Analysis of the most appropriate risk management option (RMOA)

Substance Name: tributyl O-acetylcitrate (ATBC)

EC Number: 201-067-0

CAS Number: 77-90-7

Authority: France

Date: March 2016

Cover Note

In the framework on the French National Strategy on Endocrine Disruptors in 2015, the French Competent Authority requested ANSES to evaluate the toxicological profile of ATBC and verify whether risk management measures should be necessary for this substance.
This substance has been identified in composition and migration tests performed on PVC-toys, as the substance is used as a substitute of DEHP (EC No 204-211-0).

ANSES was commissioned by the French Competent Authority (Ministry of Ecology, Sustainable Development and Energy) in the framework on the French national Strategy on Endocrine Disruptors (Stratégie Nationale sur les Perturbateurs Endocriniens or SNPE) in 2015 to evaluate the ED properties of ATBC. Indeed, ATBC is used in Toys and the French Competent Authority was willing to check the innocuity of this substance, particularly regarding its ED properties.

As no specific template existed so far, FR is used to perfrom this task on the template of a RMOA as the ultimate goal might be to identify the substance as SVHC 57(f). Consequently, France initiated a RMOA on ATBC, mainly focused on the ED properties of this substance. Based on the available data this substance is judged as low priority for further work.

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1 IDENTITY OF THE SUBSTANCE

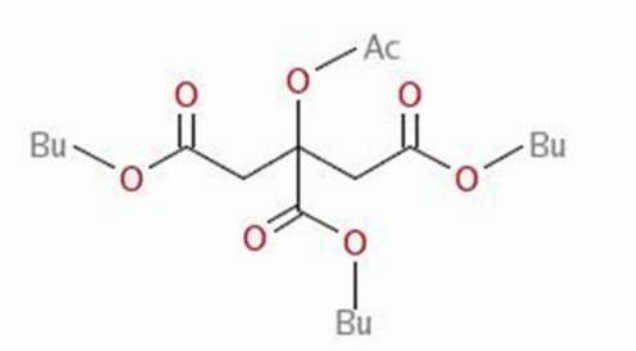
1.1 Other identifiers of the substance

Table: Other Substance identifiers

EC name (public):	tributyl O-acetylcitrate
IUPAC name (public):	tributyl 2-acetoxypropane-1,2,3-tricarboxylate
Index number in Annex VI of the CLP Regulation:	/
Molecular formula:	$C_{20}H_{34}O_8$
Molecular weight or molecular weight range:	402.5 g/mol
Synonyms:	Tributyl O-acetylcitrate tributyl 2-acetoxypropane-1,2,3-tricarboxylate 1,2,3-propanetricarboxylic acid, 2-(acetyloxy)-, tributyl ester Acetyl tributyl citrate

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



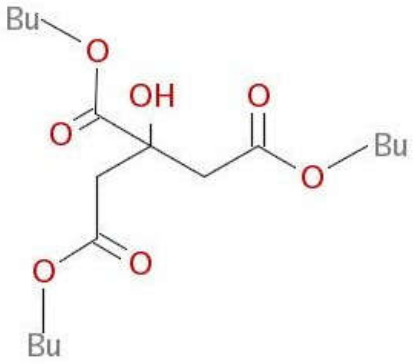
1.2 Similar substances/grouping possibilities

The tributyl citrate (TBC, EC No 201-071-2) registrant proposed in its dossier a category approach with TEC, ATEC, ATBC and ATEHC (see FR RMOA on TBC). The read-across is based on the hypothesis that the source substance TBC and the target substance ATBC have similar toxicological properties because they hydrolyse to a

common compound and non-common products predicted to have no toxicological effects.

However, in the framework of this RMOA, no read-across is used as there is specific data for ATBC.

Tributyl citrate structural formula:



2 OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Table: Completed or ongoing processes

RMOA		☐ Risk Management Option Analysis (RMOA) other than this RMOA
REACH Processes	Evaluation	☐ Compliance check, Final decision
		☐ Testing proposal
		☐ CoRAP and Substance Evaluation
	Authorisation	☐ Candidate List
		☐ Annex XIV
	Restri -ction	☐ Annex XVII[1]

[1] Please specify the relevant entry.

Harmonised C&L	☐ Annex VI (CLP) (see section 3.1)
Processes under other EU legislation	☐ Plant Protection Products Regulation Regulation (EC) No 1107/2009
	☐ Biocidal Product Regulation Regulation (EU) 528/2012 and amendments
Previous legislation	☐ Dangerous substances Directive Directive 67/548/EEC (NONS)
	☐ Existing Substances Regulation Regulation 793/93/EEC (RAR/RRS)
(UNEP) Stockholm convention (POPs Protocol)	☐ Assessment
	☐ In relevant Annex
Other processes/ EU legislation	☒ Other (provide further details below)

- ATBC is included in the regulation 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. ATBC can be used as an additive or polymer production aid. The total specific migration limit is 60 mg/kg.

- ATBC use is not forbidden in cosmetic products because not included in the regulation 1223/2009.

- EFSA authorizes ATBC as an additive in plastics intended to come into contact with food, with a a Tolerable Dose Intake (TDI) of 1.0 mg/kg bw (EFSA, 20025).

3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

3.1 Classification

3.1.1 Harmonised Classification in Annex VI of the CLP

There is no existing Harmonised Classification for ATBC.

3.1.2 Self classification

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

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)		
-		201-067-0	77-90-7	H412 , H220 , H340 , H350, H319, H315	H412, H220, H340, H350, H319, H315	-	-

H412 : Aquatic Chronic 3

H220 :Flam. Gas1

H340 :Muta.1B

H350:Carc.1B

H319 :Eye Irrit.2

H315 :Skin Irrit.2

3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

3.1.4 CLP Notification Status

Table: CLP Notifications

	CLP Notifications[2]

[2] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 3 December 2015)

Number of aggregated notifications	9
Total number of notifiers	1251

3.2 Additional hazard information

3.2.1 Existing assessments

Several hazard and/or risk assessments have already been conducted:

- In 1999, Joint FAO/WHO Expert Committee on Food Additives evaluated ATBC and, based on current intake, concluded that ATBC represented no safety concern when used as a flavouring agent (JECFA, 1999).

- In 1999, Scientific Committee on Toxicity, Ecotoxicity and the Environment published an "opinion on the toxicological charcateritics and risks of certain citrates and adipates used as a substitute for phthalates as plasticizers in certain soft PVC products" (CSTEE, 1999). The CSTEE concluded that is was not possible to estimate the relationship between exposure levels to ATBC from mouthing soft PVC toys and its NOAEL due to some data gaps. CSTEE was not able to identify migration limits for ATBC from PVC.

- In 2003, Toxicology/Regulatory Services Inc. prepared for Morflex, Inc a report entitled "Assessment of data availability and test plan for acetyl tributyl citrate" and submitted it to the US Environmental Protection Agency (US EPA) to sponsor ATBC in the High Production Volume Challenge Program (US EPA, 2003).

- In 2004, an evaluation concerning ATBC used in children's toys was done by the Scientific Committee on Toxicity, Ecotoxicity and the Environment. CSTEE conclude that there is no safety concern when young children are mouthing PVC-toys containing ATBC as plasticizer (CSTEE, 2004).

- In 2005, an evaluation concerning ATBC was done by the European Food Safety Authority (EFSA) which established a TDI (tolerable daily intake) of 1 mg/kg bw (EFSA, 2005).

- In 2008, Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) assessed the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk. Several alternative plasticizers were analyzed, among them ATBC (SCENIHR, 2008). Regarding the alternatives, for some compounds including ATBC, sufficient toxicological data is available and indicate a lower hazard compared to DEHP. However, a risk assessment of these alternative plasticizers could not be performed due to a lack of human exposure data.

- In 2010, U.S. Consumer Product Safety Commission (CPSC) published a review on exposure and toxicity data for phthalates substitutes, including ATBC (CPSC, 2010).

- In 2010, Danish Environmental Protection Agency published a report on identification and assessment of alternatives to selected phthalates (No

1341, 2010). Suitable alternative plasticisers have been identified for most applications of the phthalates including ATBC (Danish EPA, 2010).

- In 2012, ECHA published a report entitled "Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates". Available information on alternatives including ATBC was collected. Based on these informations ATBC could be an appropriate alternative to DEHP, BBP, DBP and DIBP.

- In 2014, CPSC published a report entitled "Chronic hazard advisory panel on phthalates and phthalate alternatives". According to this report and although data are somewhat limited, there is no evidence that ATBC presents a hazard to infants or toddlers from mouthing toys or child care articles containing ATBC. Therefore, the CHAP (Chronic Hazard Advisory Panel) recommends no action on ATBC. However, information on total exposure to ATBC is not available. The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to ATBC and assess the potential health risks.

- In 2015, SCENIHR does an update on its previous 2008 opinion (SCENIHR, 2015). ATBC has a low toxicity following acute oral administration. In repeated dose studies the oral NOEL was 100 mg/kg kg bw/d, based on decreased body weight, haematological and biochemical changes; increased liver weight at the higher doses. No data are available on humans. The only indication available related to leaching potential from medical devices, suggests a higher rate than DEHP. More information are necessary on this aspect to clarify human exposure in the actual conditions of use of medical device as well as on differences among oral *vs* parenteral route of exposure.

3.2.2 Current assessment

Hazards properties presented in this section are based on available data from the CSR of ATBC, as well as on previous evaluations cited above and scientific literature. It should be noticed that a detailed assessment of CSR data were not performed in the context of this RMOA.

3.2.2.1 Human health hazard assessment

Toxicokinetics

The toxicokinetics and metabolism of ATBC were studied in rats by Dow Chemical Company (1992) (as cited in Johnson *et al.,* 2002 ; US EPA, 2003 ; EFSA, 2009 et 2012 ; CPSC, 2010 ; ECHA dissemination website consulted on 19 February 2015). Groups of 4-5 male Sprague Dawley rats received a single oral dose of ^{14}C-ATBC (70 mg/kg bw, gavage). Absorption of dosed ^{14}C-ATBC from the gastrointestinal tract was rapid (half-time of 1 h) and extensive (at least 67% of ^{14}C dose absorbed). Absorbed ^{14}C-ATBC was rapidly and completely metabolized in the rat, primarily by hydrolyse to polar metabolites. Most of the absorbed radioactivity was rapidly eliminated from the blood with a half-life of 3.4 hours. Between 99% and 102% of the administrated radioactivity was recovered in the urines (59-70%), feces (25-36%), expired CO_2 (2%), tissues and carcass (0,36-1,26%) by study end (48 h). At least 9 radiolabeled metabolites were found in urines. Five were postively identified (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and acetyl dibutyl citrate). The major labeled

urinary metabolite was the monobutyl citrate. At least 3 metabolites and unchanged ATBC were identified in feces.

In vitro studies found that ATBC is metabolized by human serum and rat liver homogenates to citric, acetic, and butyric acids (Davis, 1991 ; Edlund et Sotelius, 1991 as cited in US EPA, 2003 ; CPSC, 2010 ; SCENIHR, 2008).

Based on the ADME characteristics of the compound, there is no indication for possible accumulation in human (EFSA, 2005, SCENIHR, 2008, SCENIHR, draft 2014).

No data was available for respiratory and dermal absorption. Respiratory absorption would be weak based on the low vapour pressure. Based upon the fact that the substance is a liquid with low water solubility and high logPow, absorption through the skin is unlikely.

Acute Toxicity

Lethality of ATBC by acute oral exposure is low. Five Wistar rats given a single gavage dose of ATBC at dose levels ranging from 10-30 mL/kg (approximately 10,500-31,500 mg/kg) all survived through a 21-day observation period (LD50 >31,500 mg/kg). Transient apathy was observed (Finkelstein and Gold, 1959). Twelve cats were also tested according to the same protocol (30-50 mL/kg). All 8 cats survived through an 8-week observation period (LD50 >52,500 mg/kg). The only signs observed were slight nausea and diarrhea , which subsided in less than 24 hours following dosing (Finkelstein et Gold, 1959). No deaths were observed among rats and mice of both sexes given single doses of ATBC by gavage at 25,000 mg/kg (Larionov and Cherkasova, 1977 as cited in Johnson *et al.,* 2002 et CPSC, 2010).

No sign of acute dermal toxicity was observed in guinea pigs following the application of undiluted ATBC to the skin at doses up to 1250 mg/kg (Larionov and Cherkasova, 1977 as cited in Johnson, 2002 and CPSC, 2010).

In a skin irritation study dating from 1975, ATBC was applied to 3 male albino rabbits over 4 days at a dosage of 1 mL/kg bw/d (1000 mg/kg), no signs of toxicity were noted (LD_{50} > 1 000 mg/kg) (ECHA dissemination website consulted on 19 February 2015).

The acute intraperitoneal (IP) toxicity was evaluated using Swiss albino mice (number not stated) (Meyers *et al*., 1964 as cited in Johnson, 2002). An acute IP LD_{50} of > 4 000 mg/kg was reported. Death was attributed to circulatory collapse and postictal depression. ATBC failed to induce a rapid loss of righting reflex but did cause an increase of respiratory rate. Frequently the increase in respiratory rate was accompagnied by clonic convulsions. Writhing was also observed during the first 10 minutes post injection. Similar effects were observed in Wistar rats dosed intraperitoneally with ATBC.

Mortality was not observed in groups of mice or rats dosed intraperitoneally (10,000 mg/kg bw) with undiluted ATBC (Larionov et Cherkasova, 1998 as cited in Johnson *et al.,* 2002).

Acute inhalation toxicity data are not available but ATBC is anticipated to present a very low potential for toxicity *via* inhalation route exposure because its low vapor pressure ($6{,}13.10^{-4}$ à $4{,}9.10^{-2}$ PA) and high oral LD_{50} (US EPA, 2003).

Irritation and sensitization

ATBC was tested for dermal irritation and sensitization in 59 volunteers (men and women from 21 to 60 years) in a repeated insult patch test of Draize (Hill Top Research, 1978 as cited in Johnson, 2002 and CPSC, 2010). There was no evidence of irritation in the initial patch tests, nor any reactions suggestive of contact sensitization in subsequent challenge tests (test patch (20x20 cm) moistened with 0.4 mL of ATBC to the upper arms 3 times a week for 3 weeks, challenge 48 et 96 h after application).

A guinea pig maximization test (OECD 406) showed that ATBC was not very irritating to the skin, producing only faint erythema and/or edema in response to intradermal injection, and a nonsensitizer, producing barely perceptible erythema in challenge tests (Unilever Limited, 1976 as cited in Johnson, 2002 and CPSC, 2010).

No irritant effects were noted in male albino rabbits in all of the 3 trials. Two or three male albino rabbits were exposed to 1 mL/kg ATBC (1000 mg/kg) on intact or abraded skin of abdomen (4 -18 applications, observation period : 36h to 2 weeks after last application) (study report unpublished, 1975 as cited in ECHA dissemination website consulted on 19 February 2015).

ATBC (0.1 mL) was instilled into the left conjunctival sac, with the contralateral eyes serving as controls (as cited in Johnson, 2002; CPSC, 2010). Moderate erythema was observed in two of the three rabbits within 20 minutes. The erythema subsided in one of the rabbits after 5 hours, but persisted beyond 24 hours in the other. No irritation was observed in any rabbit at 48 or 72 hours *post* instillation. Larionov and Cherkasova (1977) did not observe any ocular irritation after instilling a single drop of undiluted ATBC into the conjunctival sac of one rabbit.

ATBC does not need to be considered as a skin or eye irritant and a sensitizer.

Repeated dose studies

Oral route

In a **4-week range-finding study** in rats, ATBC caused a small decrease in both body and organ weight at the highest dose of 2,5% in the diet (equivalent to about 2700 mg/kg bw/day). No effects were observed at the lowest dose of 1% ATBC (1000 mg/kg bw/day) (CSTEE, 1999 ; SCENIHR, 2008).

Finkelstein and Gold (1959) performed a **short-term feeding study** in rats to evaluate the effect of oral exposure to ATBC on growth, hematology, and pathology. Immature Wistar rats (n = 4/sex/dose) were exposed to a diet containing 0, 5% or 10% ATBC (0; 7620; 15,240 mg/kg bw/d) for 6 weeks. The 5% ATBC diet had no deleterious effect on the growth. However, growth was reduced approximately 35% in rats fed the 10% ATBC diet. This effect may be due to frequent diarrhea. Treatment with ATBC had no effect on blood counts (measured prior to treatment and 4 and 8 weeks later) and gross or microscopic pathology (40 tissues examined at the end of the 8-week study period). The study identified a LOAEL of 15,240 mg/kg bw/d based on reduced growth (NOAEL = 7620 mg/kg bw/d) (Finkelstein et Gold, 1959 and ECHA dissemination website consulted on 19 February 2015 - study 5). Rats were administrated ATBC in the diet at doses of 0, 5 or 10% for 8 weeks (Finkelstein et Gold, 1959). No effects were observed (NOEL = 10 %). The same authors also performed a short-term feeding study on two cats. Each cat received 5 mL/kg/d ATBC (5250 mg/kg/d) *via* gavage for 2 months. The treated cats developed diarrhea and demonstrated a 30% reduction in body weight relative to controls (NOAEL < 5 mL/kg/d). No changes were observed in the appearance and behavior of the cats, or in urine, blood chemistry or blood count (Finkelstein et Gold, 1959; ECHA dissemination

website consulted on 19 February 2015 - study 6). The small group sizes in this study limit the interpretation of these results.

In a **range-finding study** for a subchronic feeding study by Jonker and Hollander (1990), Sprague-Dawley rats (n = 5/sex/dose) were exposed through the diet to ATBC (purity>98%) at doses of 0, 1000, 2700 or 5000 mg/kg/g for 14 consecutive days (Jonker and Hollanders, 1990 as cited in CSTEE, 1999 ; US EPA, 2003 et CPSC, 2010 and 2014). Transient dose-related reductions in body weights were reported among all dose groups. However, body weights among high-dose rats and mid-dose male rats remained slightly lower than control rats throughout the study. Increased cytoplasmic eosinophilia accompanied by reduced glycogen content of periportal hepatocytes was identified in the livers of two mid-dose male rats and all of the high-dose rats.

In a **90-day study** (Jonker et Hollanders, 1991 as cited Johnson, 2002; US EPA, 2003; CPSC, 2010 and ECHA, 2012), Sprague-Dawley rats (20/sex/dose) were exposed through diet to ATBC (purity 98%) at the doses of 0, 100, 300 or 1000 mg/kg bw/d. No mortality or clinic signs were observed. Slight reductions in mean body weight (non-significant) were observed in both sexes in the high dose group and females in the 300 mg/kg bw/d dose group beginning at day 28. Food consumption was slighty reduced in high-dose male rats beginning at day 28. No changes in appearance or behavior, and functional observations of motor activity, sensory activity or autonomic activity were noted. Platelet distribution and mean platelet volume were increased in both sexes at 1000 mg/kg bw/d and in males at 300 mg/kg bw/d, without any changes in platelet count or red or white blood cell parameters. These differences were not considered to be treatment-related. Any changes to the urinary composition (decreased urinary pH in males at 1000 mg/kg bw/d, fewer crystals in urine at 1000 mg/k bw/d in both sexes and in males à 300 mg/kg bw/d) were observed. Increased alkaline phosphatase activity for males in the highest dose, decreased fasting glucose for females in the two-highest doses and decreased alanine aminotransferase activity and bilirubin concentration in females at the the highest dose were measured. According to the authors, the absence of a consistent pattern for both sexes and the lack of histolopathological findings suggested thah these effects were unrelated to treatment. Increased liver relative weights for both sexes in the 1000 mg/kg bw/d dose group and in the males in the 300 mg/kg bw dose group were observed but were not associated with any evidence of hepatotoxicity (histopathological examination or biochemical analysis). A slighty increased relative kidney weight was noted for males at 1000 mg/kg bw/d. According to US EPA (2008 as cited in CPSC, 2010), liver and possibly kidney, enlargement is most likely an adaptive change occurring as a consequence of metabolic load and were not considered to be related to treatment (extensively absorbed by oral route, rapidly metabolized and excreted). All observed effects are considered to be an adaptive response, no adverse or no treatment-related, US EPA in 2008 considered a NOAEL of 1000 mg/kg bw/d (US EPA, 2008 as cited in CPSC, 2010 et 2014). In the contrary, ECHA and US EPA (2003) reported NOAEL of 300 mg/kg bw/d based on increased relative kidney weight (US EPA, 2003 ; ECHA, 2012). Authors and US EPA's claimed that hematological effects are not related to treatment and the liver and kidney effects are an adaptive response. According to ANSES, this statement cannot be challenged nor confirmed as it is not based on any scientific ground. However, it should be noted that all the following effects observed in the 90d study were observed from 300 mg/kg bw/d onward in males and only at 1000 mg/kg bw/d in females:

- Increase in platelet distribution and mean platelet volume,
- Fewer crystals in urine,
- Increased liver relative weight.

In males, at 1000 mg/kg bw/d, a decrease in urinary pH was concomitant with decreased kidney weight.

Chase and Willoughby conducted **a GLP compliant 90-days dietary study with an *in utero* exposure phase** using Han Wistar rats (Chase and Willoughby, 2002 as cited in US EPA, 2003 ; CSTEE, 2004 ; CPSC, 2014). Parental animals (F0) (n = 25/sex/dose) were treated with ATBC continuously in the diet (*ad libitum*) at target doses of 0, 100, 300 and 1000 mg/kg bw/d (purity 99.9%) for four weeks before pairing and throughout mating. F0 males were sacrified after mating. Treatment of the F0 females continued throughout gestation, littering and lactation until they were killed (PND 21). The F1 offspring were exposed *in utero* and from birth until the start of the 13-week study. During the 13-week study, the F1 animals (n = 20/sex/dose) were administered ATBC in the diet at the same target doses as the parental animals. Ten F1 males and 10 F1 females were assigned to the control and high dose group for 4-week recovery period following the 13-week treatment period.
In the 13-week study, treatment at 1000 mg/kg bw/d resulted in a slight reduction in body weight gain in both sexes (F1). Liver weights (absolute or relative, not specified) were increased and hepatic hypertrophy occurred at 1000 mg/kg bw/day in both sexes (F1). Hepatic hypertrophy resulting from an induction of metabolizing enzymes as an adaptive response to treatment is a common finding following administration of high doses of xenobiotics, and is not considered to be toxicologically significant by the authors (US EPA, 2003). Weak peroxisome proliferation was measured in males at 300 mg/kg bw/d and both sexes at 1000 mg/kg bw/d. This effect is recognized as a rodent specific effect. **Slight variations in urinary composition and in plasma electrolyte concentrations suggested an effect on renal function at the two higher dose levels.** In view of the slight nature of these changes, which were all shown to be reversible and within normal historical control ranges, and the lack of histopathological changes in the kidneys, the possible effect on renal function is considered by the authors to be due to adaptation to the excretion of high levels of ATBC and/or metabolites and is not considered to be of any toxicological significance. According to the authors, the NOAELs for systemic toxicity for ATBC were considered to be 100 mg/kg bw/d for males and 300 mg/kg bw/d for females. CSTEE agreed with authors's conclusions (CSTEE, 2004). However, according to US EPA, the NOAEL was establish at 1 000 mg/kg bw/d (US EPA, 2008 as cited in CPSC, 2010). Anses consider, like CSTEE, that the NOAEL is 100 mg/kg bw/d. Indeed, the effects observed in Han Wistar rats confirm those described in Sprague Dawley rats, in particular the kidney toxicity.

In a dose range finding study, ATBC was administered to rats (n = 3/sex/dose) *via* diet at doses of 0, 100, 1000 or 2000 mg/kg bw/d for 7 days (industrial study ECHA dissemination website consulted on 19 February 2015 – repeated study 3). Animals were observed daily for clinical signs and viability. Measurements of body weights, food consumption, necropsy with gross findings and organ weight determinations were performed. Slightly decreased body weight gain in males were observed at 2000 mg/kg bw during the first 4 days of treatment. Absolute and relative spleen weights increased at 2000 mg/kg bw in males. In females, absolute liver weights increased at 1000 and 2000 mg/kg bw (trend). According to the authors ATBC was well tolerated up to a dose level of 2000 mg/kg bw/d. Based on the results of this study and on liver effects in females, dose levels of 100, 300 and 1000 mg/kg bw/d were proposed for a **90-days study (OECD 408)**. Wistar rats (n= 10/sex/dose) were exposed daily *via* diet (Rosner, 2003). Slight biochimic changes were noted in males at the 2 highest doses such as decreased aspartate aminotransferase and lactate dehydrogenase activity, increased sodium level, decreased chloride and calcium levels, decreased globulin levels (which resulted in increased albumin/globulin ratio). In both sexes bilirubin levels decreased at 1000 mg/kg bw/d. At the highest dose, liver weights

increased in males and females accompanied by minimal hepatocellular hypertrophy (non-adverse, 2/10 females and 5/10 males). According to the authors the findings were considered to be due to hepatic metabolic adaption rather than as sign of toxicity. Anses has reservations about conclusion of the authors. Then, The highest dose of 1000 mg/kg bw can be regarded as NOAEL. CSTEE (1999) and SCENIHR (2008) considered that the NOAEL is 100 mg/kg bw/d. Based on the results of this study, 100, 300 and 1000 mg/kg bw/d were proposed for a subsequent combined chronic/carcinogenicity study (described below).

In a **combined chronic toxicity/carcinogenic study** (conducted according to 875/318/EEC; 83/571/EEC; 91/507/EEC guidelines), Wistar rats (50/sex/dose) were treated *via* diet to 0, 100, 300 or 1000 mg/kg bw/d ATBC (purity 100%) for 2 years (Sommer, 2005 cited on ECHA dissemination website consulted on 19 February 2015 - study 1). For the chronic toxicity part, a sacrifice was performed after 52 weeks. ATBC induced slight reductions in body weight (significant decrease in males at 1000 mg/kg bw/d and in females in 300 mg/kg bw/d) and food consumption in all treated groups (no clear dose-response). Changes in clinical chemistry parameters were observed :

- in males, a decrease of plasma glucose in mid and high dose groups at week 27 and in high dose group at week 53, a lower total bilirubin level in all dose groups at weeks 27 and 53, a slight increase of urea at 1000 mg/kg bw/d at week 53, a significant increase in the ALAT level in the highest dose group at weeks 27 and 53. Higher albumin and lower globulin levels were noted in the highest dose group at weeks 27 and 53, leading to a higher albumin/globulin.
- In females, a decrease in plasma glucose in the highest group at weeks 27 and 53, and a decrease in total bilirubin in 300 and 1000 mg/kg bw/d groups at weeks 27 and 53 were observed. An increase of urea level was observed in the mid and high groups at week 27 and in the highest dose group at week 53. An increase of triglycerides level and a significant decrease in the ASAT level were present at 1000 mg/kg bw/d at week 27. Lower globulin levels were observed in the mid and highest groups dose (weeks 27 and 53), leading to a higher albumin/globulin ratio in these treatment groups.

According the authors, these effects can be interpretated as adaptative changes of metabolic activation and no treatment related effect. Further, the adaptive changes were expressed by few macroscopically discernible liver changes, liver weight increase and minimal hepatocellular hypertrophy in individual animals. According to Anses, this statement is not justified because mechanisms of action are unknown and ALAT is increased in males and ASAT levels are decrease in females. However, these effects appear at high dose.Without access to the full study report, it is difficult to interpret this biochemical effects and their relationship.

Liver effects were noted such as liver weight increase in males (relative weight) and in females (absolute weight) at 1000 mg/kg bw/d. A minimal hepatocellular hypertrophy were observed in 2 males and one female in the highest dose group. The results indicated that the treatment of ATBC had no effects on survival, clinical signs, ophthalmoscopic examinations, food consumption and haematology parameters. Biochemical effects are however reported but cannot be explained without the study report. Liver seems to be the target organ. The NOAEL was at 300 mg/kg bw/d for males and 1000 mg/kg bw/d for females based on effects on body weight at 1000 mg/kg bw/d (males) and on the slight increased in liver weights and on centrilobular hypertrophy in the liver noted at 1000 mg/kg bw/d (both sexes).

After 104 weeks of treatment, mean body weights and mean body weight gains reduction were observed in males in the mid and high-dosed groups and in high-dosed group in females. Absolute and relative liver weight increased significantly

in males and females at 1000 mg/kg bw/d. An increased incidence of hepatocellular hypertrophy was noted at 1000 mg/kg bw/d in males (5/50 vs 0/50 in control) as well as a minimal to moderate single cell necrosis of hepatocytes in males (7/50 vs 0/50) and female (1/50 vs 0/50) at the highest dose. According to the authors, the relative liver weight increase along with an increased incidence of hepatocellular hypertrophy at 1000 mg/kg bw/d in males was considered to be the morphologic expression of an adaptive metabolic response rather than a toxic effect, although it is not possible to affirm and reject the possibility of a pathological effect. The origin of single cell necrosis in hepatocytes of individual high-dosed males or females remained unclear. Authors proposed a NOAEL at 300 mg/kg bw/d for males and 1000 mg/kg bw/d for females based on effects to body weights at 1000 mg/kg bw/d in males and on the slight increase in liver weights and on centrilobular hypertrophy in the liver at 1000 mg/kg bw/day in both sexes. Interestingly, it should be noted that no effects on kidney are mentionned in any report nor dissemination website reporting this long-term study.Increase of urea level was only reported transiently and only in females.

Soeler *et al.* (1950) exposed through diet 3 groups of Sherman rats (n = 20/dose, sex not specified) at concentrations of 0, 10, 100 or 1000 mg/kg bw/d ATBC (purity 99.4%) for **2 years**. There are no significant effect on body weight. Among them, 20% of the treated rats (12/60) and of control rats (8/40) died prior to scheduled sacrifice. It is likely that it was caused by pulmonary infection. Lymphoid tumors of the pleural and abdominal cavities were observed in both treated and control rats and were not considered to be treatment-related. The NOAEL is 1000 mg/kg bw/d (CPSC, 2014).

Dermal route

The application of undiluted ATBC to the skin of guinea pig did not produce pathological reactions (Larionov et Cherkasova, 1998 as cited in Johnson, 2002). At the end of the experiment, application of the test substance in fractions of 1/10 and 1/20 of maximum 2500 and 500 mg/kg dose and of the threshold and permitted dose of 12,500 mg/kg did not produce significant effects. However, the periodic application of ATBC (250 and 500 mg/kg) caused loss of body weight, a decrease in cerebral perfusion pressure, and an increase in the relative liver weight.

Intraperitoneally route

Swiss albino mice (n = 20) were exposed daily for 14 days by intraperitoneal injection of 0 or 900 mg/kg ATBC (Meyers *et al.,* 1964 as cited in Johnson *et al*., 2002 and US EPA, 2003). ATBC reduced significantly body weight gain beginning 7 days following dose initiation. There were no differences in the white blood cell counts or clotting time. Significant decrease in the erythrocyte count and hemoglobin concentration occurred. Histopathologic evaluation performed on liver, lung and kidneys do not show significant findings. Based on blood effects observed un mice, additional testing was done using albino rabbits (Meyers *et al.,* 1964 as cited in Johnson *et al*., 2002). ATBC was injected intraperitoneally daily into two rabbits (n = 2/dose) at the dose of 450 mg/kg for 14 days and at the dose of 900 mg/kg for 7 days. A decrease of red blood cell count (0.5 to 2.5 million) and a decrease in hemoglobin concentration were noted in all animals. Bone marrow smears indicated no evidence of aplastic anemia. It is therefore difficult to conclude on these effects.

Genetic Toxicity
In vitro

ATBC did not induce reverse mutation in various strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) (Ames test, OECD 471) (Gollapudi and Linscombe, 1988; Heath and Reilly, 1982; San and Wagner, 1991 as cited in Johnson *et al.,* 2002 ; CPSC, 2010 ; US EPA, 2003 et ECHA dissemination website consulted on 19 February 2015).

The registrant demonstrated absence on bacterial mutagenicity of ATBC in part with QSAR analysis (see confidential data in Annex).

ATBC did not induce mutation in L5178Y mouse lymphoma cells (Bigger and Harbell, 1991 as cited in Johnson *et al.,* 2002 ; US EPA, 2003, CPSC, 2010 and ECHA dissemination website consulted on 19 February 2015) or forward mutation at the HGPRT (Hypoxanthine-Guanine PhosphoRibosylTransferase) locus of Chinese hamster ovary (CHO) cells (Dow Chemical Company, 1991; Linscombe *et al*., 1991 as cited in CPSC, 2010) in the presence or absence of metabolic activation.

In vitro tests for chromosomal aberrations in rat lymphocyte cells gave negative results in the presence or absence of a metabolic system (Dow Chemical Company, 1988; Linscombe et al., 1991 as cited in CPSC, 2010).

In vitro gene mutation assay on mouse lymphoma cells (TK-locus), ATBC showed no evidence on mutatenic activity with or xithout metabolic activation (Bigger and Harbell, 1991 as cited in CSTEE, 1999 ; US EPA, 2003; EFSA, 2005 ; SCNEHIR, 2008).

In vivo

An assay for unscheduled DNA synthesis (UDS) in primary hepatocytes of male Han-Wistar rats treated with 800 or 2000 mg/kg of ATBC by gavage gave negative results (Fellows, 1999 as cited in CPSC, 2010 ; EFSA, 2005).

A chromosomal aberration study (OECD 475) was performed in rats (5/sex) *in vivo* after a single dose of 2000 mg/kg bw. 24 and 48 h post-treatment, bone marrow cells were collected and 100 well-spread metaphases/animal were scored for chromosomal aberrations. ATBC did nor induce chromosomal aberration (study report, 2002 as cited in ECHA dissemination website consulted on 19 February 2015)

All available data suggest that ATBC is not genotoxic except in the 4 in vitro cytotoxicity tests in mouse and mammalian cells.

Carcinogenicity

Soeler *et al.* exposed Sherman rats (20/dose, 40 control) for 2 years through diet (*ad libitum*) at concentrations of 0, 200, 2 000 or 20 000 ppm ATBC (99,4% purity) (0, 10, 100, 1000 mg/kg bw/d) (Soeler *et al*., 1950 as cited in CSTEE, 1999; Johnson *et al.,* 2002 ; US EPA, 2003 ; CPSC, 2010). A transient decrease in body weight gain was observed in animals in all treated groups (weeks 5 to 15, non significant). This reduction was not observed in a complementaty study of rats treated for one year at dietary concentration of 100 or 1000 mg/kg bw/d. Since the effect on growth was not reproducible it was considered to be an artefact. However, as the two studies have conflicting results, the artifact could also be the result of the additional study. In the main study, mortality occurred in 20% of the treated rats (12/60) and the control rats (8/40) prior to scheduled sacrifice (no significant). Necropsy of the animals that died early revealed inflammatory disease of the lungs. Pulmonary lesions (bronchitis to severe suppurative and infectious necrotizing pneumonitis) suggest an inflammatory disease of the lungs. Lymphomoid tumors of the pleural and abdominal cavities, with some infiltration of the associated organs, were observed in both control and

treated rats (see table). Therefore, these tumors were not considered to be related to treatment with ATBC. Soeler et al. conclude that the NOAEL is 1000 mg/kg bw/d.

Table : Incidence of lymphomas in ATBC-treated rats (Soeler *et al.*, 1950 as cited in CPSC, 2010)

Dose (mg/kg bw/d)	Lymphomas
0	6/40
10	1/20
100	0/20
1 000	2/20

Soeler *et al.* also fed daily two dogs to 140 mg ATBC (7-10 mg/kg/d) for 2 years (Soeler *et al*., 1950 cité dans Johnson, 2002 et CPSC, 2010). No hematology or urinalysis results were reported and no gross or microscopic abnormalities were found. However, the small number of treated dogs and lack of controls in this study limit interpretation of these results.

A combined chronic toxicity/carcinogenic study (conducted according to 875/318/EEC; 83/571/EEC; 91/507/EEC guideline) was conducted using Wistar rats (20/sex/dose). Rats were administrated doses doses of 0, 100, 300 or 1000 mg/kg bw/d in the diet for 2 years (Sommer, 2005 as cited in ECHA dissemination website consulted on 19 February 2015 - study 1). ATBC did not produce neoplastic lesions in any of the dose groups up to the highest dose (1000 mg/kg bw/d) tested and has therefore no carcinogenic potential in rats.

Toxicity to reproduction

In a **two-generation study (OECD 416)**, Sprague-Dawley rats (30/sex/dose) were exposed through diet (*ad libitum*) to ATBC at doses of 0, 100, 300 or 1000 mg/kg bw/d (purity 99.4%) (Robbins, 1994 as cited in US EPA, 2003 and CPSC, 2010 and 2014). Males F0 were exposed for 11 weeks prior to and during mating, and females were exposed for 3 weeks prior to mating, during mating and through gestation and lactation.
No effects were observed on mating, gestation or fertility of the F0 or F1 generations and no abnormalities were seen at necropsy. F1 Males and females were exposed under similar conditions from weaning for 10 weeks prior to mating. F1 females were additionally exposed through mating, gestation and lactation. No treatment-related clinical observations were noted through the study in F0 and F1 parental rats. Body weight of the F0 females in the 1000 mg/kg bw/d group was significantly reduced at the end of pregnancy (gestation day or GD 21 or 22). Body weights were also reduced in F1 parental males in the mid- and high-dose groups and appeared to be related to treatment. Water consumption of te F0 and F1 parental rats fed ATBC at a level of 1000 mg/kg bw/d was consistently lower than controls.
Slightly higher mortality and slightly reduced body weights were observed among offspring from the 300 and 1000 mg/kg bw/d dose groups compared to controls. According to the authors, it was considered that these effects were a consequence of reduced water intakes in the treated dams rather than a direct effect of treatment with ATBC. In absence of data to substantiate this statement, it cannot be challenged. No other treatment-related effects were observed among offspring. Based on these findings, 100 mg/kg bw/d appears to be a NOAEL and 300 mg/kg bw/d a LOAEL for ATBC for reductions in body weights among F1 parental males. No reproductive or developmental effects directly attributable to ATBC were observed at doses up to 1000 mg/kg bw/d.

In the **GLP compliant 13-week dietary study with an *in utero* exposure phase** described previously (Chase and Willoughby, 2002 as cited in US EPA, 2003; CSTEE, 2004; CPSC, 2014), estrous cycles, mating performance, fertility, gestation lenght and parturition were unaffected by treatment in parental animals. At the highest dose, litter size and numbers of implantations were lower than control but were in within the laboratory's historical control. Litter size, survival and growth were similar in all groups. In offspring anogenital distance and sexual maturation in both sexes and retention areolae in male were unaffected by ATBC. There were no adverse effects on sperm motility, counts or morphology. There were no effects of ATBC on any of the reproductive endpoints. Also, there were no indications of any endocrine effects of ATBC treatment. There were no findings at necroscopy of parental animals or surplus offspring that were considered to be treatment related. The NOAELs for reproduction and developmental toxicity for ATBC in this study were considered to be 300 mg/kg bw/d for parental animals and 1000 mg/kg bw/d for offsprings.

Rats and mice (strains and group sizes not reported) were exposed to ATBC (purity not reported) through diet at doses of 0, 50 or 250 mg/kg bw/d for 1 year (Larionov and Cherkasova, 1977 as cited in Johnson, 2002; US EPA, 2003 ; CPSC, 2010 and ECHA dissemination website consulted on 19 February 2015). In the ninth month of the study, animals from each group were cross-mated and embryotoxicity was evaluated. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings; eye openings; appearance of body hair and teeth; behavior; and body weight. In parental generation, no changes were observed among low-dose animals. ATBC had no significant effects on male gonads, and the spermatogenesis index in animals of the 250 mg/kg group was similar to controls. In offspring, slight changes were observed in cerebral perfusion pressure and hematology among high-dose animals, but no parameters differed significantly from controls towards the end of the study and these changes were considered by the authors to be adaptive in nature. It should be noted that effect on cerebral perfusion pressure has also been observed in guinea pig after dermal exposure. The meaning of this effect and its intensity cannot be judged based on the dissemination website. Increases in body weight, length of the newborns and placental weight were observed in the 250 mg/kg bw/d. There were no differences between groups in the fertility rate and number of animals born per pregnant female. There was a decrease in desquamatosed spermatogenic epithelium in high-dose males. However, there was no effect on fertility or litter size. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of mice and rat pups also were unaffected by treatment. No embryotoxic effect, effect on growth or fœtal development were noted. So, 250 mg/kg/d appears to be a NOAEL for both systemic and reproductive toxicity in this study. However, CPSC noted a lack of methodological details that limits interpretation of these data (CPSC, 2010).

Endocrine disruptor

ATBC is not listed as a potential endocrine disruptor in the SIN list. DHI and BKH have not closed ATBC as endocrine disruptor[3].

[3] Commission européenne (CE) DG Environnement (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption - preparation of a candidate list of substances as a basis for priority setting. Final report. RPS BKH Consulting Engineers, No. M0355008/1786Q/10/11/00 (RPS BKH Consulting Engineers, Delft)

Estrogenic activity of ATBC was measured by Nishijima *et al.* using 3 assays :

1- a yeast two-hybrid system (*Saccharomyces cerevisiae* Y190) (Nishijima *et al*., 2002) within a concentration range of 10^{-7}-10^{-3} M. After culturing for 4h at 30°C, the β-galactosidase activities were determined with or without S9. The valeurs were calculated as the rate of β-galactosidase activity divided by the β-galactosidase activity of 10^{-7} M 17β-œstradiol. The β-galactosidase activity above 0.1 shows an estrogenic activity.

2- For comparaison, ATBC was assessed for estrogenic activity with competition binding assays. ATBC was tested for its ability to displace fluorescent non-steroid estrogen (FES) form estrogen receptor-FES complex (ER-ES). With increasing concentrations of a competing ligand the FES is displaced form the complex. ATBC (10^{-7}-10^{-3} M) was added to screening buffer with ER-FES complex following by measure on Fluorescence Polarization Instrument after 60 minutes at 25°C (then converted in percent inhibition). Greater than 50% inhibition was considered estrogenic activity.

3- MCF-7 celles in the E-Screen test (10^{-9} à 10^{-4} M ATBC). A 1.5 fold increase in cell growth showed an estrogenic activity.

ATBC showed no estrogenic activity in these 3 assays.

ATBC was investigated for **estrogenic and androgenic activity** in ER and androgen receptor (AR) competitive ligand-binding assays and *in vivo* experiments (Ohta *et al.*, 2003, abstract, japonese study). Further, in *in vivo* experiments, ovariectomized Sprague-Dawley rats were observed for uterine wet weight change, uterine endometrium hyperplasia and vaginal mucosa cornification, following administration of ATBC orally (0.5 or 500 mg/kg) or subcutaneously (0.5 or 100 mg/kg). No significant response or change was observed with ATBC, either *in vitro* or *in vivo*. The results indicate that ATBC do not exert androgenic activity and confirm that there is no estrogenic activity.

Takeshita *et al.* conducted a series of *in vivo* and *in vitro* experiments to assess the action of ATBC on **SXR receptor**[4] (Takeshita *et al*., 2011). ATBC is the most potent inducer of transcription of the SXR in all five species (rabbit, rat, mice, beagle dog and human) transfected into CV-1 monkey kidney fibroblasts. ATBC did not stimulate the transcription of other human nuclear receptors including estrogen receptor (α and β), PPAR γ, thyroid hormone receptor β, progesterone receptor 1, androgen receptor and glucocorticoid receptor. The authors concluded that ATBC actived *in vitro* expression of CYP450 3A4 mediated by SXR receptor in human intestinal cells. In addition, ATBC did not induce CYP3A4 in human liver cells and human hepatocellular carcinoma.

Commission européenne (CE) DG Environnement (2002a) Endocrine Disrupters: study on gathering information on 435 Substances with insufficient data. Final report. RPS BKH Consulting Engineers, No. B4-3040/2001/325850/MAR/C2 (RPS BKH Consulting Engineers, Delft)

DHI Water & Environment (DHI) (2007) Study on enhancing the Endocrine Disrupter priority list with a focus on low production volume chemicals. DHI, No. ENV.D.4/ETU/2005/0028r (DHI, Horsholm)

[4] SRX receptor (the mice homologue of which is the PXR for pregnane X receptor) is a human nuclear receptor activated by a large number endogenous and exogenous chemicals including steroids, bile acids, and prescription drugs. Once activated, SXR form a heterodimer with RXR to activate, for example, MRP (Multidrug Resistance associated Protein) and OATP2A1 (anic anion transporting polypeptides carry bile acids as well as bilirubin and numerous hormones such as thyroid and steroid hormones across the basolateral membrane in hepatocytes, for excretion in bile). SXR is highly expressed abundantly in the liver and strongly, but much less abundantly, in intestine , where it regulates cytochrome P450 3A4, which in turn controls xenobiotic and endogenous steroid hormone metabolism.

Administration of ATBC by intraperitoneal injection during 3 days (5 or 50 mg/kg) in rats (n = 3) increased the expression of CYP3A1[5] in the duodenum and ileum but not in the liver. By gavage (2 days, 50 mg/kg), the expression of CYP3A1 is increased only at the ileum.
These *in vitro* and *in vivo* results suggest that ATBC specifically induces CYP3A in the intestine by activating SXR. Takeshita *et al.* suggest that ATBC-containing products should be used cautiously because they may alter metabolism of endogenous steroid hormones and prescription drugs.
This activating property of ATBC on PXR is coherent with the effects observed at the highest dose of ATBC in decreasing bilirubin in the two 90-days studies in rat and in the combined chronic toxicity/ carcinogenic study in males (all doses) and females(2 highest doses) at week 27 and 53 but not at week 77.

Kambia *et al.* realized docking study to specify the potential interactions of DEHP substitutes, including ATBC, with PPARα and/or γ (Peroxisome Proliferator-Activated Receptors) (Kambia *et al*., 2015). ATBC has not the shape of a classical PPAR ligand and is therefore a bit tricker to assess. According the authors, ATBC has not capacity to bind to any of the tested PPARs subtypes.

The US Tox21 program employs a broad spectrum of *in vitro* assays to use quantitative high-throughput screening (qHTS) methods to screen a large number of environmental chemicals for their potential to disturb biological pathways that may result in toxicity. The qHTS was used on 10 human nuclear receptors (AR, ERα, FXR, GR, LXRβ, PPARγ, PPARδ, RXRα, TRβ et VDR) (Huang *et al.,* 2011). Results for ATBC are in the table below (pubchem):

Bioassay	Result
qHTS assay to identify small molecule **antagonists of the androgen receptor (AR)** signaling pathway using the MDA cell line - cell viability counter screen	1 inconclusive study
qHTS assay to identify small molecule **antagonists of the peroxisome proliferator-activated receptor gamma** (PPARg) signaling pathway - cell viability counter screen	1 inconclusive study
qHTS assay for small molecule **activators of the human pregnane X receptor** (PXR) signaling pathway	1 active study
qHTS assay to identify small molecule **antagonists of** the **thyroid receptor** (TRβ) signaling pathway	1 active study, 5 inconclusives studies and 3 negative studies

ATBC is not considered as toxic for reproduction and no alert was found on potential endocrine disruption properties, in particular on estrogenic and androgenic activity. Nevertheless, some uncertainties remain. Indeed, it is not possible to conclude on the endocrine disruptor character of ATBC because there is no solid information on the other ED effects (thyroid, indirect steroid effects…). Moreover, it is not a well known ED mode of action and instead of transient biochemical modifications, its activation has not been linked to further adverse effects.

Other studies

In vitro cytotoxicity on mammalian cells (human KB cell - ID_{50}[6] = 44,7 ± 2,99 µg/mL, monkey Vero cells - ID_{50} = 39,9 ± 2,02 µg/mL, dog MDCK cells -

[5] CYP3A1 in rats corresponds to CYP3A4 in humans.

[6] ID_{50} = the concentration that reduced cell growth to 50% of control culture during 72h period of exposure

ID_{50} = 42,1 ± 2,02 µg/mL) was identified (Mochida *et al.,* 1996). A less marked cytotoxicity was observed in HeLa cells (Minimal inhibitory concentrations = 13 mg/mL for total inhibition after 24 h, 3,8 mg/mL for partial inhibition after 24 h and 5,7 mg/mL for total and partial inhibition after 7 days ; 7 days-IC_{50}[7] = 2,6 g/L) (Ekwall *et al*., 1982). Nishijima *et al.* examined cytotoxicity using human gingival fibroblasts and living skin equivalent. ATBC show a weak cytotoxicity at the concentration of 10^{-3} M without S9 mixture in human gingival fibroblasts cells (IC_{50}[8] <10mM with and without S9) and in living skin equivalent (Nishijima *et al*., 2002).

3.2.2.2 Environmental hazard

PBT assessment

PBT assessment was performed with data available on disseminate website fom ECHA and CSR from lead registrant (23/10/2013).

Abiotic degradation was evaluated by modelisation of hydrolysis (HYDROWIN program) with half-life value of 3816 years (pH 4), 3.82 years (pH 7), 139.4 days (pH 8), and 13.94 (pH 9) at 25°C (US EPA 2000). These values indicate moderate to slow hydrolysis of tributyl acety citrate under environmental conditions. Biodegradation data are controversy with 82% of degradation after 28 days with OECD 302C (MITI 1992; NITE 2009) and only 16% of degradation after 28 days with OECD 301D test (Lebertz 2009). Results from soil and compost mineralization assays show that acetyl tributy citrate can be classified as readily biodegradable based on >60% $ThCO_2$, observed in about three weeks with Respirometry test (unpublished study report 2000). This result is confirmed with 2 others studies where 60% of degradation were reached by day 52 with non specific method UML-7645 (unpublished study report 1996), or within a 10- to 14-day window following the lag phase according to EPA OPPTS 835.3300 (unpublished study report 2000). Adsorption coefficient (log Koc) is ranged between 3.167-4.943 (Koc: 1468-87 650): ATBC shows a high to very high sorption onto soil organic matter. All these data taking into consideration suggest an alert regarding P criteria of ATBC and further informations would be necessary for clarifications of aquatic biodegradation.

Bioaccumulation was evaluated by calculating using EPIWIN (v 4.0), BCFBAF (v 3.00): a BCF value of 31.57 g/L taking into account the measured log kow of 4.86. This value suggest a low potential of bioaccumulation but need to be validated by a normalized test (ex: TG 305 OECD).

Ecotoxicity studies are mainly acute tests. Algae test (OECD 201) on *Desmodesmus subspicatus* shows two EC_{50} (72h) of 74.4 mg/L and 11.5 mg/L based on growth rate and on yield, respectively. No inhibitory effects were observed on microorganisms with a EC_{10} (3h) higher than 1000 mg/l with OECD 209 (Lebertz 2009). Data available on aquatic invertebrates (*Daphnia magna*) are EC_{50} (24h) higher than 1 mg/L with OECD 202 (Ruebelt 1997) and NOEC (21d) higher or equal to 1.11 mg/L with OECD 211 (Jungbunzlauer 2010). Acute fish tests (similar to OECD 203) measured LC_{50} (96h) of 59 mg/L and in the range of 38 to 60 mg/L with *Fundulus heteroclitus* and *Lepomis macrochirus*, respectively (unpublished study report 1974). No chronic test with fish is available.

[7] IC_{50} = Inhibitory concentration

[8] IC_{50} = the concentration required to inhibit viability by 50%

According to annex requirements of Reach (Annex VII to X), almost all tests are performed except bioaccumulation, chronic fish, sediment and terrestrial assays. Concerning PBT assessment, there is a concern for P criteria due to contradictory results, a very low potential of bioaccumulation should be validated by a normalized test (not B nor vB) and no toxic effects (not T).

Regarding all these data available, ATBC can not be classified as PBT or vPvB according to criteria of annex XIII of REACH.
However, CL_{50} and EC_{50} of ATBC are ranged between 1 and 10 mg/L and its log Kow is higher than 4: ATBC could be classified as Aquatic Chronic 2 according to CLP if its persistent behavior would be demonstrated.

Endocrine disruptor

No studies dealing on the toxicity of ATBC on aquatic or terrestrial organisms are published and available on Scopus and google scholar on the date of 16/06/2015. Data available in CSR from Lead registrant, IUCLID and disseminate website from ECHA are not linked to a potential endocrine disruptor of ATBC on environment. Several regulatory website have been also consulted and no one report any endocrine disruptor concern.
Regarding endocrine disruptor concern, there is not enough data to conclude on an alert for environment.

4 INFORMATION ON (AGGREGATED) TONNAGE AND USES[9]

4.1 Tonnage and registration status

Table: Tonnage and registration status

From ECHA dissemination site		
☒ Full registration(s) (Art. 10)	☐ Intermediate registration(s) (Art. 17 and/or 18)	
Tonnage band (as per dissemination site)		
☐ 1 – 10 tpa	☐ 10 – 100 tpa	☐ 100 – 1000 tpa
☐ 1000 – 10,000 tpa	☒ 10,000 – 100,000 tpa	☐ 100,000 – 1,000,000 tpa
☐ 1,000,000 – 10,000,000 tpa	☐ 10,000,000 – 100,000,000 tpa	☐ > 100,000,000 tpa
☐ <1 . . . . . >+ tpa (e.g. 10+ ; 100+ ; 10,000+ tpa)		☐ Confidential
2 types of submission were done : - *One joint submission with a tonnage band : 10,000 – 100,000 tonnes per annum* - *One individual subsmission with a tonnage band : 100 - 1,000 tonnes per annum*		

[9] *Accessed 3 December 2015.*

4.2 Overview of uses

Table: Uses

	Use(s)
Uses as intermediate	
Formulation	Plasticizing agent,coating and paints, thinners, paint removes, fillers, putties, palsters, modelling clay, finger paints, ink and toners, non-metal-surface treatment products, washing and cleaning products including galvanic and electroplating, intermediates.
Uses at industrial sites	Plasticizing agent, non-metal-surface treatment products, intermediates. polymer preparations and compounds, coating and paints, thinners, paint removes, fillers, putties, palsters, modelling clay, finger paints, Washing and cleaning products, Metal surface treatment products, including galvanic and electroplating products, Ink and toners,adhesives and sealants, Leather tanning, dye, finishing, impregnation and care products, Lubricants, greases, release products, Paper and board dye, finishing and impregnation products: including bleaches and other processing aids, Pharmaceuticals, Polishes and wax blends, Textile dyes, finishing and impregnating products; including bleaches and other processing aids, Cosmetics, personal care products
Uses by professional workers	Plasticizing agent, Coatings and paints, thinners, paint removes, Fillers, putties, plasters, modelling clay, Non-metal-surface treatment products, Washing and cleaning products (including solvent based products), Finger paints, Metal surface treatment products, including galvanic and electroplating products, Ink and toners, Polymer preparations and compounds, Non-metal-surface treatment products, Biocidal products (e.g. disinfectants, pest control) for professional spray application outdoor, Explosives, Adhesives, sealants
Consumer Uses	Adhesives, sealants, Coatings and paints, thinners, paint removes, Fillers, putties, plasters, modelling clay, Finger paints, Non-metal-surface treatment products ,Washing and cleaning products (including solvent based products), Finger paints, Metal surface treatment products, including galvanic and electroplating products, Ink and toners, Polymer preparations and compounds
Article service life	Vehicles, paper articles, wood articles, rubber articles, plastic articles.

4.3 Additional information on uses and exposure potential

Various bibliographic websites indicate that ATBC can be used as diatary flavour in non- alcoholic beverages.

Acetyl tributyl citrate's main uses are plasticizer for vinyl, rubber and cellulosic resins, and as a flavor ingredient according to HSDB (this use is not cited in the

registration dossier of the lead registrant). ATBC can be used in production of polymeric or resinic coating for diatary contact surfaces. The more commonly used monomeric plasticisers for food packaging PVC cling-filmed include ATBC because of its properties that render it more suitable than most of the more common phthalate plasticisers (HSDB, Chemical Book, CSST, plasticisers website).
So ATBC may be found in food due to migration from food packaging.

In vinyl resins, ATBC can be found in medical plastics (pharmaceutical coatings and extra corporeal tubing), animal ear tags, and children's toys (HSDB, 2008).
Regarding the phthalate substitutes in toys, the plastifiers used in toys these last few years are in particular citrates like ATBC (used as a DEHP substitute) (plasticisers website, CPSC).

To assess the risks of certain citrates and adipates used as a substitute for phthalates as plasticizers in certain soft PVC products, a a study examined the extraction of ATBC from PVC disks cut from a custom molded ball and two commercially available toys has been performed with human volunteers (5 males and 5 females, 18 to 30 years old) (Bestari et al., 2002). Volunteers were instructed to move the disks in their mouths, draw upon, apply pressure with the tongue or lightly chew the disks for four consecutive 15 minute intervals. All saliva was collect for subsequent analysis of ATBC content.

The mean rates for ATBC migration rates were 1.53, 1.75 and 2.19 µg/min for the custom molded ball, a yellow rubber duck toy and a blue shampoo bottle top, respectively. The two largest subjects had considerably higher migration rates than the other volunteers, the highest migration rate was found to be 10.1 µg ATBC/min (CSTEE 2004).

Using the highest ATBC migration rate of 10.1 µg/min at a maximum mouthing duration of 180 min for a child weighing 8.0 kg and assuming that all the extracted ATBC is swallowed, an estimated worst-case daily intake via oral exposure becomes 227 µg per kg bodyweight.

5 JUSTIFICATION FOR THE RISK MANAGEMENT OPTION

ATBC is an alternative to phthalates in various applications, especially in sensitive ones like medical devices or toys. In the framework on the French National Strategy on Endocrine Disruptors in 2015, the French Competent Authority requested ANSES to evaluate its toxicological profile and verify whether risk management measures should be necessary for this substance.

It should first be recognized that ATBC is a pretty well studied substance for which few recent long term studies have been provided. All the requirements as described in the annexes VII, VIII, IX & X appear to be fulfilled (see preliminary analysis in annex I).

ATBC is not considered as toxic for reproduction and no alert was found on potential endocrine disruption properties, in particular on estrogenic and androgenic activity. However, there is a concern for activation of the PXR pathway but it is currently unclear which adverse effects this may lead to. So, it is not possible to conclude on the endocrine disruptor character of ATBC because there is no solid information on the other ED effects (thyroid, …).
Danish EPA, Swedish chemical agency (KEMI) and Ireland agree with France's conclusions based on the current available data (following ED Expert Group

discussions the 2-3 September 2015). In particular, Ireland considers that PXR/ SXR interaction is not endocrine disruption.

Regarding environment, ATBC is not considered as PBT nor vPvB. No alert for endocrine disruptor endpoint has been identified. However, ATBC could be classified as Aquatic Chronic 3 according to CLP if its persistent behavior would be demonstrated. Contradictory results on aquatic biodegradation suggest an alert regarding P criteria of ATBC and further informations would be necessary for clarifications.

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 not already regulated by specific EU legislation that provides a pressure for substitution?	x	

The presently available information indicates no alert on potential endocrine disruption properties of ATBC.
Nevertheless, some uncertainties remain:

- For human health, there is no solid information for some ED effects (thyroid, …);
- Concern on the activation of the PXR pathway but it is currently unclear which adverse effects this may lead to;
- There is not enough ED data to conclude an alert for environment;
- Contradictory results on aquatic biodegradation suggest an alert regarding persistence of the substance also the available data seems to show no bioaccumulation.

Based on the available studies, it seems unclear which data to request to diminish the existing uncertainty. Moreover based on the data on exposure (CSTEE, 2004), children exposure *via* toys appear negligible. This substance is therefore judged as low priority for further work.

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ANNEX I - CONFIDENTIAL INFORMATION

Genetic toxicity

OECD QSAR toolbox was applied (according to Mekeyan et al., 2004) on category members including 5 nearest neighbours of ATBC (CAS Nos. 14882-94-1, 133960 -21-1,2068-78-2, 60197-67-3,21259-20-1) to demonstrate absence on bacterial mutagenicity. LogKow was taken into account (for the target compound, log Kow is 4.29 and the 5 neighbours were within the log Kow range of 2.18 -6.21). ATBC was predicted to be negative for bacterial reverse mutation.

Table 1: preliminary analysis of REACH requirements as described in the annexes VII, VIII, IX & X

Toxicological information required	Study (species)	Reference study	Data quality (Klimisch) (US EPA, 2003)
Toxicokinetics	in vivo GLP study (rat)	Dow Chemical Company (1992)	1B
	in vitro studies	Davis (1991)	2A
		Edlund et Sotelius (1991)	2A
Acute toxicity – oral route	in vivo study (Wistar rats and cats)	Finkelstein and Gold (1959)	2A
	In vivo study (rats and mice)	Larionov and Cherkasova (1977)	
Acute toxicity – dermal route	in vivo study (guinea pigs)	Larionov and Cherkasova (1977)	
Acute toxicity – Intraperitoneal route	in vivo study (Swiss albino mice)	Meyers *et al*. (1964)	
	in vivo study (rats and mice)	Larionov and Cherkasova (1977)	
Acute toxicity – inhalation route	no data	-	
Skin irritation or skin corrosion	In vivo study (male albino rabbits)	study report unpublished (1975)	
Eye irritation	In vivo study (rabbits)	Larionov and Cherkasova (1977)	
	In vivo study (rabbits)	Unpublished study (1975)	
Skin sensitization	Guinea pig maximisation test (OECD 406)	Unilever Limited (1976)	
	Draize test (Human)	Hill Top Research (1978)	
Repeated dose toxicity – oral route	4-week range-finding study – rats	As cited in SCENIHR, 2008	
	6-week and 8-week study (Wistar rats)	Finkelstein and Gold (1959)	2A
	2 months study (cats)	Finkelstein and Gold (1959)	2A
Repeated dose toxicity – dermal route	(guinea pig)	Larionov et Cherkasova (1998)	

Toxicological information required	Study (species)	Reference study	Data quality (Klimisch) (US EPA, 2003)
Repeated dose toxicity – Intraperitoneal route	14-day study (Swiss albino mice and rabbits)	Meyers *et al.* (1964)	2A
Repeated dose toxicity – 90 days – oral route	90-day study (SD rats) (GPL, OECD 408)	Jonker et Hollanders (1991)	1A
	90-day industrial study (OECD 408) (Wistar rats)	Unpublished study	
	13-week dietary study with an in utero exposure (Han Wistar rats)	Chase and Willoughby (2002)	1A
Repeated dose toxicity - >12 months	Combined chronic toxicity/carcinogenic study (Wistar rats)	Sommer (2005)	
Reproductive toxicity	1-year embryotoxicity study (rats and mice)	Larionov and Cherkasova (1977)	2D
	two-generation study (SD rats) (GPL, OECD 416)	Robbins (1994)	2C
	13-week dietary study with an in utero exposure (Han Wistar rats) (GPL, OCDE 408, US EPA OPPTS 870.3100, EC Method B26)	Chase and Willoughby (2002)	1A
Carcinogenicity study	combined chronic toxicity/carcinogenic study (Wistar rats)	Sommer (2005)	
	2-year study (Sherman rats)	Soeler *et al.* (1950)	2D
Mutagenicity – in vitro : ames test	Ames test (GPL)	Gollapudi and Linscombe, 1988	1A
		Heath and Reilly, 1982	2D
		San and Wagner, 1991	2C
Mutagenicity – in vitro cytogenicity in mammalian cells or in vitro micronucleus study; in vitro gene mutation study in mammalian cells	mammalian cell gene mutation assay (L5178Y mouse lymphoma cells) (OECD 476)	Bigger and Harbell, 1991	2A
	forward mutation assay (HGPRT TK+/- gene mutation of CHO cells)	Dow Chemical Company, 1991	1A
	In vitro chromosomal aberration assay (rat lymphocyte cells) (OECD 473)	Dow chemical, 1988	1A
	cytotoxicity on mammalian cells study	Mochida *et al.* (1996)	
		Ekwall *et al*. (1982)	

Toxicological information required	Study (species)	Reference study	Data quality (Klimisch) (US EPA, 2003)
		Nishijima *et al*. (2002)	
Mutagenicity – in vivo	In vivo/in vitro unscheduled DNA synthesis	Fellows, 1999	2C
	chromosomal aberration study (OECD 475)	Study report (2002)	