood samples were analysed for total and plasma proteins. Immunotoxicity was assessed by measurements of the weights of lymphoid organs, immuno-histochemical evaluation of spleen, thymus, lymph nodes, and the humoral response to the T-lymphocyte-dependent antigen sheep red blood cell (SRBC). A reduced growth rate of animals was detected only at the high dose level. The weights of lymphoid organs varied in a non-dose-dependent way. The other results showed no significant effect on the weight and histopathology of the organs and lymph nodes, or on humoral

response. In addition, it was noted that there was no difference in behaviour between treated animals and controls.

An application (up to 1000 mg/kg/day) of TPP on the intact or abraded skin of rabbits, 5 times a day for 3 weeks, showed no gross or microscopic effects on the spleen, thymus or lymph nodes. (ATSDR, 2012)

### **3.2.1.7 Neurotoxicity**

Neurotoxicity is regarded as a potential adverse effect of many organophosphates. Therefore triphenylphosphate was tested for neurotoxicity *in vivo* and *in vitro*. It is recognized that the rat is a poor model for delayed effects compared to the hen.

The oldest results regarding triphenylphosphate were those being reported by Smith *et al.* (1932) who treated 4 hens orally with doses of 500 to 2000 mg/kg bw without any effects. These results have since been confirmed by several other tests with doses varying from 500 to 10000 mg/kg bw followed by observations from a few days to three weeks. There was no signs of paralysis, no histopathological changes in examined nervous tissues or behavior immediately after or during observation periods. The activity of plasma acetylcholinesterase, which was determined in a number of these studies, was decreased to reach up to 87% of the control activity (Study summarized in OECD SIDS, 2002). The major weakness of many of these studies is that there are no reports of the purity of the tested samples.

Studies on other species have been reported. Two of them, which have been reported with details, are summarized below.

In a 4 months study in rats, the authors determined the influence of dietary treatment with triphenyl phosphate at levels of 161, 345, 517, and 711 mg/kg bw, on the nervous system of male rats (10 per group). In addition to standard clinical observations, the neurotoxicity was assessed in open field, accelerating rotarod, forelimb grip strength and negative geotaxis examinations. These parameters were determined 4 times at the end of each month of treatment. Additionally body weights and food consumption were recorded weekly. No adverse effects were noted in any of the neurotoxicity parameters. Body weights were dose dependently reduced at 345 and 711 mg/kg bw triphenyl phosphate (Sobotka *et al.* 1986).

In a 15-day repeated dose dermal study, rabbits (10/sex/group) were treated on clipped, intact (half of the animals) and abraded skin (half of the animals), five times per week for three weeks with doses of 0, 100 and 1000 mg/kg bw/day under open conditions. No mortality, clinical symptoms, or changes in body weight, hematology, clinical chemistry, necropsy, organ weights and histopathology reported. Yet, decreased cholinesterase levels in plasma, erythrocytes and brain were reported (Monsanto, 1979).

In conclusion, a decrease of cholinesterase activity has been reported, but no other neurotoxicity effect has been recorded in these (old) studies. However, the relevance of the available data to assess the delayed neuropathy of TPP is questioned due to the few endpoints assessed and the too short duration of assays in neurotoxicity studies available.

### **3.2.1.8 Repeated toxicity studies**

There are few studies on the repeated toxicity of TPP. They are summarized below.

In an old study summarized in OECD SIDS (2002), rats (3 groups of 5 male animals per dose) were treated by dietary administration of TPP for 35 days. Doses were 0,



0.5 and 5% (estimated doses: ~ 350 - 3500 mg/kg bw/day) in the diet at the beginning of the study. The animals exposed to the high dose refused food and lost weight. Therefore the dose was reduced to 0.1% after three days. Parameters recorded were clinical observations, body weight (3 times/week), food consumption, and hematology (hemoglobin content, cell volume, red cell count, total and differential white cell count). At the end of the treatment period, 2/5 rats were kept for a further 14-day recovery period. All animals were killed and subjected to gross necropsy. Organ weights (kidneys and livers) were recorded (no further examinations - clinical chemistry, histopathology, urinalysis - reported). Treatment caused a slight depression of body weight gain and an increase of liver weight at a level of 0.5% (estimated dose: ~ 350 mg/kg bw/day) in the diet. No findings were recorded in clinical observation, hemoglobin content, cell volume, red cell count, total and differential white cell count and at necropsy. At the concentration of 0.1% in the diet (estimated dose: ~ 70 mg/kg bw/day), no significant effect could be observed (= NOEL). Although the initial dosage was too high, we can question if the dosing chosen on the third day was not too low compared to those of more recent studies (Sutton et al., 1960).

In the 15-day dermal study mentioned in 3.1.2.7, rabbits exposed to TPP (at 100 or 1000 mg/kg-day), no effects were reported, except the decrease of plasma cholinesterase level (OECD SIDS, 2002).

In a recent unpublished study according to OECD 408 (Van Otterdijk FM, 2015, summarized in the CSR report), Wistar rats (10/sex/dose) were treated during 90 days with TPP for 90 consecutive days by dietary administration at dose levels of 0, 300, 1500 and 7500 ppm. The mean estimated dose over the study period was 0, 20, 105, and 583 mg/kg bw/day for males and 0, 22, 117, and 632 mg/kg bw/d for females. According to the authors (no other precision given):

- No treatment-related mortality occurred, and no toxicologically relevant clinical signs were noted;
- The magnitude of liver weight was increased at 7500 ppm (approximately 30 and 21% decrease for males and females, respectively).
- Histopathological findings in the liver consisted of centrilobular hepatocellular hypertrophy of the liver in males at 1500 and 7500 ppm and in females at 7500 ppm, accompanied by enlargement and red brown discolouration of the liver and higher liver weight at necropsy at 7500 ppm;
- Changes in clinical biochemistry parameters consisted of higher total proteins and calcium levels in males at 7500 ppm, and higher cholesterol concentration in males and females at 7500 ppm, and in males also at 1500 ppm.

Based on the liver effects, particularly centrilobular hypertrophy observed at 1500 ppm in line with the increase in liver weight at 7500 ppm, a no observed adverse effect level (NOAEL) of 20 and 22 mg/kg (for males and females respectively) was established. In the particular case of this dossier, the reporters considered that the centrilobular hypertrophy identified, as the first effect impacting the liver, is significant, especially when considering the other effects observed on rodents and in fish.

### **3.2.1.9 Carcinogenicity study**

Only one study was found with a design that does not correspond to long-term carcinogenic study. This is a mouse lung adenoma test on male strain A/St mice (sensitive strain) (Theiss et al. 1977). The mice (20 per group) received intraperitoneal injections (3 times per week, during 3 weeks) of 20, 40 or 80 mg/kg of TPP (purity: 95-99.9 %). Adenomas were seen only in the 80 mg/kg group with

no significant increase of incidence compared to negative controls. Positive control (urethane) induced tumors in mouse with 100% survival, attesting sensitivity of the biological model.

No study available for long-term carcinogenicity.

### **3.2.1.10 Reproduction study**

Fertility and developmental toxicity were examined in a dietary study in Sprague-Dawley rats at doses of 0, 166, 341, 516 or 690 mg/kg bw/day (Welsh et al. 1987). Forty males and 40 females per group were treated for 3 months. Upon completion of the subchronic phase of the experiment, animals receiving identical diets were cohabitated in a 1:1 sex-ratio in the afternoon. The following morning, females were examined for the presence of sperm. The day of finding sperm was designated as day 0 of gestation. The animals continued to receive the test diets throughout mating and gestation. On day 20 of gestation, dams were examined externally and then sacrificed by carbon dioxide asphyxiation.

Body weights were measured and food cups were weighed on days 7 and 14 and before cesarean sections on day 20 of gestation. Daily observations were made on the dams and any changes in the general appearance, health or behavior of the animals were noted. A laparotomy was performed on each followed by an examination of the major organs. Ovaries were removed and examined for numbers of corpora lutea. Uterine blood vessels were clamped off and the entire gravid uterus was excised and weighed. The number and the position of fetuses (viable or dead) and resorption sites (early or late) were recorded. Fetuses were examined individually for gross abnormalities. For each fetus, uterine position, sex, weight and crown-rump were recorded. Runts were defined as any fetus weighing less than 70% of the average weight of the male or female controls.

No significant signs of parental toxicity were detected. There were no effects on pregnancy rate, number of viable fetuses and implants, corpora lutea, implants, implantation efficiency, number of early and late deaths, or average percent resorbed. There were no significant differences between treated groups and controls in the incidence of specific sternebral variations or in the average number of sternebral variations per litter.

It should be noted that male and female pups from all treated groups tended to weigh more than their respective controls. However, the difference was significant only for males in the 341 and 690 mg/kg bw/day groups. Furthermore, all treated groups had significantly more fetuses exhibiting moderate hydroureter and enlarged ureters (in the region adjacent to the kidney) than the control group, but the incidence of these variations seems not related to dose since a greater proportion of fetuses were affected in the two lowest dose levels than in the two highest levels. The authors explained this by the fact that the reference incidence in the controls was also high and there was no clear dose response. The significance of these effects remains unclear. Incidence of specific soft-tissue variations in foetuses are summarized in the following table.

**Incidence of Specific Soft-Tissue Variations in Fetuses**

Variation	Dose Level (%)				
	0	0.25	0.50	0.75	1.00
	No. Examined <sup>a,b</sup>				
	187(28)	243(38)	216(34)	206(34)	212(34)
Hydroureter, Severe	5(2)	1(1)	4(3)	4(3)	2(2)
Hydroureter, Moderate	13(12)	31(20)*	29(20)*	34(22)**	27(15)*
Enlarged Ureter (proximal to kidney), Severe	1(1)	2(2)	1(1)	3(2)	
Enlarged Ureter (proximal to kidney), Moderate	3(3)	13(11)*	20(13)***	11(9)*	11(6)*
Ectopic Kidney	2(2)	1(1)	1(1)	4(4)	
Hemorrhage	6(6)	8(6)	10(9)	6(6)	6(6)
Abnormal Inferior Vena Cava		1(1)			1(1)
Bronchiectasis		1(1)		1(1)	
Microrchidia			1(1)		
Cryptorchid Testes			1(1)		
Dilated Anterior Vena Cava			1(1)		

<sup>a</sup>No. of litters in parentheses.

<sup>b</sup>Asterisks indicate probability levels associated with differences between control and test value (\* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ).

(According to Welsh et al., 1987)

**3.2.1.11 Metabolic studies on parents and their offspring**

Patisaul et al. (2013) published the results of an exploratory study to evaluate accumulation, metabolism, and endocrine disrupting effects of a commercial mixture of flame retardants (named "FM550") in rats exposed across gestation and lactation. This mixture is made of 4 RFs: 2-ethyl-2,3,4,5 tetrabromobenzoate (TBB), bis (2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) (sum total of TBB and TBPH is approximately 50% of the total flame retardants), isopropylated triphenylphosphate (IPTPP), and triphenylphosphate (TPP). Pregnant Wistar rats were administered 0, 0.1 or 1 mg/kg/day in the diet during gestation and through lactation (GD8 - PND 21). The main effects observed were:

- Maternal toxicity: Increased serum total thyroxine (TT4) levels in the high dose dams compared to controls was reported (+65%,  $p \leq 0.05$ ). There was no significant change in triiodothyronine (T3) levels in dam serum. Decreased hepatic carboxylesterase activity was also reported in dams in the high dose group. It should be noted that the measurement of TSH concentration was not performed.
- Developmental toxicity: female offspring in the high dose group displayed a significantly earlier vaginal opening when compared to controls. A statistically significant increase in weight was reported in both males and females in the high dose group at PND 120. This effect persisted through PND 180 to PND 220 with high dose males and females having significantly higher weights than same sex controls. Left ventricular (LV) free wall thickness was significantly increased in male offspring in the high dose group; there were no changes in LV thickness in females at any dose.

This study does not allow to attribute these effects to one or more components of the "FM550" mixture.

Green et al. (2017) examined the metabolic impact of a perinatal exposure to TPP on type 2 diabetes onset and adipose accumulation in UCD-type 2 diabetes mellitus rats. This rat model is presented as closely mimicking the pathophysiology and progression of type 2 diabetes mellitus (T2DM) in humans. To that purpose, the authors administered 170 µg/animal/day of TPP (or ethanol in the control groups) to pregnant dams (n=8 per group) in maternal food from gestational day (GD) 8.5 to weaning (postnatal day 21). Two protocols were developed in two different studies (A and B). In study A, the authors examined whether TPP was a developmental obesogen in both sexes. In study B, the aim was to determine, using weight-matched males, if TPP accelerated the onset of T2DM (Green et al. 2017).

The authors first evaluated that perinatal exposure to TPP was not overtly toxic with respect to the length of gestation, litter size, sex-ratio, or the body weight of the dams or pups at weaning. For both studies, body weight and non-fasting glucose were recorded weekly. Energy expenditure was evaluated through measurement of food intake (weekly) and core body temperature (biweekly). T2DM was diagnosed from two consecutive weekly non-fasting glycaemia of  $\geq 200$ mg/dl according to ADA (American Diabetes Association) diagnostic guidelines.

Study A: The authors demonstrated that perinatally-exposed females exhibited enhanced body weight starting at week 9 and from week 12 until sacrifice by 3.5 months. Cumulative energy intake was also significantly enhanced. Similar effects were described in males although not significant. Thus, elevated body weight was at least partially explained by increased caloric intake in females. In addition, there was increased body adiposity as revealed by the weight of multiple fat pads in both sexes particularly the inguinal and mesenteric fat pads, and males accumulated more fat in these areas than females. It could involve a Pparg-mediated mechanism because there was a strong and significant enhancement of Pparg mRNA levels in males' fat pads. In females, the elevation did not reach significance. Despite enhanced body weight/fat pads, it was found that perinatal exposure to TPP did not modify glucose and lipid homeostasis. This was assessed through handling metabolic tests (glucose tolerance and insulin sensitivity tests) and measuring fasting plasma levels of triglyceride, cholesterol and free fatty acids. The authors further demonstrated significant increases in fasting leptin, the hormone of satiety, in both sexes consistent with the increased fat pads. However, no effect was recorded on thyroid hormones or adiponectin whose levels are inversely correlated with fat pads. The authors further demonstrated that the increase in adiposity was not linked to changes in various hypothalamic lipid mediators (endocannabinoids, oxylipins...). In conclusion, it is stated that TPP could act as a developmental obesogen and an increased appetite is the most likely cause for enhanced body weight specifically in females. Enhanced adiposity correlates with enhanced leptin levels coherent with an obesity-associated leptin resistance, which is more evident in males than in females.

Study B: This part of the paper is only dealing with male as female UCD-type 2 diabetes mellitus rats are protected from diabete by estrogens. In these rats, the incidence of T2DM is about 43% in females while it reaches 92% in males. Importantly, as obesity is a known risk factor for diabetes and to avoid weight as a confounding factor, the authors selected males perinatally exposed to TPP or ethanol (controls) weighing between 350 and 400 g at 8 weeks of age because they start developing T2DM at approximately 23 weeks of age. In these conditions, the authors demonstrated increase in the incidence of T2DM. For example, by 26 weeks, 79% of male rats perinatally exposed to TPP had developed T2DM while only 33% of the vehicle treated developed T2DM. These effects were independent of adiposity (no changes in fat pads) and energy balance (no change in food intake or body temperature). Neither plasma levels of insulin, thyroid hormones, leptin

and adiponectin nor plasma levels of triglycerides or of cholesterol were modified by exposure to TPP.

However it was observed enhanced plasma levels of free fatty acids, indicative of enhanced lipolysis which is possibly related to the described acceleration of the onset of T2DM. Indeed, enhanced lipolysis results in lipotoxicity and it is a marker of insulin resistance. The authors also present contradictory data regarding the HOMA IR index not consistent with the plasma levels of insulin and measures. This leads to conclude that this study fails to demonstrate a diabetogene effect of TPP.

In conclusion, the study highlights that perinatally exposure to TPP triggers metabolic disturbances characterized by enhanced weight gain and enhanced adiposity to be connected with increased plasma levels of leptin and possibly with leptin resistance explaining the higher food intake. As such, TPP may be considered as a developmental obesogen as stated by the authors. In addition, it is suggested that TPP may accelerate the onset of T2DM but this assumption requires more data.

### **3.2.1.12 Endocrine disruptor studies**

Cano-Sancho et al., 2017, assessed the effects of TPP and its metabolite diphenyl phosphate (DHP) on the adipogenic differentiation of 3T3-L1 cells, glucose uptake and lipolysis in differentiated 3T3-L1 adipocytes *in vitro*. According to the authors, the whole data set provide evidence that both TPP and its metabolite DHP act as endocrine disruptors on the regulation of adipogenic differentiation and lipolysis by impairing noradrenergic mechanisms and, in the case of TPP, also by mimicking the insulin signalling pathway and stimulating glucose uptake (Cano-Sancho, Smith, and La Merrill 2017).

In the study of Kojima et al., 2013, using cell-based reporter gene assays with CHO-K1 and COS-7 cells, the authors characterized the agonistic and/or antagonistic activities of 11 OPFRs (included TPP) against human nuclear receptors: estrogen receptor (ER $\alpha$  and ER $\beta$ ), androgen receptor (AR), glucocorticoid receptor (GR), thyroid hormone receptor (TR $\alpha$ 1 and TR $\beta$ 1), retinoic acid receptor (RAR) RAR $\alpha$ , retinoid X receptor (RXR $\alpha$ ), pregnane X receptor (PXR), peroxisome proliferator-activated receptor (PPAR $\alpha$  and PPAR $\gamma$ ). TPP (purity > 97%) showed ER $\alpha$  and ER $\beta$  agonistic activity, AR antagonistic activity, GR antagonistic activity and PXR agonistic activity. TPP did not show agonistic or antagonistic activity towards TR $\alpha$ / $\beta$ , or agonistic activity towards RAR $\alpha$ , RXR $\alpha$  or PPAR $\alpha$ / $\beta$ .

In their conclusion, the authors indicate that the effects of these eleven OPFRs were dependent on their side chain structures, and that TPP and TCP, in particular, elicit pleiotropic effects on nuclear receptors (Kojima et al. 2013).

In the study of Kojima et al., 2016, the agonistic and/or antagonistic activities of 12 primary OPFR-metabolites (including those of TPP, meta and para hydroxyl phenyl diphenyl phosphate (p-HO-DHP) towards 10 human nuclear receptors were examined using cell-based transcriptional assays. The results showed that the TPP metabolites exhibited more potent estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$  agonistic activity than their parent, TPP, whereas DHP and HO-DHP did not show any ER $\alpha$  agonistic activity. Thus, HO-m-TPP and-p-HO-TPP showed ER $\alpha$ - and ER $\beta$ -mediated estrogenic activity greater than 20% than that of the E<sub>2</sub>-induced maximal activity. The order of the relative ER $\alpha$  agonistic activity of these compounds was HO-p-TPP > HO-m-TPP > TPP > DHP = HO-DHP. In addition, these metabolites also showed ER $\beta$  antagonistic activity at high concentrations and exhibited pregnane X receptor (PXR) agonistic activity as well as androgen receptor (AR) and glucocorticoid receptor (GR) antagonistic activities at levels similar to those of TPP (Kojima et al. 2016).

In the study of Krivoshiev et al., 2016, MCF-7 flow-cytometric proliferation assay was used to establish potential estrogen-disrupting effects of 12 currently-used flame retardants (FRs), including TPP. The results showed that TPP is one of the five FRs that have a statistically significant estrogenic activity (Krivoshiev et al. 2016).

Schang et al., 2016, in order to determine the effects of OPFRs on testosterone production (which is synthesized mainly by Leydig cells in the testis), compared the *in vitro* effects of 7 OPFRs, including TPP, and of BDE-47, on MA-10 mouse Leydig tumors cells. The results showed that TPP significantly reduced MA-10 cell mitochondrial activity, significantly increased superoxide production, and had no effect on basal progesterone production (Schang, Robaire, and Hales 2016).

A study investigated the thyroid hormone-disrupting activity of nine frequently detected OPFRs (including TPP). TPP (purity > 99.5%) showed neither agonist nor antagonist properties in either dual-luciferase gene reporter assay for thyroid receptor  $\beta$  (TR $\beta$ ) and thyroid hormone dependent cell proliferation of a rat pituitary tumour cell line GH3 cell proliferating test (Zhang et al. 2016).

### **3.2.1.13 Summary and discussion of health data**

Several *in vitro* tests carried out with TPP and/or its metabolites, including hydroxylated metabolites (DPHP, p-OH-TPP, m-OH-DPHP), show that these substances possess an evident potential of endocrine-disrupting effects via ER $\alpha$  / ER $\beta$ , AR, GR, and PXR activity. The *in vitro* study of Kojima et al., 2016, showed that the order of the relative agonistic activity of TPP and its metabolites on ER $\alpha$  was: HO-p-TPP > HO-m-TPP > TPP > DPHP = HO-DPHP.

There are only 2 toxicokinetic studies. Both are *in vitro* studies. The first shows that TPP is degraded by hydrolysis in rat liver homogenate to DPHP as the major product. The second (which explored the metabolite formation of TPP in primary human hepatocytes) shows that TPP is metabolised into DPHP and mono and di-hydroxylated TPP, as the major metabolites, and that quantification of biotransformation products revealed that DPHP corresponded to less than half of the depletion of TPP.

A recent study in the Human (Preston et al. 2017) found evidence that exposure to TPP may be associated with increased total thyroxine (TT4) levels (especially in women).

The available data indicate that TPP is not irritating to the skin and not active in mutagenicity and genotoxicity tests.

The majority of the few *in vivo* available studies on the repeated toxicity did not investigate a wide variety of parameters. However, the results show rather low or even no toxic effects for the key parameters studied: clinical observations, clinical chemistry, hematology, immunotoxicity, body weight gain, organ weight, histopathology, and parameters of reproduction and development.

The available neurotoxicity data show a clear decrease in cholinesterase activities in plasma and brain, but without revealing any behavioural or histological effects.

The study of Welsh et al., 1987, appears to show no treatment-related effects on fertility or fetal development in rats treated up to 690 mg/kg/day during gametogenesis, after mating, and until day 20 of gestation. However, this study reported an increase in weight of pups and an increase in the incidence of soft-tissue malformations (moderate hydroureter and enlarged ureters), even if these effects were not dose-related.

The study of Green et al., 2017, highlights that perinatally exposure to TPP triggers metabolic disturbances characterized by enhanced weight gain and enhanced adiposity to be connected with enhanced plasma levels of leptin, the hormone of satiety, and possibly with leptin resistance explaining enhanced food intake. In addition, TPP may as well accelerate the onset of T2DM although some data regarding the HOMA IR index should be interpreted with caution. As such, TPP may be considered a developmental obesogen as stated by the authors. These results are in agreement with the data from the *in vitro* study of Cano-Sancho et al., 2017, which shows that TPP, and its metabolite DPHP, act as endocrine disruptors on the regulation of adipogenic differentiation and lipolysis.

### 3.2.2 Environment data

Environmental hazards properties presented are based on available data from the chemical safety report (CSR) of TPP. Scientific public papers available dealing on e-fate and ecotoxicity were also considered in the evaluation of the substance.

#### 3.2.2.1 E-fate and Ecotoxicity of TPP

TPP is, in its pure form, a white or colourless crystalline solid with a melting point comprised between 49°C to 52°C at an atmospheric pressure of 101.3 kPa and a boiling point of 413-414°C. According to data, TPP exhibits a water solubility of 1.9-2.1 mg/L at 25°C, and has a low vapour pressure of 0.000835 Pa at 25°C and an estimate Henry's Law Constant value of 0.21 Pa m<sup>3</sup>/mol at 20°C and 0.41 Pa m<sup>3</sup>/mol at 25°C.

If the substance is released to air, according to the estimation program AOP win, TPP is expected to undergo phototransformation in air with an estimated half-life of 11.8 hours. TPP absorbs ultraviolet rays with a maximum wavelength of 261 nm, and therefore is susceptible to direct photolysis by sunlight.

If released into water, TPP could undergo hydrolysis. According to the CSR, results of an hydrolysis test (EU Method C.7) performed at pH 5, 7 and 9 and a temperature of 25°C, the substance presents a half-life value  $\geq$  28, 19 and 3 d, respectively. These results indicate a moderate stability to hydrolysis in the environment and a rapid hydrolysis in alkaline environment. No transformation products were detected in the test. However, the TPP contains phenolic group expected to be released by hydrolysis. This is in accordance with results obtained from the prediction tool EAWAG-BBD: Pathway prediction systems (PPS), which shows that the first degradation products of TPP is phenol and diphenyl phosphate.

Concerning biodegradation, screening tests are presented in the CSR. Results from a ready biodegradability test (OECD 301C, oxygen consumption) show that TPP exhibits a degradation rate of 83-94% after 28 days, revealing that the substance is readily biodegradable. In an inherent biodegradability test (OECD 303A), TPP exhibited a degradation rate of 93% after 20 days. Moreover, according to EPIsuite (MCI method) the substance presents an estimated log Koc of 4.03 at 20°C, but considering the log Kow of 4.6, the log Koc is corrected and estimated to be of 3.24. This value may be underestimated, as highlighted by UK during their assessment of the TPP (Brooke et al. 2009). In soil, under aerobic conditions (one soil, loamy sand), TPP DT50 is of 37 days and, after 101 days, the degradation rate was 80-84%. The degradation in soil leads to the generation of carbon dioxide and diphenyl hydrogen phosphate. Under anaerobic conditions in the same test system, the DT50 was 21 days and, after 102 days, the degradation rate was 68 %. The degradation products were carbon dioxide, phenol and diphenyl hydrogen phosphate. By running PBT profiler, a concern emerges for persistence in sediments and soils with a calculated half-life of 340 and 75 days, respectively. Thus, it is

expected a tendency of adsorption of TPP onto suspended solids and sediments. Nevertheless, by comparing these values with the criteria fixed in annex XIII of REACH regulation, TPP is neither considered P nor vP.

Regarding bioaccumulation, a BCF value of 420 L/kg was calculated based on [<sup>14</sup>C]-carbon labelling (Muir, Yarechewski, and Grift 1983). However, according to the TGD estimation based on log Kow, the BCF value is of 1720 L/kg. In conclusion, considering the BCF values estimated, TPP is neither considered B nor vB.

For ecotoxicity assessment, acute and long term toxicity data are available in both CSR and literature. Those reported in the CSR are based on tests with fish, invertebrates (*Americamysis bahia*) and algae (*Anabaena flos-aquae*) and long term data are solely based on tests with fish (*Oncorhynchus mykiss*). For the short term toxicity test, the highest value corresponds to a 84% inhibition of the nitrogenase activity when exposed to 0.1 mg/L. The 96 h LC50 is 0.4 mg/L for fish, and is comprised between 0.18 and 0.32 mg/L for invertebrates. These data allow to classify the TPP as Aquatic acute 1 toxic H400.

Regarding long-term data an alert exist on the toxicity of TPP with a fish ChV<sup>3</sup> of 0.007 mg/L according to PBT profiler. For their evaluation, the registrant used a 30-days EC10 of 0.037 mg/L in fish (*Oncorhynchus mykiss*). Nevertheless, another data, a 90-days NOEC ≥ 0.0014 mg/L in fish (*Oncorhynchus mykiss*), is available and not used by the registrant with the justification that *Oncorhynchus mykiss* present the same response than *Pimephales promelas* in an acute test (LC50 of 0.6 and 0.4 mg/L, respectively) but not in a long term test. Due to this difference, the registrant justified the fact that this value is not used because these species seems to be too sensitive and thus, this lowest value should be considered as an artefact. This justification is judged inappropriate because the two different fish species can have a different sensitivity in long term experiment explaining these differences. It's justifying the use of this value for the assessment of long-term toxicity. The whole dataset allow to classify the TPP as Aquatic chronic 1 toxic H410.

**In conclusion, TPP can be classified as Aquatic acute 1 H400 and Aquatic chronic 1 H410.**

### 3.2.2.2 Endocrine disruptor characteristic of TPP for the environment

Regarding endocrine disruptor concern, TPP is listed in the TEDX list of potential endocrine disruptors. TPP was also identified in the EDSP for endocrine disruptor bioactivity. Literature review on the potential of the TPP as endocrine disruptor is reported in table 6.

**Table 6: Endocrine disruptor potential of TPP**

Methodology	Results	Reference
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<sup>3</sup> Estimated chronic value according to ECOSAR/EPI (EPIWIN/EPISUITE) Estimations Programs Interface for Windows, Version 1.11.



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Dual Luciferase Reporter Gene Assay Yeast two Hybrid Assay E-Screen Assay	<p>↑ Activation of ER<math>\alpha</math> in a dose-dependent response (REC<sub>20</sub> of 2.7 x 10<sup>-7</sup> M = 32.6 ppb) in CHO-K1</p> <p>↑ Activation of ER<math>\alpha</math> in a dose-dependent response (REC<sub>20</sub> of 6.5 x 10<sup>-7</sup> M)</p> <p>↑ Activation of ER<math>\alpha</math> in a dose-dependent response (REC<sub>20</sub> of 1 x 10<sup>-6</sup> M) in MCF-7 cells</p> <p>Tight binding affinity for hER<math>\alpha</math> in docking approach, agonist effect</p>	(Zhang et al. 2014)
Zebrafish ( <i>Danio rerio</i> ), 21 day exposure	<p><b>Female</b></p> <p>↓ In Egg number, spawning event and hatchability (0.2 and 1 mg/L)</p> <p>↑ in plasma E2 levels, E2/11-KT ratio (1 mg/L), E2/T ratio, VTG (0.2 and 1 mg/L), <i>LH<math>\beta</math></i>, <i>LHR</i> and <i>FSHR</i> genes (ovary), <i>HMGRA</i>, <i>StAR</i>, <i>17<math>\beta</math>HSD</i>, <i>CYP17</i>, <i>CYP19A</i></p> <p>↓ In Testosterone, 11-KT, <i>GnRH2</i>, <i>GnRH3</i>, <i>FSH<math>\beta</math></i>, <i>HMGRB</i></p> <p><b>Male</b></p> <p>↑ in plasma E2 levels and E2/11-KT ratio(0.2 mg/L), VTG (1 mg/L), <i>GnRH1</i>, <i>GnRH2</i>, <i>CYP11A</i>, <i>CYP17</i>, <i>CYP19A</i></p> <p>↓ In E2/T ratio (0.04 and 0.2 mg/L), <i>GnRH3</i>, <i>FSH<math>\beta</math></i>, <i>LH<math>\beta</math></i>, <i>LHR</i> genes (testes), <i>HMGRA</i>, <i>StAR</i>, <i>17<math>\beta</math>HSD</i></p>	(Liu et al. 2013)
Zebrafish ( <i>Danio rerio</i> ), 120 day exposure	<p>↓ In Gonadosomatic index of female (5 and 500 <math>\mu</math>g/L)</p> <p>↓ In condition factor in male (500 <math>\mu</math>g/L)</p> <p>↑ in plasma E2 levels (5 and 500 <math>\mu</math>g/L for female and 5 <math>\mu</math>g/L for male)</p> <p>↑ in plasma cortisol (5 and 500 <math>\mu</math>g/L for male and 500 <math>\mu</math>g/L for female)</p> <p>↓ In 11-KT (500 <math>\mu</math>g/L for female and 5-500 <math>\mu</math>g/L for male)</p> <p>↑ in <i>fsH<math>\beta</math></i> (50 and 500 <math>\mu</math>g/L), <i>lh<math>\beta</math></i> and <i>gnrh3</i> (5 and 500 <math>\mu</math>g/L), <i>era</i>, <i>pomc</i>, <i>mr</i>, <i>T4</i>, <i>T3</i>, <i>trhr2</i> in female brain 500 <math>\mu</math>g/L</p> <p>↑ in <i>lhr</i>, <i>star</i>, <i>CYP19a</i> in female ovary 500 <math>\mu</math>g/L (only <i>star</i> at 50 <math>\mu</math>g/L, 2 fold)</p> <p>↑ in <i>pomc</i> and ↓ in <i>trh</i> for male brain (500 <math>\mu</math>g/L)</p> <p>↓ in <i>star</i>, <i>CYP17</i> in male testes 500 <math>\mu</math>g/L and ↑ in <i>fsHr</i>, <i>lhr</i>, <i>3<math>\beta</math>hsd</i>, <i>17<math>\beta</math>hsd</i></p>	(Liu et al. 2016)
Zebrafish embryos/larvae ( <i>Danio rerio</i> ), 120 hpf	<p>↓ In hatching and survival (100 and 500 mg/L)</p> <p>↑ in <i>CYP1A</i>, <i>NCOR2</i>, <i>CYP1B1</i>, <i>PPAR<math>\alpha</math></i>, <i>PPAR<math>\gamma</math>c1a</i>, <i>LPL</i>, <i>IL6</i>, <i>PPAR<math>\gamma</math></i>, <i>TR<math>\alpha</math></i>, <i>RelA</i>, <i>TGF<math>\beta</math>1</i>, <i>HSP90aa1</i>, <i>11<math>\beta</math>HSD</i>, <i>EGFR</i> (2mg/L)</p> <p>↓ In MR and HPSE</p>	(Liu et al. 2013)
H295R hormone transcript (48h) MVLN Luciferase Assay (72h) Zebrafish ( <i>Danio rerio</i> ), (14 days)	<p>↑ in E2, T, <i>CYP11A1</i>, <i>CYP11B2</i>, <i>CYP19A1</i> (1 mg/L), E2/T ratio (0.1 and 1 mg/L)</p> <p>↓ In <i>SULT1E</i>, <i>SULT2A1</i> (1 mg/L)</p> <p>↓ In affinity of E2 for ER (0.001 mg/L), ER antagonism</p> <p><b>Male</b></p> <p>↑ in E2 levels, E2/T ratio, E2/11-KT ratio, VTG (0.04, 0.2, 1 mg/L), <i>CYP17</i>, <i>CYP19A</i> (1 mg/L)</p> <p>↓ In T, 11-KT (1 mg/L)</p> <p><b>Female</b></p> <p>↑ in E2 levels, E2/11-KT ratio, <i>CYP17</i>, <i>CYP19A</i> (1 mg/L)</p> <p>↓ In VTG (1 mg/L)</p>	(Liu et al. 2012)
Zebrafish embryos/larvae ( <i>Danio rerio</i> ), (7 days) GH3 (rat pituitary)	<p><b>GH3</b></p> <p>↑ in <i>tsh<math>\beta</math></i>, <i>tra</i>, <i>tr<math>\beta</math></i>, <i>dio1</i> (100 <math>\mu</math>g/L TPP)</p> <p><b>FRTL-5</b></p> <p>↑ in <i>nis</i> (3, 10 mg/L), <i>tpo</i> (10 mg/L), <i>nkx2.1</i> (1 and 10 mg/L)</p> <p>↓ In <i>tshr</i>, <i>tg</i> (1 mg/L),</p> <p><b>Female</b></p> <p>↑ in malformation rate (500 <math>\mu</math>g/L), T3, T4, <i>ttr</i> (40, 200, 500 <math>\mu</math>g/L), <i>tra</i> (200 <math>\mu</math>g/L), <i>dio1</i> (500 <math>\mu</math>g/L), <i>nis</i>, <i>tg</i>, <i>ugt1ab</i> (200, 500 <math>\mu</math>g/L)</p> <p>↓ In <i>crh</i>, <i>tr<math>\beta</math></i> (500 <math>\mu</math>g/L)</p>	(Kim et al. 2015)

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FRTL-5 (rat thyroid follicular) <sup>4</sup>		
Battery of assay	<i>C. Elegans</i> larval development 0.9 µM (decreased growth and modified morphology) Zebrafish embryonic development 2 µM (malformations such as edema, small head and eyes, curved spines) Acute neurotoxicity in rat neural network activity 16.3 µM (decreased of extracellular action potentials)	(Behl et al. 2015)
<i>C. elegans</i> Development	Impact larval development at 0.16 µM, reproduction at 6.30 µM, feeding at 40 µM Inhibition of mitochondrial membrane potential at 0.6 µM as a sign of larval development arrest	(Behl et al. 2016)
Zebrafish embryos (OECD 236)	↓ in heart rate (0.50 and 1.0 mg/L), cardiac muscle cells, ventricle and atrium walls thickness, BMP4, NKX2-5, TBX5 genes, ↑ in SV-BA distance (0.10 – 1.0 mg/L), blocking cardiac looping. Most sensitive window for TPP effect on heart fonction is 0-48 hpf.	(Du et al. 2015)
Zebrafish embryos (72 hpf)	↑ pericardial area (6.25 to 50 µM) ↓ body length (25-50 µM), <i>cyp26a1</i> , in RARα, β, γ, TPP act as antagonist RAR inducing developmental toxicity	(Isales et al. 2015)
Japanese medaka ( <i>Oryzias latipes</i> ) (5 days test)	↓ in hatchability (dose and time dependant), relative average speed (625 µg/L), heart rate, body length, relative average speed depending on light phase (125; 625 µg/L) ↑ in time to hatch, gross abnormality rate, body length (625 µg/L) Inhibition of AChE activity (125; 625 µg/L) with down-regulation of <i>ache</i> trancription Down-regulation of 5 biomarkers genes for developmental neurotoxicity ( <i>gap43, a1tubulin, mbp, shha, syn2a, elavl3</i> )	(Sun et al. 2016)

As indicated by this unexhaustive review of scientific literature, there is substantial evidence that TPP could interfere with endocrine systeme *in vitro* and *in vivo*. The observed effects indicate an estrogenicity of TPP in female and male zebrafish, with increased plasmatic concentrations of E2 (Liu et al. 2012; Liu et al. 2016; Liu et al. 2013) and, in some cases, of VTG (Liu et al. 2013; Liu et al. 2012). Decrease in plasmatic concentrations of 11-ketotestosterone in both sexes were also observed (Liu et al. 2012; Liu et al. 2016; Liu et al. 2013). Moreover, in zebrafish and Japanese medaka, TPP had a negative effects on the egg number and their hatchability in a dose- and time-dependent manner (Liu et al. 2013; Liu et al. 2013; Sun et al. 2016). Sex-dependent changes in transcriptional profiles of several genes of the hypothalamic-pituitary-gonad (HPG), hypothalamic-pituitary-interrenal (HPI) and hypothalamic-pituitary-thyroid (HPT) axes where also observed (Liu et al. 2013; Kim et al. 2015; Liu et al. 2013). In particular, *era*, *trh*, *fshβ*, *T3*, *T4* were genes whose expression was modulated after exposure to TPP. *In vitro* assays also reported increase in the activation of ERα activity after exposure to TPP (Zhang et al. 2014).

**In conclusion, there is significant evidence that TPP can interfere with endocrine system and impaired reproduction by impacting egg production and hatchability in zebrafish and could be considered as being an ED for environment.**

<sup>4</sup> *In vitro* tests realized at the same time than a test with zebrafish embryos/larvae.

### 3.2.2.3 Other effects

**Table 7: Neurotoxic, metabolic and heart development effects of TPP**

Methodology	Results	Reference
Zebrafish (120 hpf)	Default of acclimatation to dark/light phases (64 $\mu$ M), hypoactivity Death detected at 0.64 $\mu$ M at 24 hpf and 0.0064 $\mu$ M at 120 hpf $\uparrow$ in edemas	(Noyes et al. 2015)
Zebrafish embryos/larvae (120 hpf)	Change in locomotor activity, $\downarrow$ activity dark phase, $\uparrow$ activity in light phase (0.4-4 $\mu$ M) Signs of neurotoxicity	(Jarema et al. 2015)
Zebrafish embryos (96 hpf)	$\downarrow$ In body length (1 $\mu$ M TPP from 5.25 to 96 hpf). $\uparrow$ in Pericardial edema, effect on developing heart (2-4 $\mu$ M TPP from 5.25 to 96 hpf). Blocking heart two-chamber (atrium-ventricle) looping at 24-48 hpf at 4 $\mu$ M, resulting in tube-heart phenotype (dioxin-like phenotype) Pharyngula is the most sensitive stage on heart embryogenesis, exposure result on altered cardiac function. TPP induced cardiotoxicity through AHR-independent pathway $\downarrow$ In <i>cyp1a1</i> .	(McGee et al. 2013)
Zebrafish liver (7 days)	<b>Metabolomic effects:</b> disruption in liver: 19 SCMs were significantly changed; involved in carbohydrate metabolism (glucose, UDP-glucose, glycolate, fumarate, succinate, and lactate), lipid and fatty acid metabolism (choline, acetylcarnitine, esterified cholesterol, arachidonic acid [ARA], timnodonic acid [EPA], linoleic acid and fatty acids , amino acid metabolism (glutamate, glutamine and leucine), and osmolyte metabolism (TMAO, dimethylamine [DMA]). <b>Transcriptional effect:</b> 471 and 364 DEGs in 0.050 mg/L and 0.300 mg/L TPP, affected the expression of genes related to carbohydrate and lipid metabolism and to the DNA damage repair system (like p53 signaling pathway). Blood test: $\downarrow$ glucose, pyruvate, triglyceride and total cholesterol in 0.050 mg/L and 0.300 mg/L TPP. <b>Histopathological liver changes:</b> vacuolization, enlarged sinusoidal vessels, pyknotic nuclei and loss of nuclei,	(Du et al. 2016)

Exposure of zebrafish to TPP can impair the heart development (Du et al. 2016; Isales et al. 2015; McGee et al. 2013). The exposition of fish embryo to TPP impacted the embryo development and led to the generation of cardiac malformations (McGee et al. 2013; Kim et al. 2015; Behl et al. 2015). These developmental malformations were characterized by modification of the cardiac muscle wall thickness, the cardiac looping and, in zebrafish and Japanese medaka, by a decreased heart rate (McGee et al. 2013; Sun et al. 2016; Isales et al. 2015). TPP can also lead to the generation of cardiac/pericardiac edemas (McGee et al. 2013; Noyes et al. 2015). The substance exhibits neurotoxic effects described by modification of the locomotion in zebrafish embryo and perturbing the dark/light adaptation mechanisms and the activity (Sun et al. 2016; Noyes et al. 2015; Jarema et al. 2015). Moreover, TPP impacts the liver of fish as identified through impairment of the metabolisms of glucides and lipids.

### 3.2.2.1 Environmental concentrations

TPP has been detected in surface and drinking water (up to 8  $\mu$ g/L) and wastewaters (up to 3  $\mu$ g/L) in several countries (Liu et al. 2016).

These concentrations were relatively close to the concentrations used in the different ecotoxicological tests and at which adverse effects were observed, indicating that the observed effects may occur in the environment.

### 3.3 General conclusion

This substance was presented and discussed during the 10<sup>th</sup> EDEG (Helsinki, November 2017). Two presentations were given on this substance by UK and FR. UK is currently conducting a substance evaluation while FR performed a RMOA analysis.

Data indicate that the main metabolic pathway of TPP is the formation of DPHP and mono- and di-hydroxylated TPP. During EDEG-10, the importance of considering these metabolites were discussed. Indeed, hydroxylated metabolites were mainly detected *in vitro*. *In vivo*, they were not detected. Only a recent study found hydroxylated metabolites in fish. It was also specified that phenols, for which there is available information on ED properties, should be considered as relevant metabolites as they can be formed by cleavage.

#### Regarding Human Health:

Data reported are in favor of a low acute human toxicity with no irritation and no sensitisation effects. Even if there is significant structural alert based on QSAR methodology, the available experimental data do not show any *in vitro* genotoxic effects. For the effects on the environment, there is a concern that TPP can be both bioaccumulable and persistent in soils and sediments due to log Kow and Koc values and to modelisation (PBT profiler). Nevertheless, TPP is not identified as a PBT because available experimental data does not fulfil PBT criteria. Experimental data allow to classify the TPP as Aquatic acute tox cat. 1 H400 and Aquatic chronic tox cat. 1 H410. The main discussion point on the environment was on the opportunity to perform an OECD 210 fish early-life stage toxicity test to investigate systematic toxicity before performing any ED investigation or to perform an OECD 234 fish sexual development test allowing to evaluate both systematic and ED concerns meanwhile. The preference of the EDEG was to perform an OECD 234 fish sexual development test.

There is a real possibility that TPP, such as demonstrated in the literature for triphenylphosphite, engender delayed neurotoxicity.<sup>5</sup> The available neurotoxicity data in animals treated with TPP (hens and rodents) show a decrease in cholinesterase activities in plasma and brain, but without behavioural or observable histological effects. Nevertheless, these data are not sufficient to conclude on the neurotoxicity of TPP because the studies might not be long enough to ascertain that TPP induce delayed neuropathy. In addition, recent studies performed in different fish species indicate of a potent toxicity for the brain reflected by modification of the locomotion and dark/light adaptation *in vivo*.

*In vivo* data on reprotoxicity in rodents are lacking and available data are limited. An early study on reproduction showed an increase in the weight of the pups and in the incidence of fetal soft-tissue malformations (moderate hydroureter and enlarged ureters), but these effects do not appear to be dose-related. In fishes, more recent data were available, indicating an impact of TPP on spawning events, egg production and their hatchability in a dose- and time-dependent manner.

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<sup>5</sup> (Kato et al. 1990; Carrington and Abou-Donia 1988; Padilla, Grizzle, and Lyerly 1987; Tanaka Jr. et al. 1990)

Developmental impact of TPP was recorded, leading to cardiac malformations, edemas and embryos malformations, also reflected by modifications on the expression of some of the related genes and proteins on fishes. Cardiac malformations were also reported in rat study (Patisaul et al. 2013).

TPP interacts with nuclear receptors (ER $\alpha$  and ER $\beta$  agonistic activity, AR antagonistic activity, GR antagonistic activity, and PXR agonistic activity) *in vitro* and there are some *in vitro*, *in vivo* and *in silico* informations on possible effects of TPP on thyroid maternal hormones also reflected by modifications on the expression of some of the related genes and proteins on fishes. However, these data are insufficient to conclude due to the lack of *in vivo* data on adverse effects from "accepted" models. During EDEG-10, it was claimed by industry that the effects observed should be of low concern because a recent human study (Preston et al 2017) was negative. While discussing this study, it was admitted that the observations were not directly correlated to the substance exposure because the variability within a person is much higher than within the group; it was reminded that several hormones were measured (how it was done and the potential limits of the measures were not discussed), and T4 only was modified.

Moreover, perinatal exposure to TPP triggers metabolic disturbances characterized by enhanced weight gain and enhanced adiposity that could be connected with enhanced plasma levels of leptin and possibly with leptin resistance explaining an enhanced food intake. As such, TPP may be considered as a developmental obesogen. In addition, it has been suggested that TPP may accelerate the onset of T2DM but this assumption has to be confirmed by additional data. The impact of TPP *in vivo* was also highlighted in fish where TPP impacted liver metabolism, especially the glucides and lipids metabolism.

Regarding the concern for metabolic disorder, it was emphasized during EDEG-10 discussions that the model used in the study raising the concern for obesogenicity was a diseased model. They are genetically predisposed to get diabetes and to get fatter. The ECETOC expert, supported by IE expert, expressed doubts about the validity and robustness of the study by Green et al (2017) on which FR based the concern for the obesogenicity potential of the substance. Too low doses were tested and no rationale for the choice of doses were provided. In addition, according to this expert, as ethanol was used as the vehicle the study should be rather considered as a 'mixture' study. He also pointed out that in all other studies, the animal lost weight. It was discussed the possibility to develop methods to clarify the concern on obesogenicity, based on methodologies developed by pharmaceutical industry to test anti-obesogens or with a dedicated assay looking at food intake and energy consumption. He stated that models to investigate obesogenicity are available as they were developed for pharmaceuticals. NL expert supported this view. CHEM Trust expert stated that the expert group should stress the need to develop an OECD guideline to clarify the metabolic effects. The CHEM Trust expert raised the concern that there is exposure to the substance in the early life stages at levels of ng/g of lipids via breast milk. She was pleased to hear that a modified EOGRTS is currently being conducted by NTP, which will provide useful information. Indeed, the pup weight changes in the study currently on-going at NTP will be very useful information.

FR expert suggested that NTP could be contacted to add additional parameters to the U.S. NTP study currently being conducted, as it is only at the stage of range finding. DK expert supported the FR expert's view that obesogenicity is a concern and should be followed up. FR expert added that there are transcriptomic and metabolomics studies in fish available in the literature showing that the substance has effects on the metabolic pathway (e.g. effects on fatty acid changes and glucose synthesis). In conclusion, UK expert indicated that their preference is currently to wait for the study report from the NTP study before doing any further

testing on human health. Meanwhile, NTP was contacted. FR proposed NTP to add the following parameters:

- on conscious animal:
  - food intake and food efficiency
  - energy expenditure by indirect calorimetry,
  - body mass composition (% fat mass by RMN analysis) once a week or at least at the end of the experiment,
  - glucose oral tolerance (GTT) (to be done one week before the sacrifice).
- at sacrifice:
  - insulin, leptin, adiponectin, ghrelin, triglycerides and free fatty acid (blood),
  - white and brown adipose tissue weight (at sacrifice) and Ucp1 and Ucp2 expression activity in brown and white adipose tissue to search for beinging effect,
  - gene expression analysis such as hypothalamic NPY and AGRP, POMC, MC4R, insulin and leptin receptor in case of indications of changes in food behavior.

We received an answer from NTP mentioning that they always measure food intake if the chemical is given in the diet, or that it can be done easily whatever the route. They also mentioned that clinical chemistry, dissection of the white and brown adipose tissue and gene expression is easy to incorporate. However, NTP also mentioned that these data will have to be awaited for the next 5 years as it will take at least that long to start the study and collect tissues.

In summary, the data available indicate a trend for:

- neurotoxicity of TPP;
- toxicity to environment that enables classifying TPP as aquatic acute and chronic toxic category 1;
- effects on reproduction and development in fishes, but *in vivo* data on rodents are needed to firmly conclude on the effect on human health;
- possible toxicity on circulating thyroid hormones;
- metabolic disturbance, impacting food intake and being a developmental obesogen.
- effects on soft tissues, particularly on the heart, so far only observed in fish.

### Regarding specifically the Environment:

Different views were expressed in support of both the UK and FR proposals, i.e. to perform OECD 210 or OECD 234 respectively. AT expert expressed preference for OECD 234. DK expert valued both proposals. He stressed the importance of the choice of the right concentrations for the test and to take into consideration any metabolite formed. He indicated that a good compromise could be to increase the test concentrations in the OECD 234 to the level of the OECD 210 (5 concentrations of the substance). NL expert was in favour of performing OECD 234 as he considers that there is already a lot of information on the adverse effects caused by the substance, and what is needed is a confirmatory test. Cefic expert indicated that the choice of the test depends on the question to be answered, be it clarification of the systemic toxicity or ED effects. DK expert responded that OECD 234 can answer both questions related to systemic toxicity and ED effects. FR expert supported DK expert's intervention and re-stated their preference for OECD 234. UK welcomed feedback on their proposal and they will consider it thoroughly. UK agreed that metabolites should be looked at more closely.

IE expert stressed that there are already a lot of data available for human health, therefore the concern should focus on environment.

Conclusions:

The effects described in the available studies appear to be limited in rodents and do not allow to draw definitive conclusions on potential hazards of TPP on human health. Environmental data show endocrine disruptor potential of TPP. Nevertheless, these data are insufficient to conclude that TPP is an endocrine disruptor according to the OECD conceptual framework for testing and assessment of endocrine disruptors (data allow to reach the level 3 of the OECD conceptual framework on endocrine disruptor (OECD 2012)). Additional *in vivo* data for human health and at the populational level for environment are required to fulfil the ED-definition. There may be enough data but the results of the NTP study should be awaited before doing anything.

Different views were expressed regarding the Environment, the majority of ED Experts Group was in favour of conducting OECD 234.

General issues:

The ED experts group discussed the need to have an appropriate model to investigate the obesogenicity concern. Further discussion at general level on this topic was encouraged by the group.

## 4 INFORMATION ON (AGGREGATED) TONNAGE AND USES<sup>6</sup>

### 4.1 Tonnage and registration status

**Table: Tonnage and registration status**

From ECHA dissemination site	
Registrations	<input checked="" type="checkbox"/> Full registration(s) (Art. 10)  <input type="checkbox"/> Intermediate registration(s) (Art. 17 and/or 18)
Total tonnage band for substance (excluding volume registered under Art 17 or Art 18, or directly exported)	100-1,000 tpa

### 4.2 Overview of uses

TPP is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

<sup>6</sup> Please provide here the date when the dissemination site was accessed.

This substance is used in the following products: adhesives and sealants and cosmetics and personal care products.

Other release to the environment of this substance is likely to occur from: indoor use and outdoor use resulting in inclusion into or onto a materials (e.g. binding agent in paints and coatings or adhesives).

The following information is extracted from ECHA dissemination website:

**Table: Uses**

	<b>Use(s)</b>
<b>Uses as intermediate</b>	-
<b>Formulation</b>	Formulation of plastic and rubber preparations Formulation of flame retardant/plasticizer preparations and cosmetics
<b>Uses at industrial sites</b>	Production of plastic and rubber articles (conversion)
<b>Uses by professional workers</b>	Use of adhesives and sealants Laboratory chemical
<b>Consumer uses</b>	Use of adhesives and sealants Cosmetic products containing triphenyl phosphite

*The table above could include available non-confidential information on tonnages for the listed uses.*

## 5 JUSTIFICATION FOR THE RISK MANAGEMENT OPTION

### **Conclusions of the analysis of the most appropriate risk management options:**

On our point of view, it is necessary to request further data in the frame of substance evaluation. This is justified by the signals highlighted by the existing data on the neurotoxic effect, the possibility of metabolic and/or reprotoxic and developmental effects, and effects on soft tissues. The discussion on the additional data to request will be possible after the end of the evaluation by the UK Chemical Agency (Health and Safety Executive), which is ongoing.

To remove the uncertainties on environmental effects, Anses proposes to perform an OECD 234 Fish sexual development test for environment or a OPPTS 850.1500 Fish life cycle toxicity on two generations. For toxicity, developmental effects need to be assessed due to uncertainties and lack of data in rodent on risk assessment for human health.

Therefore, the strategy on whether to evaluate both environmental and/or human health endocrine properties of TPP will be discussed at MSC level following UK evaluation.



**Table: SVHC Roadmap 2020 criteria**

	Yes	No
a) Art 57 criteria fulfilled?		x?
b) Registrations in accordance with Article 10?	x	
c) Registrations include uses within scope of authorisation?*	x	
d) Known uses <u>not</u> already regulated by specific EU legislation that provides a pressure for substitution?	x	

## 6 REFERENCES

- Behl, Mamta, Jui-Hua Hsieh, Timothy J. Shafer, William R. Mundy, Julie R. Rice, Windy A. Boyd, Jonathan H. Freedman, et al. 2015. "Use of Alternative Assays to Identify and Prioritize Organophosphorus Flame Retardants for Potential Developmental and Neurotoxicity." *Neurotoxicology and Teratology* 52 (Pt B): 181–93. doi:10.1016/j.ntt.2015.09.003.
- Behl, Mamta, Julie R. Rice, Marjo V. Smith, Caroll A. Co, Matthew F. Bridge, Jui-Hua Hsieh, Jonathan H. Freedman, and Windy A. Boyd. 2016. "Comparative Toxicity of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers to *Caenorhabditis elegans*." *Toxicological Sciences* 154 (2): 241–52. doi:10.1093/toxsci/kfw162.
- Brooke, D N, M J Crookes, P Quarterman, and Simon Burns. 2009. "Environmental Risk Evaluation Report: Triphenyl Phosphate (CAS No. 115-86-6)." SCHO0809BQUK–E–P. <http://nora.nerc.ac.uk/8615/>.
- Cano-Sancho, German, Anna Smith, and Michele A. La Merrill. 2017. "Triphenyl Phosphate Enhances Adipogenic Differentiation, Glucose Uptake and Lipolysis via Endocrine and Noradrenergic Mechanisms." *Toxicology in Vitro: An International Journal Published in Association with BIBRA* 40 (April): 280–88. doi:10.1016/j.tiv.2017.01.021.
- Carrington, Clark D., and Mohamed B. Abou-Donia. 1988. "Triphenyl Phosphite Neurotoxicity in the Hen: Inhibition of Neurotoxic Esterase and of Prophylaxis by Phenylmethylsulfonyl Fluoride." *Archives of Toxicology* 62 (5): 375–80. doi:10.1007/BF00293626.
- Du, Zhongkun, Guowei Wang, Shixiang Gao, and Zunyao Wang. 2015. "Aryl Organophosphate Flame Retardants Induced Cardiotoxicity during Zebrafish Embryogenesis: By Disturbing Expression of the Transcriptional Regulators." *Aquatic Toxicology* 161: 25–32. doi:10.1016/j.aquatox.2015.01.027.
- Du, Zhongkun, Yan Zhang, Guowei Wang, Jianbiao Peng, Zunyao Wang, and Shixiang Gao. 2016. "TPhP Exposure Disturbs Carbohydrate Metabolism, Lipid Metabolism, and the DNA Damage Repair System in Zebrafish Liver." *Scientific Reports* 6 (February). doi:10.1038/srep21827.
- Green, Adrian J., James L. Graham, Eduardo A. Gonzalez, Michael R. La Frano, Syrago-Styliani E. Petropoulou, June-Soo Park, John W. Newman, Kimber L. Stanhope, Peter J. Havel, and Michele A. La Merrill. 2017. "Perinatal Triphenyl Phosphate Exposure Accelerates Type 2 Diabetes Onset and Increases Adipose Accumulation in UCD-Type 2 Diabetes Mellitus Rats."

- Reproductive Toxicology* (Elmsford, N.Y.) 68 (March): 119–29. doi:10.1016/j.reprotox.2016.07.009.
- Hinton, D. M., J. J. Jessop, A. Arnold, R. H. Albert, and F. A. Hines. 1987. "Evaluation of Immunotoxicity in a Subchronic Feeding Study of Triphenyl Phosphate." *Toxicology and Industrial Health* 3 (1): 71–89. doi:10.1177/074823378700300103.
- Isales, Gregory M., Rachel A. Hipszer, Tara D. Raftery, Albert Chen, Heather M. Stapleton, and David C. Volz. 2015. "Triphenyl Phosphate-Induced Developmental Toxicity in Zebrafish: Potential Role of the Retinoic Acid Receptor." *Aquatic Toxicology* 161: 221–30. doi:10.1016/j.aquatox.2015.02.009.
- Jarema, Kimberly A., Deborah L. Hunter, Rachel M. Shaffer, Mamta Behl, and Stephanie Padilla. 2015. "Acute and Developmental Behavioral Effects of Flame Retardants and Related Chemicals in Zebrafish." *Neurotoxicology and Teratology*, Investigating the neurotoxicity of past, present, and future flame retardants, 52, Part B (November): 194–209. doi:10.1016/j.ntt.2015.08.010.
- Katoh, Kiyoshi, Nobuhiro Konno, Toru Yamauchi, and Masaaki Fukushima. 1990. "Effects of Age on Susceptibility of Chickens to Delayed Neurotoxicity Due to Triphenyl Phosphite." *Pharmacology & Toxicology* 66 (5): 387–92. doi:10.1111/j.1600-0773.1990.tb00767.x.
- Kim, Sujin, Joeun Jung, Inae Lee, Dawoon Jung, Hyewon Youn, and Kyungho Choi. 2015. "Thyroid Disruption by Triphenyl Phosphate, an Organophosphate Flame Retardant, in Zebrafish (*Danio Rerio*) Embryos/Larvae, and in GH3 and FRTL-5 Cell Lines." *Aquatic Toxicology* 160 (March): 188–96. doi:10.1016/j.aquatox.2015.01.016.
- Kojima, Hiroyuki, Shinji Takeuchi, Toshihiro Itoh, Mitsuru Iida, Satoshi Kobayashi, and Takahiko Yoshida. 2013. "In Vitro Endocrine Disruption Potential of Organophosphate Flame Retardants via Human Nuclear Receptors." *Toxicology* 314 (1): 76–83. doi:10.1016/j.tox.2013.09.004.
- Kojima, Hiroyuki, Shinji Takeuchi, Nele Van den Eede, and Adrian Covaci. 2016. "Effects of Primary Metabolites of Organophosphate Flame Retardants on Transcriptional Activity via Human Nuclear Receptors." *Toxicology Letters* 245 (March): 31–39. doi:10.1016/j.toxlet.2016.01.004.
- Krivoshiev, Boris V., Freddy Dardenne, Adrian Covaci, Ronny Blust, and Steven J. Husson. 2016. "Assessing In-Vitro Estrogenic Effects of Currently-Used Flame Retardants." *Toxicology in Vitro: An International Journal Published in Association with BIBRA* 33 (June): 153–62. doi:10.1016/j.tiv.2016.03.006.
- Liu, Chunsheng, Qiangwei Wang, Kang Liang, Jingfu Liu, Bingsheng Zhou, Xiaowei Zhang, Hongling Liu, John P. Giesy, and Hongxia Yu. 2013. "Effects of Tris(1,3-Dichloro-2-Propyl) Phosphate and Triphenyl Phosphate on Receptor-Associated MRNA Expression in Zebrafish Embryos/Larvae." *Aquatic Toxicology* 128–129 (March): 147–57. doi:10.1016/j.aquatox.2012.12.010.
- Liu, Xiaoshan, Kyungho Ji, and Kyungho Choi. 2012. "Endocrine Disruption Potentials of Organophosphate Flame Retardants and Related Mechanisms in H295R and MVLN Cell Lines and in Zebrafish." *Aquatic Toxicology* 114–115: 173–81. doi:10.1016/j.aquatox.2012.02.019.
- Liu, Xiaoshan, Kyungho Ji, Areum Jo, Hyo-Bang Moon, and Kyungho Choi. 2013. "Effects of TDCPP or TPP on Gene Transcriptions and Hormones of HPG Axis, and Their Consequences on Reproduction in Adult Zebrafish (*Danio Rerio*)." *Aquatic Toxicology* 134–135: 104–11. doi:10.1016/j.aquatox.2013.03.013.
- Liu, Xiaoshan, Dawoon Jung, Areum Jo, Kyungho Ji, Hyo-Bang Moon, and Kyungho Choi. 2016. "Long-Term Exposure to Triphenylphosphate Alters Hormone

- Balance and HPG, HPI, and HPT Gene Expression in Zebrafish (*Danio Rerio*)." *Environmental Toxicology and Chemistry* 35 (9): 2288–96. doi:10.1002/etc.3395.
- McGee, Sean P., Alex Konstantinov, Heather M. Stapleton, and David C. Volz. 2013. "Aryl Phosphate Esters Within a Major PentaBDE Replacement Product Induce Cardiotoxicity in Developing Zebrafish Embryos: Potential Role of the Aryl Hydrocarbon Receptor." *Toxicological Sciences* 133 (1): 144–56. doi:10.1093/toxsci/kft020.
- Meeker, John D., and Heather M. Stapleton. 2010. "House Dust Concentrations of Organophosphate Flame Retardants in Relation to Hormone Levels and Semen Quality Parameters." *Environmental Health Perspectives* 118 (3): 318–23. doi:10.1289/ehp.0901332.
- Monsanto. 1979. Three week dermal toxicity study of Santicizer-154 and triphenyl phosphate in rabbits. Bio dynamics Inc.; East Millstone; 08873 NJ, USA, Report Nr: 78-2171 ; 18 JUNE 1979, OTS 0 519 475.
- Muir, D. C. G., A. L. Yarechewski, and N. P. Grift. 1983. "Environmental Dynamics of Phosphate Esters. III. Comparison of the Bioconcentration of Four Triaryl Phosphates by Fish." *Chemosphere* 12 (2): 155–66. doi:10.1016/0045-6535(83)90159-5.
- Noyes, Pamela D., Derik E. Haggard, Greg D. Gonnerman, and Robert L. Tanguay. 2015. "Advanced Morphological — Behavioral Test Platform Reveals Neurodevelopmental Defects in Embryonic Zebrafish Exposed to Comprehensive Suite of Halogenated and Organophosphate Flame Retardants." *Toxicological Sciences* 145 (1): 177–95. doi:10.1093/toxsci/kfv044.
- OECD SIDS. 2002. Triphenyl phosphate (CAS N°: 115-86-6). SIDS Initial Assessment Report.
- OECD. 2012. "Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption." Guidance Document 150. Organisation for Economic Co-operation and Development. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2012\)22&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2012)22&doclanguage=en).
- Padilla, Stephanie S., Thomas B. Grizzle, and Donald Lyerly. 1987. "Triphenyl Phosphite: In Vivo and in Vitro Inhibition of Rat Neurotoxic Esterase." *Toxicology and Applied Pharmacology* 87 (2): 249–56. doi:10.1016/0041-008X(87)90286-9.
- Patisaul, Heather B., Simon C. Roberts, Natalie Mabrey, Katherine A. McCaffrey, Robin B. Gear, Joe Braun, Scott M. Belcher, and Heather M. Stapleton. 2013. "Accumulation and Endocrine Disrupting Effects of the Flame Retardant Mixture Firemaster® 550 in Rats: An Exploratory Assessment." *Journal of Biochemical and Molecular Toxicology* 27 (2): 124–36. doi:10.1002/jbt.21439.
- Preston, Emma V., Michael D. McClean, Birgit Claus Henn, Heather M. Stapleton, Lewis E. Braverman, Elizabeth N. Pearce, Colleen M. Makey, and Thomas F. Webster. 2017. "Associations between Urinary Diphenyl Phosphate and Thyroid Function." *Environment International* 101 (April): 158–64. doi:10.1016/j.envint.2017.01.020.
- Sasaki, K., T. Suzuki, M. Takeda, and M. Uchiyama. 1984. "Metabolism of Phosphoric Acid Triesters by Rat Liver Homogenate." *Bulletin of Environmental Contamination and Toxicology* 33 (3): 281–88.
- Schang, Gauthier, Bernard Robaire, and Barbara F. Hales. 2016. "Organophosphate Flame Retardants Act as Endocrine-Disrupting Chemicals in MA-10 Mouse Tumor Leydig Cells." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 150 (2): 499–509. doi:10.1093/toxsci/kfw012.

- Smith M. I., E. W. Engel, E. F. Stohlman. 1932. Further studies on the pharmacology of certain phenol esters with special reference to the relation of chemical constitution and physiologic action. *Nat. Inst. Health Bull.* 160, 1-53.
- Sobotka, T. J., R. E. Brodie, A. Arnold, G. L. West, and M. W. O'Donnell. 1986. "Neuromotor Function in Rats during Subchronic Dietary Exposure to Triphenyl Phosphate." *Neurobehavioral Toxicology and Teratology* 8 (1): 7-10.
- Sun, Liwei, Hana Tan, Tao Peng, Sisi Wang, Wenbin Xu, Haifeng Qian, Yuanxiang Jin, and Zhengwei Fu. 2016. "Developmental Neurotoxicity of Organophosphate Flame Retardants in Early Life Stages of Japanese Medaka (*Oryzias Latipes*)." *Environmental Toxicology and Chemistry* 35 (12): 2931-40. doi:10.1002/etc.3477.
- Sundkvist, Anneli Marklund, Ulrika Olofsson, and Peter Haglund. 2010. "Organophosphorus Flame Retardants and Plasticizers in Marine and Fresh Water Biota and in Human Milk" 12 (4): 943-51. doi:10.1039/B921910B.
- Sutton W. L., C. J. Terhaar, F. A. Miller, R. F. Scherberger, E. C. Riley, R. L. Roudabush, D. W. Fassett. 1960. Studies on the industrial hygiene and toxicology of triphenyl phosphate. *Arch Environ Health* 1: 45 - 58. Studie summarized in OECD SIDS, 2002.
- Tanaka Jr., Duke, Steven J. Bursian, Ellen J. Lehning, and Richard J. Aulerich. 1990. "Exposure to Triphenyl Phosphite Results in Widespread Degeneration in the Mammalian Central Nervous System." *Brain Research* 531 (1-2): 294-98. doi:10.1016/0006-8993(90)90788-D.
- Theiss, Jeffrey C., Gary D. Stoner, Michael B. Shimkin, and Elizabeth K. Weisburger. 1977. "Test for Carcinogenicity of Organic Contaminants of United States Drinking Waters by Pulmonary Tumor Response in Strain A Mice." *Cancer Research* 37 (8 Part 1): 2717-2720.
- US-EPA. 2015. Flame retardant used in flexible polyurethane foam: An alternatives assessment update.
- Van den Eede, Nele, Ingrid de Meester, Walid Maho, Hugo Neels, and Adrian Covaci. 2016. "Biotransformation of Three Phosphate Flame Retardants and Plasticizers in Primary Human Hepatocytes: Untargeted Metabolite Screening and Quantitative Assessment." *Journal of Applied Toxicology: JAT* 36 (11): 1401-8. doi:10.1002/jat.3293.
- van Otterdijk FM. 2015. 90-Day oral toxicity study with triphenyl phosphate by dietary administration in the rat. Testing laboratory: WIL Research Europe B. V., 5231 DD's-Hertogenbosch, The Netherlands. Report no.: Project 505940. Owner company: LANXESS Deutschland GmbH, Germany. Report date: 2015-04-30. Summarized In CSR report (2015).
- Welsh, J. J., T. F. Collins, K. E. Whitby, T. N. Black, and A. Arnold. 1987. "Teratogenic Potential of Triphenyl Phosphate in Sprague-Dawley (Spartan) Rats." *Toxicology and Industrial Health* 3 (3): 357-69. doi:10.1177/074823378700300308.
- Zhang, Quan, Chenyang Ji, Xiaohui Yin, Lu Yan, Meiya Lu, and Meirong Zhao. 2016. "Thyroid Hormone-Disrupting Activity and Ecological Risk Assessment of Phosphorus-Containing Flame Retardants by in Vitro , in Vivo and in Silico Approaches." *Environmental Pollution* 210 (March): 27-33. doi:10.1016/j.envpol.2015.11.051.
- Zhang, Quan, Meiya Lu, Xiaowu Dong, Cui Wang, Chunlong Zhang, Weiping Liu, and Meirong Zhao. 2014. "Potential Estrogenic Effects of Phosphorus-Containing Flame Retardants." *Environmental Science & Technology* 48 (12): 6995-7001. doi:10.1021/es5007862.