CPSC Staff Statement on University of Cincinnati Report
"Toxicity Review for Acetyl Tri-n-butyl Citrate (ATBC)"[1]

October 2018

The U.S. Consumer Product Safety Commission (CPSC) contracted with the University of Cincinnati to conduct toxicology assessments for six dialkyl o-phthalate (o-DAP) substitutes: acetyl tri-n-butyl citrate (ATBC); bis(2-ethylhexyl) adipate (DEHA); di-2-ethylhexyl terephthalate (DEHT); 1,2-cyclohexanedicarboxylic acid, dinonyl ester, branched and linear (DINX); trioctyltrimellitate (TOTM); and 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate (TPIB). The reports will be used to inform staff's assessment of products that may contain these compounds and is the first step in the risk assessment process.

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition. First, it must be "toxic" under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial personal injury or substantial illness during or as a proximate result of any customary or reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards of products under the FHSA.

The first step in the risk assessment process is hazard identification, which consists of a review of the available toxicity data for the chemical. If it is concluded that a substance may be "toxic", then a quantitative assessment of exposure and risk is performed to evaluate whether a specified product may be considered a "hazardous substance".

The toxicity review for ATBC follows.

[1] This statement was prepared by the CPSC staff, and the attached report was produced by the University of Cincinnati for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

TOXICITY REVIEW FOR
ACETYL TRI-N-BUTYL CITRATE
(ATBC)

Contract No. CPSC-D-17-0001
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1 Introduction

This report summarizes available data on the identity, physicochemical properties, manufacture, supply, use, toxicity, and exposure associated with acetyl tri-n-butyl citrate (ATBC). It is an update of a previous contractor report to CPSC (Versar, 2010).

Literature searches for physico-chemical, toxicological, exposure, and risk information were performed in November 2017 using the CAS number and synonyms (see Appendix 1 for the full list of search terms), and using the following databases:

- EPA SRS
- PUBMED
- RTECS
- TSCATS (included in TOXLINE)
- TOXNET databases, including
    - TOXLINE
    - CCRIS
    - DART/ETIC
    - GENE-TOX
    - HSDB

Searches of the PubMed and Toxline databases covered all dates through the date of the search (November, 2017). However, studies dated up to 2007 were screened out of the library during the screening process using the Endnote files, as the current report supplements and updates a staff report prepared in 2010 (Versar, 2010). Other databases and websites were also used to identify additional key information, particularly authoritative reviews. Searches for authoritative reviews addressing general toxicity and physicochemical information were conducted with the following databases using the CAS number for ATBC and synonyms. These sites included:

- ANSES Information on Chemicals (https://www.anses.fr/en)
- ChemIDPlus (https://chem.nlm.nih.gov/chemidplus/)
- ECHA Information on Chemicals (https://echa.europa.eu/information-on-chemicals)
- EFSA (https://www.efsa.europa.eu/)
- EPA (https://www.epa.gov/)
- EPA chemistry dashboard (https://comptox.epa.gov/dashboard)
- EPA IRIS (https://www.epa.gov/iris)
- FDA (https://www.fda.gov/)
- Google (www.google.com)
- Health Canada (https://www.canada.ca/en/health-canada.html)
- IARC (https://www.iarc.fr/)

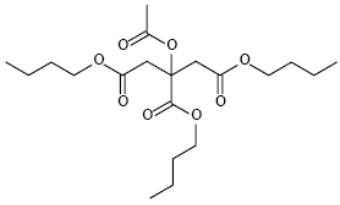
- INCHEM (http://www.inchem.org/)
- JEFCA (http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/)
- NICNAS (https://www.nicnas.gov.au/)
- NTP (https://ntp.niehs.nih.gov/)
- OECD (http://www.oecd.org/)
- WHO (http://www.who.int/en/)

New studies identified in the primary literature included repeated dose, chronic/carcinogenicity and reproductive studies, as well as data on exposure and mechanism of action, as well as reviews. Several of the key toxicity studies were unpublished and not available as the primary studies. Therefore, these studies were evaluated based on authoritative reviews and data compilations, including U.S. EPA (2008), OECD (2002), U.S. EPA (2014), and ECHA (2018).

2 Physico-Chemical Characteristics

Physical-chemical properties and identification information for this compound are highlighted in Table 1.

Table 1: Physicochemical Properties and Identification Information for Acetyl tri-n-butyl citrate

Chemical Name	Acetyl tri-n-butyl citrate
Synonyms	2-(Acetyloxy)-1,2,3-propanetricarboxylic acid, tributyl ester; 2-Acetoxy-1,2,3-propanetricarboxylic acid tributyl ester; 2-Acetyltributylcitrate; Acetyl tributyl citrate; Acetyltributyl citrate; Acetylcitric acid, tributyl ester; ATBC; Citric acid, tributyl ester, acetate; Citroflex A; Tributyl acetyl citrate; Tributyl acetylcitrate; Tributyl citrate acetate; Tributyl O-acetylcitrate
CAS Number	77-90-7
Structure	 SCENIHR, 2007
Chemical Formula	$C_{20}H_{34}O_8$
Molecular Weight	402.5 g/mol
Physical State	Liquid
Color	Colorless

Melting Point	-59°C (measured pour point) (The Morflex Inc, 2003)
Boiling Point	326°C (measured) (The Morflex Inc, 2003)
Vapor Pressure	0.052 mm Hg at 20°C (measured) (The Morflex Inc, 2003)
Water Solubility	<100 mg/L at 25°C (measured); 5 mg/L at 25°C (measured) (SRC, 2012)
Log K_{ow}	4.92 at 22 ^{o}C (The Morflex Inc, 2003)
Flashpoint	113°C (closed cup, PubChem, 2018)
Density	1.046 at 25°C (PubChem, 2018)
K_{oc} (the organic carbon normalized solid-water partition coefficient)	1800 (Versar, 2010)
Henry's Law Constant	5.5×10^{-3} atm-m^{3}/mole (estimated) (U.S. EPA 2012, Episuite)
BCF	250 L/kg (Versar, 2010)
Sources	As cited by U.S EPA, 2014 unless otherwise stated

See Appendix B for more detail on the characteristics.

ATBC is an ester of citric acid with chemical formula $C_{20}H_{34}O_8$. ATBC is a colorless, transparent liquid that is soluble in alcohol and ether. It is soluble in water at 5 mg/L (temperature not specified), and has an estimated K_{oc} value of 1800, indicating a readiness to adsorb to suspended solids and sediments. Volatilization from moist soil surfaces is not expected to be an important fate process based upon its estimated Henry's Law constant of 5.5×10^{-3} atm-m^3/mole. Additionally, ATBC is not expected to volatilize from dry soil surfaces based upon its low vapor pressure (HSDB, 2008).

If released into air, an estimated vapor pressure of 0.052 mm Hg at 20^{o}C indicates that ATBC will exist in both the vapor and particulate phases in the ambient atmosphere. The vapor-phase ATBC will be degraded by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 27 hours. Particulate-phase ATBC will be removed from the atmosphere by wet and dry deposition (HSDB, 2008). An estimated BCF of 250 suggests that the potential for bioconcentration in aquatic organisms is moderate (HSDB, 2008).

3 Manufacture, Supply, and Use

Manufacture and Supply

ATBC is a high production volume chemical (OECD, 2009; HPVIS, 2014; as cited by Bui et al., 2016). The aggregated production and/or import volume in the United States was between 1 and 10 million pounds during calendar year 2005 (U.S. EPA, 2014).

Use

ATBC is a popular plasticizer for polyvinyl resins and is used as an alternative to phthalates in children's products (Bui et al., 2016). ATBC is used in the manufacture of pharmaceutical drugs and toys, and has commercial uses as a solvent in paints, inks, and nail enamel (HSDB, 2018). Additional uses include in medical tubing and blood bags, as an ingredient in cosmetics, as pesticide inerts, and as a component of adhesives, coatings, and ink formulations (SCENIHR, 2016). ATBC has a variety of food-related uses. It is permitted as a food additive and food contact substance; it is a flavor additive in non-alcoholic beverages, is used in paper/paperboard in contact with fatty food, and is used in the production of food-contact surfaces of resinous and polymeric coatings (Sheftel, 2000; Burdock, 1995; FDA, 2002a, b; all as cited by Versar, 2010).

CPSC (2014) reported that ATBC was present in just over half of the toys and child care articles tested by CPSC (Dreyfus, 2010, as cited by CPSC, 2014). Bui et al (2016) reported that ATBC has been detected in foods, household products, nail products, and toys (Kawakami et al., 2011; FCPSA, 2008; Tsumura et al., 2002; Zygoura et al., 2011; all as cited by Bui et al., 2016) and was measured in sake (highest concentration 7.3 µg/g) (Tsumura et al., 2002; as cited by Bui et al., 2016).

4 Toxicokinetics

Metabolism studies in rats (oral feeding studies) and in rat liver homogenates reveal that ATBC is extensively absorbed and rapidly metabolized and excreted (CTFA, 1998b; Dow Chemical Company, 1992; Davis, 1991; Edlund and Ostelius, 1991; all as cited by Versar, 2010). The toxicokinetics and metabolism of ATBC were studied in male Sprague-Dawley rats by Dow Chemical Company (1992, as cited by Versar, 2010; ECHA, 2018). In this study, the rats were gavaged with radiolabeled ATBC in corn oil at 70 mg/kg.

Absorption

In the Dow study, absorption of ATBC from the gastrointestinal tract was rapid (half-time of 1 hour, peak blood levels 2-4 hours after dosing) and extensive (at least 67% of the administered dose).

Metabolism

ATBC is quickly and almost completely metabolized, primarily by hydrolysis to polar metabolites including acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and acetyl dibutyl citrate (two isomers), along with several other unidentified metabolites. *In vitro* studies found that ATBC is metabolized by human serum and by rat liver homogenates to citric, acetic, and butyric acids (Davis, 1991; Edlund and Ostelius, 1991). In the urine, at least 9 metabolites were identified; the major metabolite was thought to be monobutyl citrate (Dow Chemical Company, 1992). In the feces, unchanged ATBC represented about 7% of the dose, but at least 3 metabolites were also present. In a more recent study, Alves et al. (2017) found that metabolism by human liver and/or human intestinal microsomes resulted in the formation of

acetyl dibutyl citrate (two isomers), tributyl citrate, and dibutyl citrate, and suggested that these metabolites could be used for biomonitoring.

Elimination

In the *in vivo* rat study, most of the absorbed radioactivity was rapidly eliminated from the blood with a half-life of 3.4 hours (Dow Chemical Company, 1992). SCENIHR (2016) reported biphasic clearance from blood, with a 39 hour half-life for the slower phase. However, that review noted that the second phase could be an artifact resulting from radioactivity entering intermediate metabolic pathways. Approximately 99% of the administered radioactivity was eliminated within 48 hours of dosing, primarily in the urine (59-70%) and feces (25-36%), with a small amount (2%) expired as CO_2. Only 0.4-1.3% remained in the carcass at 48 hours.

These data indicate that the bioaccumulation potential for ATBC is low.

5 Hazard Information[2]

5.1 Acute Single Dose Toxicity

5.1.1 Acute Oral 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 (LD_{50} >31,500 mg/kg) (Finkelstein and Gold, 1959). Cats were also tested in this study. All 12 cats given a single gavage dose of ATBC at dose levels ranging from 30 to 50 mL/kg (approximately 31,500-52,500 mg/kg) survived through an 8-week observation period (LD_{50} >52,500 mg/kg) (Finkelstein and Gold, 1959). Shortly following dosing in this study, the oily dosing material began to leak from the rectums of both rats and cats. Rats appeared sluggish following dosing, but recovered during the course of the observation period. Cats showed signs of nausea and developed diarrhea, which subsided in less than 24 hours following dosing. Hematology and urinalysis examinations conducted at 2-week intervals for 2 months on two cats dosed with 52,500 mg/kg did not reveal any treatment-related changes (Finkelstein and 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).

5.1.2 Acute Dermal Toxicity

No data were located on the acute dermal lethality of ATBC. However, Johnson (2002, citing Larionov and Cherkasova, 1998), reported that “periodic” dermal application of ATBC to the

[2] Where available, this report provides significance level p values in all sections. However, secondary references used as data sources often reported only that a change was significant without reporting the p level, or just reported an effect without noting if it was statistically significant. If no p level is reported in this text, the p level was not available in the cited secondary reference, but the significance is presumed to be statistical.

skin of guinea pigs at 250 or 500 mg/kg was associated with loss of body weight, a decrease in cerebral perfusion pressure, and an increase in the liver weight. Additional details on the exposure scenario or the results were not available.

5.1.3 Acute Inhalation Toxicity

No data were located on the acute inhalation toxicity or lethality of ATBC.

5.1.4 Irritation/Sensitization

The dermal irritation of ATBC was evaluated in three repeated dose studies (Anonymous, 1975, as cited by ECHA, 2018). All three studies evaluated the effects of repeated dermal exposure to 1000 mL/kg, in groups of three (without abrasion) or two (with abrasion) male albino rabbits. In the first study, irritation was evaluated daily and 36 hours after 4 daily applications to intact skin. In the second and third studies, rabbits with intact skin or abraded skin were treated 6 days/week for a total of 18 applications, and irritation was evaluated daily and for 2 weeks after the last application. ATBC was not irritating to the skin of rabbits in these three studies. In contrast, SCENIHR (2016) reported that ATBC produced moderate dermal irritation in rats. No additional details were provided, nor was the source of these positive results cited.

In an eye irritation study for which details were not available to the dossier preparer (Anonymous, 1975, as cited by ECHA, 2018), ATBC was administered at 0.1 mL to the eyes of three male albino rabbits, and the eyes were observed at 20 minutes and 3-72 hours. No information was available on the scoring system used. Moderate erythema was reported within 20 minutes in two of the rabbits, while the third was apparently unaffected. This erythema was also present at 3 hours, but had subsided in one rabbit by 5 hours. One rabbit still had moderate erythema at 24 hours, while the other two were negative. No irritation was seen at 48 and 72 hours. From these results, ATBC was considered slightly irritating to the eyes.

In a modification of the Magnusson and Kligman guinea pig maximization test, guinea pigs were injected intradermally with ATBC and complete Freunds adjuvant and then supplemented 7 days later by dermal application of ATBC to the injection sites. The animals were challenged 14 days later with dermal patch exposure for 24 hours at a remote location. This was followed by an additional challenge after one more week. Contact sensitization was evaluated 24 hours and 48 hours after removal of the challenge patch. Study details were limited, but no sensitization was observed in any guinea pigs (Anonymous, 2001a, as cited by ECHA, 2018).

Animal study results have been confirmed by the results of human tests for irritation and sensitization conducted using the Draize skin test in 59 male and female volunteers (aged 21-60 years) (Hill Top Research, 1978, as cited by Johnson, 2002; ECHA, 2018[3]). In this study, 0.4 mL

[3] ECHA cited this study as an anonymous 2001 study, but 2001 is likely the date of the dossier from which the data were extracted.

of the test solution was applied to the arms of the subject for 24 hours, 3 times/week for 3 weeks. The patch sites were scored prior to patch applications and at 48 and 96 hours after applications. No irritation or contact sensitization was observed in any human subject.

No other human data on the health effects of ATBC were located.

5.2 Repeated Dose Toxicity

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. Twenty-one day-old Wistar rats (mixed sex groups, 4/dose) were allowed free access to a diet containing 0, 5% or 10% ATBC for up to 6 weeks. Doses were approximately 0, 7620 or 15,240 mg/kg-day, using U.S. EPA (1988) reference values for weanling Wistar rats. Body weight gain among rats fed the 5% ATBC diet exceeded controls. However, body weight was reduced by approximately 35% in rats fed the 10% ATBC diet. High-dose rats also had frequent diarrhea, which may have influenced the (decreased) weight gain. In a separate experiment using the same protocol, treatment with ATBC had no effect on blood counts (measured prior to treatment and at 4 and 8 weeks) 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-day and NOAEL of 7620 mg/kg-day, based on decreased body weight gain and diarrhea. This study was limited by the small sample size and incomplete study protocols.

Finkelstein and Gold (1959) also performed a short-term ATBC feeding study on two cats. Each cat received 5 mL/kg-day ATBC (approximately 5250 mg/kg-day) via gavage for 2 months. An additional two cats served as controls. Treated cats developed diarrhea and their final body weight at 2 months was reduced by 30% relative to controls. No changes were observed in the appearance and behavior of the cats, or in urine, blood chemistry or blood count. The small group sizes in this study limit interpretation of these results.

In an initial palatability and range-finding study, rats of an unspecified strain (3/sex/dose) were administered ATBC in the diet for 7 days (target doses of 0, 100, 1000 or 2000 mg/kg-day; Anonymous, 2001b, as cited by ECHA, 2018). At 7 days, body weight gain was slightly decreased in males and food consumption was slightly increased in females at the high-dose. Absolute and relative spleen weights were increased in males at the high dose, and absolute and relative liver weights were increased in females at ≥1000 mg/kg-day. Further details were not available for this study.

In another range-finding study, Sprague-Dawley rats (5/sex/dose) were administered ATBC (purity>98%) in the diet at doses of 0, 1000, 2700 or 5000 mg/kg-day for 14 consecutive days (Jonker and Hollanders, 1990, as cited in U.S. EPA, 2008, as cited in Versar, 2010). No rats died during the study. Transient dose-related reductions in body weights were reported among all dose groups. Body weights among high-dose rats and mid-dose male rats remained slightly lower than control rats throughout the study (sic). Food consumption remained lower among high-dose males throughout the study as well. Increased cytoplasmic eosinophilia and reduced glycogen

content in periportal hepatocytes were observed in two mid-dose male rats and all of the high-dose rats. No further details of this study were available.

Based on the results of the range-finding study, Sprague-Dawley rats (20/sex/dose) were administered ATBC (purity >98%) in the diet *ad libitum* at doses of 0, 100, 300 or 1000 mg/kg-day for 13 weeks (OECD Guideline 408; Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014). Actual doses were reported as 0, 101, 302 and 996 mg/kg-day for males and 0, 100, 296 and 999 mg/kg-day for females. No mortality or adverse clinical signs were observed. Slight, non-significant reductions in mean body weights were noted among mid-dose female rats and in male and female high-dose rats. Food consumption was slightly reduced in high-dose male rats, but food efficiency was not affected. There were no changes in rat appearance or behavior, and functional observations of motor activity, sensory activity or autonomic activity revealed no treatment-related effects. Hematology, clinical chemistry and urinalysis results were unremarkable (Versar, 2010). Although some changes in these measurements were reported by U.S. EPA (2008), the magnitude and statistical significance of these apparent changes were not reported and the U.S. EPA (2014) did not consider them to be toxicologically significant. High-dose males had decreased urinary pH, and fewer crystals were seen in the urine in mid- and high-dose males. The U.S. EPA (2008) did not consider these changes to be related to treatment because of the absence of effects in both sexes for specific parameters and the lack of any corresponding histopathological changes. Relative liver weights were increased among mid-dose male rats and high-dose rats of both sexes. In addition, there was a slight increase in the relative kidney weights of high-dose male rats. Statistical significance and magnitude of these changes were not reported. Gross necropsy and histopathology did not reveal any treatment-related effects in the liver, kidneys or other organs.

Versar (2010) concluded that the increased relative liver, and possibly kidney weight (described as enlargement in that report) is most likely an adaptive change occurring as a consequence of metabolic load. This assessment agrees that the increased relative organ weights are not adverse, and notes that they might be related to enzyme induction, possibly with a contribution from the slight decrease in body weight. Further evaluation is not possible in the absence of the primary data.

Thus, although Jonker and Hollanders (1991) observed slight changes in mean body weights, food consumption, hematology, clinical chemistry and urinalysis in at least on sex at the mid-dose and in both sexes of high-dose rats, these changes are either not considered to be adverse or not related to treatment with ATBC. Increased relative organ weights among high-dose rats were not accompanied by any biochemical or histopathological changes indicative of liver or kidney damage. Based on the findings summarized in U.S. EPA (2008) for the subchronic study performed by Jonker and Hollanders (1991), the high dose of 1000 mg/kg-day appears to be a subchronic NOAEL due to the absence of toxicologically significant findings[4].

[4] U.S. EPA (2008, 2014) identified the mid dose of 300 mg/kg-day as a NOAEL and the high dose of 1000 mg/kg-day as a LOAEL, based on decreased body weight and organ weight changes. The basis for this judgment is unclear, since the changes in body weight were described as slight and nonsignificant.

In another range-finding study (OECD Guideline 408), Wistar rats (10/sex/dose) were treated with ATBC in diet for 13 weeks (target doses of 0, 100, 300, or 1000 mg/kg-day; Anonymous, 2003, as cited by ECHA, 2018). There were no clinical signs of toxicity, and no effect on body weight, feed consumption, hematology, or urinalysis. Sodium was significantly increased, while chloride and calcium were significantly decreased (all $p<0.01$) in the mid- and high-dose males, but the changes were <10% and not clearly dose-related. Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were also significantly decreased ($p<0.01$) in mid- and high-dose males. These changes in clinical chemistry were not toxicologically meaningful. A statistically significant ($p<0.01$), dose-related decrease in bilirubin was observed in both sexes at the high dose. In the absence of other signs of toxicity, the decrease in bilirubin may reflect the induction of liver metabolic enzymes. Absolute and relative liver weights were significantly increased at the high dose of both sexes ($p<0.01$ or $p<0.05$). This finding was accompanied by hepatocellular hypertrophy in both rat sexes (males 1/10, 0/10, 6/10, and 5/10; females 0/10, 0/10, 1/10, and 2/10 in the controls, low-, mid-, and high-dose groups, respectively). The ECHA dossier noted that the histopathology evaluation was limited, but did not provide further details. Overall, the only treatment-related effects were increased liver weight and associated hypertrophy. In the absence of other liver damage, these are considered adaptive changes due to enzyme induction (Hall et al., 2012; U.S. EPA, 2005). Therefore, the high dose of 1000 mg/kg-day is a study NOAEL for systemic effects and liver effects.

5.3 Chronic Toxicity/Carcinogenicity

In a GLP-compliant (75/318/EEC; 83/571/EEC; 91/507/EEC guideline[5]) combined chronic toxicity/carcinogenicity study, Wistar rats (50/sex/dose) were fed ATBC in diet at nominal doses of 0, 100, 300, or 1000 mg/kg-day (Anonymous, 2005, as cited by ECHA, 2018). There were no treatment-related clinical signs of toxicity or increased mortality. Final body weight was significantly decreased ($p<0.01$) by 11% and 16% in mid- and high-dose males, respectively, and by 13% in high-dose females. Food consumption was reduced in mid- and high-dose males, but this decrease was not statistically significant. ECHA (2018) stated that the absolute weight of several organs was significant decreased "due to lower body weight." In contrast, relative liver weight was significantly increased ($p<0.01$) by 18% in high-dose males and by 16% in high-dose females. Complete histopathology evaluation was conducted on the control and high-dose rats, and at lower doses for target organs (e.g., the liver). Treatment-related histopathology was limited to the liver and was seen only in high-dose animals. Moderate centrilobular hypertrophy was seen in 5/50 high-dose males, and minimal to moderate single cell necrosis of hepatocytes was seen in 7/50 males and 1/50 females in the high-dose group. These lesions were not reported in any other dose group. No treatment-related neoplastic lesions were observed.

As noted in Section 5.2, increased liver weight and associated hypertrophy are considered adaptive changes related to enzyme induction. However, the single-cell necrosis is a potentially adverse effect. Based on these findings, the NOAEL is 300 mg/kg-day, based on decreased body weight in males and females and single-cell necrosis in males at 1000 mg/kg-day. ATBC was not

[5] Reported by Danish EPA (2014) as comparable to OECD Guideline 452.

carcinogenic in this assay. The decreased body weight in mid-dose males was not considered adverse due to the concomitant decrease in food consumption, perhaps due to palatability[6]. The decreased body weight at the high dose may also be secondary to decreased food consumption, but the decrease was larger and thus potentially adverse.

The carcinogenicity study included additional satellite groups of 20 rats/sex/dose. These animals underwent clinical chemistry, urinalysis and hematology evaluation at 26 and 52 weeks, and were sacrificed at 52 weeks for the chronic toxicity portion of the study. At 52 weeks, body weight was decreased by 7% and 15% in mid- (not statistically significant) and high-dose males ($p<0.01$), and by 11% ($p<0.05$) and 8% (not significant) in mid- and high-dose females, respectively. Evaluation of the clinical chemistry data is complicated by the absence of reporting of quantitative data, information on statistical significance, and degree of change. Serum urea was "slightly" increased in high-dose males at week 53, in mid- and high-dose females at week 26, and in high-dose females at week 52. This change does not appear to be toxicologically significant, in light of the small magnitude of change in males and absence of progression with time. High-dose males had a significant (not further explained) increase in the alanine aminotransferase level at weeks 27 and 53, but aspartate aminotransferase was *decreased* in high-dose females at week 27, and not week 53. Males of all exposure groups excreted higher urine volumes at week 53. At weeks 27 and 53, urinary pH was lower in mid- and high-dose males, possibly as a direct physical result of excretion of the acidic monobutyl citrate metabolite. There were however, no treatment-related crystals in the liver or kidney stones. Relative liver weight was significantly increased by 24% in high-dose males and absolute liver weight was significantly increased by 16% in high-dose females. These increases were accompanied by minimal centrilobular hepatocellular hypertrophy in 2/20 males and 1/20 female at the high dose. There were no other histopathological findings. As noted for the 2-year study, the liver changes were adaptive. Therefore, in males the NOAEL at the 52-week sacrifice was 300 mg/kg-day and the LOAEL was 1000 mg/kg-day, based on decreased body weight. No LOAEL was identified in females, in light of the smaller decrease and absence of a dose-response in body weight.

Three groups of Sherman rats (20 rats/dose) (sex not specified) were allowed free access to a diet containing ATBC (99.4% purity) at concentrations of 200, 2000 or 20,000 ppm for 2 years (Soeler et al., 1950). Target doses were 10, 100 and 1000 mg/kg-day. A fourth group of 40 rats was fed the control diet. Appearance and behavior of treated rats were similar to controls. During weeks 5 to 15, all treated groups exhibited a transient non-significant reduction in growth rate. In order to evaluate body weight further, Soeler et al. (1950) fed two additional groups of 10 rats each a diet containing ATBC at target doses of 100 or 1000 mg/kg-day for one year. A third group of 20 rats was fed the control diet. Similar reductions in body-weight gain were not observed in this study. Since the apparent effect on growth was not reproducible, the author did not consider it to be an effect of ATBC treatment. In the 2-year study, mortality occurred in 20% of the treated rats (12/60) and the control rats (8/40) prior to study termination. Necropsy of the

[6] Food consumption was decreased by 6.1%, while terminal body weight was decreased by 11%. Although this magnitude of change in final body weight would be considered adverse, the chemical-related decrease was less than 5%, after accounting for the decrease due to decreased food consumption. This decrease is substantially less than the 10% usually considered adverse in a chronic study.

affected rats revealed inflammatory disease in the lungs. Pulmonary lesions ranged from bronchitis to severe suppurative and infectious necrotizing pneumonitis. This suggested possible infection among the test animals. As shown in Table 2, lymphomas were observed in both control and treated rats. Based on the higher tumor incidence in control rats, these tumors are not considered to be related to treatment with ATBC. In conclusion, Soeler et al. (1950) did not observe any significant treatment-related effects in rats exposed to ATBC up to 1000 mg/kg-day in the diet for 2 years. Therefore, the NOAEL for this study is 1000 mg/kg-day. This study is of limited value as a cancer bioassay because group sizes were relatively small (20 per treated group and 40 in controls), 20% of animals died early from infection, and doses did not approach the maximum tolerated dose [MTD] so were inadequate (Soeler et al., 1950).

Table 2. Incidence of Lymphomas in ATBC-Treated Rats[a]

Dose (mg/kg-day)	Lymphomas
0	6/40[b]
10	1/20
100	0/20
1000	2/20

[a]Soeler et al. (1950)

[b]Compiled from Table IV of the reference; the text of the reference reports the control incidence as 4/40

In another experiment, Soeler et al. (1950) fed two mongrel dogs gelatin capsules containing 140 mg ATBC daily (approximately 7-10 mg/kg-day) for 2 years. No unusual hematology or urinalysis results were observed 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.

5.4 Reproductive Toxicity

A two-generation study that was compliant with Good Laboratory Practice (GLP) (but not OECD or EPA test guidelines) was summarized as a robust summary in U.S. EPA (2008) and in ECHA (2018) based on Robbins (1994). Sprague-Dawley rats (30/sex/dose) were administered ATBC (purity 99.4%) continuously in the diet at target doses of 0, 100, 300 or 1000 mg/kg-day. Males 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. Male and female pups from each F1 generation litter were exposed under similar conditions beginning at weaning for 10 weeks prior to mating. F1 females were additionally exposed through mating, gestation and lactation. Only tissues that appeared abnormal at necropsy were evaluated for histopathology,

and so small lesions could have been missed. Actual doses were within 10% of target doses in both the F0 and F1 rats.

No treatment-related clinical signs were observed in parental rats of either generation. Body weights of high-dose F0 females were significantly reduced at the end of pregnancy (GD21 or 22), but not at other times. Body weights were also reduced in F1 parental males from the mid- and high-dose groups in a manner that appeared to U.S. EPA (2008) to be treatment-related. No further information was provided. Water consumption among high-dose rats from both the F0 and F1 generations was consistently lower than concurrent controls throughout the study. No effects were observed on mating, gestation or fertility of the F0 or F1 generations and no physical abnormalities were seen at necropsy. “Slightly” higher pup mortality and “slightly” reduced pup body weights were observed among offspring from the 300 and 1000 mg/kg-day dose groups compared to controls; no further details were available. U.S. EPA (2008) suggests that these effects were a consequence of reduced water consumption among treated dams rather than a direct effect of treatment with ATBC. No other developmental abnormalities were observed among offspring. Based on the findings summarized in U.S. EPA (2008) of the two-generation study performed by Robbins (1994), 100 mg/kg-day appears to be a NOEL and 300 mg/kg-day a LOEL for ATBC for reductions in body weights among F1 parental males; in the absence of information on the magnitude of the change, it cannot be determined whether 300 mg/kg-day is a LOAEL. No reproductive or developmental effects directly attributable to ATBC were observed at doses up to 1000 mg/kg-day. The study is limited by the absence of sperm evaluations, and by the absence of a complete histopathology evaluation of reproductive tissues.

A 1-generation reproduction study (OECD Guideline 408) was described in a robust summary provided in U.S. EPA (2008) and ECHA (2018), and based on Chase and Willoughby (2002). Parental Han Wistar rats (25/sex/dose) were exposed to Citroflex A-4 (ATBC, 99.9% purity) continuously in the diet (target doses of 0, 100, 300 or 1000 mg/kg-day) for 4 weeks prior to and during mating. F0 females were additionally exposed during gestation and lactation until the offspring were weaned on lactation day 21. Groups of F1 offspring (20/sex/dose) were exposed to ATBC continuously in the diet for 13 weeks, at the same target doses as the parental animals. An additional 10 F1 males and 10 F1 females were assigned to the control and high-dose group for a 4-week recovery period following the 13-week treatment period. It is not clear from the available information whether there was a complete histopathological evaluation of reproductive tissues. Actual doses were within 3% of target doses. Although the general condition of parental animals was unaffected by treatment, it was noted that high-dose parental females had an increased incidence of yellow staining in the perigenital and sacral regions during treatment. No other effects were observed among F0 rats, and in particular no effects were observed on estrous cycles, mating performance, fertility or gestation, or on sperm parameters (motility, counts or morphology). ECHA (2018) reported slight reductions in body weight gain at 1000 mg/kg-day in the F0 rats, but the changes were apparently neither biologically nor statistically significant, since they were not mentioned by U.S. EPA (2008). Further details were not available. ECHA (2018) and U.S. EPA (2008) reported slight changes in urinary composition and plasma electrolyte concentrations, but the latter noted that the observed changes were within normal historical control ranges and were reversible, and there were no corresponding histopathological

changes. ECHA (2018) suggested that the changes were related to adaptation to the excretion of high levels of the test material and/or metabolites, and thus not toxicologically significant. ECHA (2018) reported "weak" peroxisome proliferation at 300 mg/kg-day in males and at 1000 mg/kg-day in both sexes. Hepatocyte hypertrophy was also reported at 1000 mg/kg-day (presumably in both sexes).

There was no effect on sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males, sperm assessments) of the F1 rats. The number of implantations and litter size among high-dose rats were slightly lower than the control group, but within the laboratory's historical control ranges. U.S. EPA (1998) reported the same effects on body weight gain, liver weight and peroxisome proliferation for the F1 generation as seen in F0 rats. ECHA (2018) reported that the F1 generation was also evaluated for neurobehavioral effects, hematology, blood chemistry, peroxisome proliferation and histopathology, but did not report any effects. It is not clear whether there were no effects, or whether effects from the 13-week study were reported as a separate repeated dose toxicity study.[7]

As discussed above, hepatic hypertrophy and increased liver weight resulting from the induction of metabolizing enzymes is an adaptive response (Hall et al., 2002; U.S. EPA, 2005), and peroxisome proliferation is not relevant to humans (Felter et al., 2018). U.S. EPA (2008) and ECHA (2018) also reached these conclusions. Based on these results, this study identifies a NOAEL of 1000 mg/kg-day for systemic and reproductive toxicity.

In a study summarized from the Russian literature, Larionov and Cherkasova (1977) administered ATBC (purity not reported) as a milk solution continuously in the diet to groups of male and female mice and rats (strains and group sizes not reported) at target doses of 0, 50 or 250 mg/kg-day for 1 year. During the ninth month of the study, animals from each group were cross-mated and embryotoxicity was evaluated. Slight, transient changes were observed in body weights, cerebral perfusion pressure, and hematology among high-dose animals, but no parameters differed substantially from controls towards the end of the study and these changes were considered by the researchers to be adaptive in nature. No changes were observed among low-dose animals. No effects were observed in male gonads from treated rats or mice, and the spermatogenesis index for high-dose rats and mice was within the range for control animals. There was a decrease in desquamatosed spermatogenic epithelium in high-dose males. However, there was no effect on fertility or litter size. Offspring from high-dose animals weighed slightly more than offspring from control animals and were slightly longer on average. The physiological development of mice and rat pups (including timing for eye opening and the appearance of body hair) was unaffected by treatment. Based on the available report, 250 mg/kg-day appears to be a NOAEL for systemic and reproductive and developmental toxicity in this study. However, the lack of methodological details limits interpretation and consideration of these data.

Rasmussen et al. (2017a) evaluated the effects of ATBC on female mice at low doses, with the goal of more closely mimicking human exposure. Groups of 7-8 cycling CD-1 mice, aged 60

[7] It is possible that the 13-week study is the study summarized in Section 5.3 as Anonymous (2005, as cited by ECHA, 2018). However, that study summary states that only 10/sex/dose were exposed, and does not mention a recovery phase.

days were monitored for their estrous cycle for 20 days prior to beginning exposure. Beginning on postnatal day 88, the mice received either tocopherol-stripped corn oil, or 5 or 10 mg/kg-day ATBC (via oral instillation into their cheek pouch) for 15 days. After 15 days of dosing, the females were mated with a proven breeder male. Cohabitation with the male continued until gestation day 18, after which the females were separated and allowed to litter. Ovarian sections were evaluated to count and classify ovarian follicles and corpora lutea. There was no dose- or time-related effect on body weight gain, although there were a few days when the high dose was significantly ($p<0.05$) less than control. ATBC dosing did not affect the estrous cycle length compared to the controls, and there was no effect on the time spent in different stages of the cycle. There was a significant ($p<0.05$) decrease in cycle length between pre- and post-dosing at 5 mg/kg-day. Similar less significant differences were seen in the other two groups. There was no effect on reproductive indices including: days to conception, gestation length, litter size and weight, implantation sites, corpora lutea, implantation loss, and fetal sex ratio. There was no effect on maternal gross organ morphology or organ weight of the uterus, kidney, adrenals, liver or ovaries. There was an increase in relative spleen weight ($p<0.05$) at 5 mg/kg-day, but not at 10 mg/kg-day. There was a dose-related decrease in healthy ovarian follicles (significant, $p<0.05$ at 10 mg/kg-day), although there was no corresponding increase in atretic follicles. The toxicological significance of this finding is unclear, since there was no impact on fertility. Furthermore, the existing well-conducted reproductive toxicity studies (Robbins, 1994, as cited by U.S. EPA, 2008; ECHA, 2018; Chase and Willoughby, 2002, as cited by U.S. EPA, 2008; ECHA, 2018) found no effect on fertility at doses up to 1000 mg/kg-day, although it is noted that they were conducted with rats, not mice, and so are not directly comparable. Rasmussen and colleagues (2017b) also conducted an *in vitro* study evaluating ovarian follicle growth, as described in Section 5.7.

5.5 Prenatal, Perinatal, and Post-natal Toxicity

The only available developmental toxicity data are from the F1 pup evaluations summarized in Section 5.4 from the reproductive toxicity studies and from Larionov and Cherkasova (1977). No studies have conducted a full evaluation of developmental toxicity, including histopathological evaluation for skeletal and visceral alterations.

5.6 Genotoxicity

Available data suggest that ATBC is not genotoxic. As summarized by Versar (2010), ATBC did not induce reverse mutation in various strains of *Salmonella typhimurium* (Gollapudi and Linscombe, 1988; Heath and Reilly, 1982; San and Wagner, 1991), forward mutation in L5178Y mouse lymphoma cells (Bigger and Harbell, 1991) or forward mutation at the HGPRT locus of Chinese hamster ovary (CHO) cells (Dow Chemical Company, 1991; Linscombe et al., 1991) in the presence or absence of metabolic activation. ATBC was also negative in *in vitro* tests for chromosomal aberrations in rat lymphocyte cells (Dow Chemical Company, 1988; Linscombe et al., 1991) and an assay for unscheduled DNA synthesis in primary hepatocytes of male Han-Wistar rats treated with 800 or 2000 mg/kg of ATBC by gavage (Fellows, 1999)..

5.7 Mechanistic Studies

In a study related to the *in vivo* study described in Section 5.4, Rasmussen et al. (2017b) evaluated the effects of *in vitro* exposure of ovarian antral follicles to ATBC. Antral follicles were isolated from female CD-1 mice on PND 32-37 and treated with vehicle control, or ATBC at 0.001 - 1000 μg/mL for 24-72 hours. Exposure to ATBC had no effect on the growth rate of the follicles and no effect on follicle viability. The authors reported a statistically significant ($p<0.05$) increase in the percentage of non-growing follicles at 0.01 μg/mL, but no significant differences at lower or higher doses. The authors also reported increased DNA fragmentation at the same concentration, but did not test other concentrations of ATBC. The toxicological implications of these findings are uncertain in the absence of a dose-response.

Ohta et al. (2003, as cited by SCENIHR, 2016) investigated estrogenic and androgenic properties of ATBC *in vitro* and estrogenic activity *in vivo*. This study found no evidence of estrogenic activity in ligand-binding assays *in vitro* or *in vivo* in ovariectomized Sprague-Dawley rats that received single oral doses of 0.5 or 500 mg/kg ATBC.

Strajhar et al. (2017) investigated the effect of ATBC on production of steroids and gene expression in human adrenal H295R cells, a cell line that is used for the validated OECD test guideline 456 to identify potential endocrine disrupting chemicals based on changes in testosterone and estradiol production. Increased expression of CYP11B2 and CYP21A2 were observed following exposure to ATBC. Exposure to ATBC also increased or tended to increase CYP11B1 expression, as well as increase levels of corticosterone, aldosterone, and progesterone at the highest concentration tested (10 μM). The authors stated that this concentration is unlikely to occur *in vivo*, but that their study results may be useful for hazard identification, particularly in the context of exposure to mixtures.

In another mechanistic study, Takeshita et al. (2011) evaluated the activation of the steroid and xenobiotic receptor (SXR) (also known as pregnane X receptor [PXR]) by several chemicals, including ATBC. The study authors noted that SXR is expressed at high levels in the liver and intestine, and regulates the expression of the metabolic enzyme CYP3A4. In the study, ATBC activated the expression of SXR in CV-1 monkey kidney fibroblasts cotransfected with a human SXR expression vector and a reporter gene. Increased expression of CYP3A4 was also seen in a cell line derived from a human colon epithelial tumor (S174T), in which SXR is endogenously expressed. Increased expression of CYP3A4 was not seen in ATBC-exposed human liver cells. These results suggest that ATBC may be able to induce metabolism in cultured cells. Rasmussen et al. (2017a) noted that SXR receptor and its transcriptional target are expressed in the ovary, and hypothesized that the effects on ovarian follicles reported by Rasmussen et al. (2017a, 2017b) may be related to altered CYP3A4 altering steroid levels.

5.8 Mode of Action

Hepatic Peroxisome Proliferation

Overall, the weight of evidence from a large number of studies supports the existence of an ATBC-induced PPARα-dependent MOA for liver effects, and for liver tumor formation in rodent models (Corton et al., 2014; Felter et al., 2018). As described by Felter et al. (2018), the key events for this MOA are: 1) activation of PPARα, 2) alteration of cell growth pathways, 3) alteration in hepatocyte fate including increased cell proliferation and decreases in apoptosis, and 4) clonal expansion leading to tumors. In rodents, peroxisome proliferation is a well-studied MOA for effects on the liver, and for the formation of liver tumors. Peroxisome proliferators, such as DEHP, cause liver-related changes that include increased relative liver weights due to hepatocellular hypertrophy and proliferation, increased replicative DNA synthesis, increased number and size of peroxisomes (ultrastructural effects), and induced peroxisomal and microsomal fatty acid-oxidizing enzymes, among other changes. In humans, activation of PPARα does not lead to increased relative liver weights, oxidative enzyme induction or other responses typically associated with sustained PPARα activation observed in wild-type mice (Felter et al., 2018; Ito et al., 2012). The weight of evidence supports the conclusion that a PPARα MOA is either "not relevant" or "unlikely to be relevant" in humans (Felter et al., 2018).

ATBC has been evaluated in chronic assays in Wistar rats in a study conducted according to modern test guidelines (Anonymous, 2005, as cited by ECHA, 2018), as well as in a study in Sherman rats conducted under older test methods (Soeler et al., 1950). There was no increase in tumors, particularly liver tumors, in either study, consistent with the idea that the peroxisome proliferative activity of ATBC is weak. However, most, if not all, of the liver-related effects of ATBC are related to peroxisome proliferation, including increased liver weight and hepatocellular hypertrophy, and possibly enzyme induction.

MOA for Kidney Effects

No data were located that systematically investigated the MOA for effects of ATBC on the kidney, and there were no discussions of such an MOA in any of the authoritative assessments reviewed. However, the data presented in this assessment suggest that an initial hypothesis of an MOA is possible. In an *in vitro* study with adrenal cells (Strajhar et al., 2017), ATBC induced CYP11B2 (aldosterone synthase) and CYP21A (a hydroxylase involved in the biosynthesis of the steroid hormones aldosterone and cortisol). Induction of aldosterone can lead to increased sodium retention. Increased sodium was reported in a range-finding 13-week study in Wistar rats (Anonymous, 2003, as cited by ECHA, 2018), although there was no significant change in sodium in another 13-week study, conducted in Sprague Dawley rats up to 1000 mg/kg-day (Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014). Increased aldosterone would be expected to increase hydrogen ion excretion, decreasing the pH of the urine. Decreased urinary pH was seen in the subchronic study with Sprague-Dawley rats at 1000

mg/kg-day (Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014) and in interim evaluations of Wistar rats exposed to 300 or 1000 mg/kg-day for 1 year (Anonymous, 2005, as cited by ECHA, 2018). However, decreased pH would generally lead to *increased* uric acid stones, while *decreased* crystals were seen in the Jonker and Hollanders (1991) study, and there was no effect on stones in the 1-year study or its chronic counterpart. Thus, most of the data are consistent with this proposed MOA, but there are some inconsistencies. Additional evaluation of the hypothesized key events, including *in vivo* evaluation of CYP11B2, CYP21A, and aldosterone levels would be useful, to see if they are affected at the doses associated with the pH changes. The significance of this hypothesized MOA is unclear, in the absence of adverse effects on the kidneys or on metabolic balance.

5.9 Lowest Hazard Endpoints by Organ System and Exposure Duration

Available toxicity studies demonstrate that the repeat dose toxicity of ATBC is low. The primary observed effects were decreased body weight gain and increased liver weight. For some studies, insufficient information was available to assess whether the magnitude of the decrease in body weight was sufficient to be considered adverse.

Decreased body weight (magnitude not reported) was observed in one subchronic study in Sprague-Dawley rats at 1000 mg/kg-day (Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014), but not in another study in Wistar rats at the same dose (Anonymous, 2003, as cited by ECHA, 2018). Decreased body weight of >10% was seen in male and female Wistar rats at 1000 mg/kg-day in a chronic study (Anonymous, 2005, as cited by ECHA, 2018). Terminal body weight was also increased by 11% in male rats at 300 mg/kg-day, but at least some of the decrease may have been due to decreased food consumption, which occurred at this dose and at 1000 mg/kg-day. In a 2-generation reproductive toxicity study (Robbins, 1994, as cited by U.S. EPA, 2008 and ECHA, 2018), decreased body weight of an unspecified magnitude was seen in males at 300 mg/kg-day, and in females at the end of gestation at 1000 mg/kg-day, but not at other times. In a one-generation reproductive toxicity study, there were slight decreases in body weight that apparently did not reach statistical or biological significance, even at the high dose of 1000 mg/kg-day (Chase and Willoughby 2002, as cited by U.S. EPA, 2008 and ECHA, 2018).

Increased relative liver weight was seen in the subchronic and chronic studies at 1000 mg/kg-day (Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014; Anonymous, 2003, as cited by ECHA, 2018; Anonymous, 2005, as cited by ECHA, 2018). In the Wistar rat studies, increased relative liver weight was accompanied by hepatocyte hypertrophy. Increases in liver weight (and in some cases hepatocyte hypertrophy) may have been secondary to decreased body weight (for the changes in liver weight), enzyme induction, and/or peroxisome proliferation. The increased relative liver weight was generally considered non-adverse in the absence of other findings (Hall et al., 2012).

No reproductive toxicity was seen in rats in a 2-generation study (Robbins, 1994, as cited by U.S. EPA, 2008 and ECHA, 2018) at doses up to 1000 mg/kg-day, but the study is limited by the absence of evaluation of sperm parameters, and by the absence of a complete histopathological evaluation of reproductive tissues. There was also no reproductive toxicity at doses up to 1000 mg/kg-day in a 1-generation study that included sperm evaluation of the F1 generation (Chase and Willoughby 2002, as cited by U.S. EPA, 2008 and ECHA, 2018). In contrast, Rasmussen et al. (2017a) reported a decrease in healthy ovarian follicles in mice at 10 mg/kg-day, but no effect on reproductive indices. The toxicological significance of the decreased follicles is unclear.

No standard developmental toxicity studies are available. No evidence of developmental toxicity or effect on sexual maturation was seen in the 1-generation reproductive toxicity study at doses up to 1000 mg/kg-day (Chase and Willoughby 2002, as cited by U.S. EPA, 2008 and ECHA, 2018), and no developmental toxicity was observed in the 2-generation study at doses up to 1000 mg/kg-day (Robbins, 1994, as cited by U.S. EPA, 2008 and ECHA, 2018). However, neither study included a complete histopathological evaluation for visceral or skeletal abnormalities.

ATBC was not carcinogenic in either a GLP-compliant combined chronic toxicity/carcinogenicity study in rats (Anonymous, 2005, as cited by ECHA, 2018), or in an older study with Sherman rats that used small group numbers (Soeler et al., 1990). ATBC has not been tested for carcinogenicity in a second species.

5.10 Uncertainties and Data Gaps

Several uncertainties of varying importance were identified in this assessment.

Database:

The overall database on ATBC is fairly complete, including many of the key studies (repeated dose, carcinogenicity, multi-generation reproductive toxicity). High-quality studies are available only in rats, although there is one non-guideline cat study and one nonstandard reproductive toxicity study in mice. Data were available primarily for the oral route. No inhalation data were available, and the only dermal data were irritation and sensitization studies. Sensitization has been evaluated in both humans and guinea pigs, and irritation has been evaluated in rabbits and humans.

Key data gaps are the lack of standard developmental toxicity studies, and the lack of high-quality repeated dose toxicity data in a second species. Most of the studies summarized in this assessment were available only in secondary sources or from robust summaries, without primary data, making it difficult to independently evaluate the toxicological significance of the reported effects. There is some additional uncertainty regarding the extent of the sperm evaluation in the 1-generation study. It is not clear whether a complete evaluation of reproductive tissues has been conducted in the context of a reproductive toxicity study. Such an evaluation has been conducted in the context of a 2-year study.

Hazard:

Body weight: There is uncertainty in the identification of effect levels for changes in body weight. In many cases, information was available only on statistical significance, not on the magnitude of change. In other cases, the decreased body weight was accompanied by decreased food consumption and it is not clear how much, if any, of the decrease is related to poor palatability of the chemical in the diet.

Kidney: As noted in the context of the MOA discussion, the data are consistent with the suggestion that ATBC alters aldosterone levels, with an impact on serum levels of sodium and urinary pH. However, there is uncertainty in the hypothesized MOA, in the absence of *in vivo* data on enzyme levels and levels of aldosterone, particularly at doses in the range of the observed effects. In addition, the significance of such changes is unclear, in the absence of kidney stones or any adverse impact on pH balance of the blood.

Reproductive toxicity: The significance of the effects on ovarian follicles reported by Rasmussen et al. (2017a, 2017b) is unclear, particularly in the absence of effects on reproductive function at doses orders of magnitude higher.

Carcinogenicity: ATBC was negative in a well-conducted carcinogenicity study in rats, but it has not been tested for carcinogenicity in a second species.

Table 3. Summary of NOAELs/LOAELs Identified for ATBC by Organ System

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
Wistar rat (M&F) (4 mixed sex/dose) Finkelstein and Gold, 1959	6 weeks Diet 0, 5% or 10% About 0, 7620 or 15,240 mg/kg-day	Systemic	NOAEL = 7620 LOAEL = 15,240	Reduced body weight gain and diarrhea	Study is limited by incomplete methods compared to modern test guidelines
Sprague-Dawley rat (M&F) (20/sex/dose) OECD guideline 408	13 weeks Diet (% not reported) Actual doses M: 0, 101, 302, 996 mg/kg-day	Systemic	NOAEL = 996 (M) LOAEL = None NOAEL = 999 (F) LOAEL = None	No adverse effects	Study is limited by the absence of quantitative details in the available secondary sources. Changes in serum chemistry not considered treatment related. Changes in body weight were

[8] All effect levels as identified by the authors of this assessment. Effect levels identified by previous assessments are in the comments column

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008a; U.S. EPA, 2014	F: 0, 100, 296, 999 mg/kg-day	Liver	NOAEL = 996 (M) LOAEL = None NOAEL = 999 (F) LOAEL = None	Increased relative liver weight among mid-dose males and high-dose rats of both sexes	"slight" and not statistically significant. U.S. EPA (2008a, 2014) identified the mid dose as a NOAEL, based on decreased body weight and organ weight changes at the high dose
Wistar rat (M&F) (10/sex/dose) OECD Guideline 408 Anonymous, 2003, as cited by ECHA, 2018	13 weeks Diet (% not reported) Target doses: 0, 100, 300, 1000 mg/kg-day	Systemic	NOAEL = 1000 LOAEL = None	None	Sporadic non-adverse changes in clinical chemistry This is a range-finding study for the 2-year Wistar rat bioassay; limited histopathology
		Liver	NOAEL = 1000 LOAEL = None	Increased liver weight and hypertrophy	
Wistar rat (M&F) (20/sex/dose) GLP compliant	52 weeks Diet Target:	Systemic	NOAEL = 300 (M) LOAEL = 1000 (M) NOAEL = 1000 (F) LOAEL = None (F)	Decreased body weight	Changes in clinical chemistry were slight or were sporadic and did not progress, and so were not considered adverse.

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
Anonymous, 2005, as cited by ECHA, 2018	0, 100, 300, 1000 mg/kg-day	Liver	NOAEL = 1000 LOAEL = None	Increased liver weight and hypertrophy were considered adaptive	This is a satellite study to the 2-year bioassay
Wistar rat (M&F) (50/sex/dose) GLP compliant Anonymous, 2005, as cited by ECHA, 2018	2 years Diet Target: 0, 100, 300, 1000 mg/kg-day	Systemic	NOAEL = 300 LOAEL = 1000	Decreased body weight	Well-conducted study. Body weight was significantly decreased ($p<0.01$) by 11% and 16% in mid- and high-dose males, respectively, and by 13% in high-dose females. Food consumption was also reduced in mid- and high-dose males, but by less than the decrease in body weight
		Liver	NOAEL = 300 (M) LOAEL = 1000 (M) NOAEL = 1000 (F) LOAEL = None	Single cell necrosis of hepatocytes. Increased liver weight and hypertrophy were considered adaptive	
		Cancer	NOAEL = 1000 LOAEL = None	No treatment-related tumors	
Sherman rat 20/dose (sex not specified) 40 controls Additional 10/dose exposed to 100 or 1000, and 20 controls for 1 year Soeler et al., 1950	2 years Diet 0, 200, 2000 or 20,000 ppm Target doses: 0, 10, 100, 1000 mg/kg-day	Systemic	NOAEL = 1000 LOAEL = None	Decreased body weight gain not reproducible	Study is limited by incomplete testing and documentation relative to modern methods. Animals that died early had lung inflammation and pneumonitis, suggesting a possible infection.
		Cancer	NOAEL = 1000 LOAEL = None	No tumors	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
Reproductive/Developmental Toxicity					
Sprague-Dawley rat (M&F) (30/sex/dose) Robbins, 1994, as cited by U.S. EPA, 2008a; ECHA, 2018	2-generation, F0 beginning 11 weeks premating (M) or 3 weeks premating (F); during mating and lactation periods for 2 generations Diet Target doses: 0, 10, 100, 1000 mg/kg-day	Systemic	NOEL = 100 (M) LOEL = 300 (M) NOAEL = 1000 (F) LOAEL = None (F)	Decreased body weights of unspecified magnitude among F1 parental males	Slightly higher pup mortality and slightly reduced pup body weights were considered secondary to reduced maternal water consumption. Study is limited by the absence of evaluation of sperm parameters, and by the absence of a complete histopathology evaluation of reproductive tissues.
		Reproductive	NOAEL = 1000 LOAEL = None	No adverse effects	
		Developmental	NOAEL = 1000 LOAEL = None	No adverse effects	
Han Wistar rat (M&F) (25/sex/dose) Chase and Willoughby, 2002, as cited by U.S. EPA, 2008a; ECHA, 2018	1-generation, F0 beginning 4 weeks premating, during mating and lactation period, and continuing for 13 weeks after lactation Diet Target doses: 0, 100, 300 or 1000 mg/kg-day	Systemic	NOAEL = 1000 LOAEL = None	No adverse effects	Increased liver weight, hypertrophy and peroxisome proliferation in F0 and F1 rats not considered adverse. Assessment included evaluation of sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males), sperm assessments in F1 rats, and of sperm or estrous cycle in F0 rats. The number of implantations and litter size among high-dose rats were
		Reproductive	NOAEL = 1000 LOAEL = None	No adverse effects	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
					slightly lower than the control group, but they were within the laboratory's historical control ranges, and not considered adverse by U.S. EPA (2008a).
CD-1 mouse (F) 7-8/dose Rasmussen et al. 2017a	Oral into cheek pouch 15 days of dosing, followed by mating and parturition 0, 5, 10 mg/kg-day	Reproductive	NOEL = 5 LOEL = 10	Decreased number of healthy ovarian follicles, in absence of effect on atretic follicles	No effect on estrous cyclicity, fertility, corpora lutea No microscopic evaluation of fetuses. Sporadic decreases in body weight. Relative spleen weight was significantly increased at 5 mg/kg-day, but not 10 mg/kg-day
		Developmental	NOAEL = 10 LOAEL = None	No effect on litter size and weight or fetal sex ratio	
		Systemic	NOAEL = 10 LOAEL = None	No adverse effect	
Rat (strain not specified) (M&F) #/dose not specified Larionov and Cherkasova, 1977	1 year including mating and gestation, with mating after 9 months Diet Target doses: 0, 50 or 250 mg/kg-day	Systemic	NOAEL = 250 LOAEL = None	No adverse effect	Only the study summary is available; no primary data were presented. The study is limited by the lack of details on methods and results. There was no separate reporting for rats and mice
		Reproductive	NOAEL = 250 LOAEL = None	No adverse effect	

6 Exposure

The use of ATBC in consumer products has been described in Section 3 of this report.

The general population may be exposed to ATBC via dermal contact with consumer products, oral contact via mouthing of products (e.g., children's toys), by the ingestion of food or beverages containing this compound, or by ingestion of foods stored in plastic materials containing ATBC (Versar, 2010; HSDB, 2018).

CPSC (2014) estimated infant exposure to plasticizers from mouthing soft plastic objects (except pacifiers) and determined that ATBC, out of the four phthalate substitutes evaluated, had the highest overall average exposure, and that exposure was comparable to that estimated for DINP. Based on mean migration rate and mean mouthing duration, the mean exposure was estimated at 2.3, 1.5, and 1.4 μg/kg-day for babies aged 3 - <12 months, 12 months - <24 months, and 24 - <36 months, respectively. The highest upper bound exposure was 5.1 μg/kg-day (babies aged 3 - <12 months), based on mean migration rate and 95th percentile mouthing duration. CPSC (2014) noted that the migration rate for ATBC generally increased with increasing concentration.

Bui et al. (2016) reported estimated intake rates for ATBC calculated by Stuer-Lauridsen et al. (2001, as cited by Bui et al., 2016) using the EASE (Estimation and Assessment of Substance Exposure) model: 4.36 x 10^{-3} μg/kg-day (inhalation, oral and dermal intake (specific population and activity not specified) and 60 μg/kg-day in children using teething rings (inhalation and dermal uptake). Bui et al. (2016) also reported an estimate of 0.02 μg/kg-day from dietary uptake as a food additive (; ECDGE, 2000; as cited by Bui et al., 2016). Bui et al. (2016) noted that the intake rates for alternative plasticizers are not based on biomonitoring data and that important uptake routes may not have been included due to lack of studies measuring exposure.

Subedi et al. (2017) reported on the levels of ATBC in indoor dust in samples from 11 childcare facilities in 7 U.S. states, 5 salons in 3 states, and 11 homes in 5 states. The overall range of concentrations was 45 – 4860 μg/g. Levels in salons were 3-10 times those in homes and child care facilities. The authors estimated the daily intake from the dermal route to be ≤1 ng/kg-day for all age groups; the highest estimated oral intake was 1340 ng/kg-day for infants in childcare facilities.

Versar (2010) reported on a review by Sheftel (2000), in which the migration of ATBC from several products was documented. Sheftel found ATBC has a higher leaching rate than that of DEHP, as determined from a study of its migration from PVC films. Migration of ATBC from food packaging material to cheese that had been wrapped in ATBC-plasticized vinylidene chloride copolymer films was reported to be 6.1 ppm, or 2.0-8.0 mg/kg in the cheese itself, after exposure to the film for 5 days at temperatures of 5ºC. The ATBC concentration in similarly wrapped cake (after 5 days at 5ºC) was reported to be 3.2 ppm. Migration of ATBC from plasticized vinylidene chloride-vinyl chloride copolymer film into fatty or water rich foods was found to be as low as 0.4 mg/kg after minimal contact during microwave cooking of a soup, and

up to 79.8 mg/kg for use of the film during the microwave cooking of peanut-containing cookies. Migration of ATBC from plasticized polyvinylidene chloride-polyvinyl chloride films during microwave heating was determined to be 73.9 mg/L into olive oil after heating for 10 minutes, and 4.1 mg/L into water after heating for 8 minutes (Sheftel, 2000, as cited by Versar, 2010). ATBC was also determined to have a higher leaching rate from medical tubing than DEHP (Welle et al., 2005, as cited by SCENIHR, 2016).

Dreyfus (2010) measured a mean migration rate of 4.4 µg/cm^2/min migration rate of ATBC into simulated saliva (range 0.4 to 14.0 µg/cm^2/min, SD 4.38) (Dreyfus, 2010, as cited by CPSC, 2014). The high extractability of ATBC results in higher migration into aqueous solutions (and thus potentially higher exposure) than seen with some other phthalate replacements.

Versar (2010) reported that occupational exposure to ATBC may occur through inhalation and dermal contact at workplaces where the compound is produced or used. The National Institute for Occupational Health and Safety (NIOSH) NOES Survey, 1981-1983, statistically estimated that 106,668 workers (98,183 females) may be exposed to ATBC in the U.S. (NIOSH, 1983; as cited by Versar, 2010). U.S. EPA reported that six facilities in the U.S reported estimates of workers exposed to ATBC from fewer than ten up to 99 per plant, but indicated that this number may be greatly underestimated (HSDB, 2018).

Biomonitoring

Alves et al. (2017) detected ATBC in 49% of fingernail samples and detected its major *in vitro* metabolites in up to 95% of samples in a group of about 60 Norwegian volunteers. They did not detect ATBC in urine, but one ATBC metabolite was detected in 3% of urine samples. The authors noted that detection in fingernails implies that the ATBC is either retained for a long duration in the body, or there is ubiquitous exposure to ATBC.

7 Discussion

7.1 Toxicity Under FHSA

ATBC does not fit the designation of "acutely toxic" under the Federal Hazardous Substances Act (FHSA) (16 CFR§1500.3(c)(2)(i)(A)) following single oral exposures. **Acute LD_{50} values for ATBC in rats were reported to be >30,000 mg/kg (Finkelstein and Gold, 1959).** It is **not known whether ATBC fits the designation of "acutely toxic" under the FHSA** following dermal or inhalation exposure in the absence of related acute lethality studies.

ATBC was not irritating to the skin of rabbits in three repeated dose studies (Anonymous, 1975, as cited by ECHA, 2018), and was slightly irritating to the eyes of rabbits (Anonymous, 1975, as

cited by ECHA, 2018). No irritation or sensitization was observed in a study of patch testing of 59 male and female volunteers (Hill Top Research, 1978, as cited by Johnson, 2002; ECHA, 2018). There was also no sensitization observed in a modification of the Magnusson and Kligman guinea pig maximization test (Anonymous, 2001a, as cited by ECHA, 2018).

Sufficient animal data exist to support the conclusion that ATBC can be considered "toxic" under the FHSA due to its toxicity following subchronic and chronic exposures.

Decreased body weight was observed following subchronic (Jonker and Hollanders, 1991, as cited in U.S. EPA, 2008; U.S. EPA, 2014) and chronic exposures (Anonymous, 2005, as cited by ECHA, 2018).

Based on the results of standard 2-generation (Robbins, 1994, as cited by U.S. EPA, 2008 and ECHA, 2018) and 1-generation (Chase and Willoughby 2002, as cited by U.S. EPA, 2008 and ECHA, 2018) reproductive toxicity studies in rats, it appears that ATBC is not a reproductive toxicant. However, there are some uncertainties in this conclusion, in light of the observation by Rasmussen et al. (2017a) of a decrease in healthy ovarian follicles in mice at doses well below those that did not impair reproductive function in mice and rats.

The available data are insufficient to assess the developmental effects of ATBC. No standard developmental toxicity studies are available that evaluated the fetuses histopathologically. However, no developmental toxicity or effects on sexual maturation were seen in a 1-generation reproductive toxicity study (Chase and Willoughby 2002, as cited by U.S. EPA, 2008 and ECHA, 2018) or in a 2-generation reproductive toxicity study (Robbins, 1994, as cited by U.S. EPA, 2008 and ECHA, 2018).

There is sufficient evidence to support the conclusion that ATBC is not a direct acting genotoxicant. ATBC did not induce gene mutations in bacteria (Gollapudi and Linscombe, 1988; Heath and Reilly, 1982; San and Wagner, 1991), or mammalian cells (Bigger and Harbell, 1991; Dow Chemical Company, 1991; Linscombe et al., 1991), and was negative in *in vitro* tests for chromosomal aberrations in rat lymphocyte cells (Dow Chemical Company, 1988; Linscombe et al., 1991).

ATBC was not carcinogenic in a GLP-compliant combined chronic oral toxicity/carcinogenicity study in rats (Anonymous, 2005, as cited by ECHA, 2018), but it has not been tested for carcinogenicity in a second species.

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APPENDIX 1

Search Terms Used

"2-(Acetyloxy)-1,2,3-propanetricarboxylic acid, tributyl ester" OR "2-Acetoxy-1,2,3-propanetricarboxylic acid tributyl ester" OR "2-Acetyltributylcitrate" OR "Acetyl tributyl citrate" OR "Acetyltributyl citrate" OR "Acetylcitric acid, tributyl ester" OR "ATBC" OR "Citric acid, tributyl ester, acetate" OR "Citroflex A" OR "Tributyl acetyl citrate" OR "Tributyl acetylcitrate" OR "Tributyl citrate acetate" OR "Tributyl O-acetylcitrate" OR 77-90-7

APPENDIX 2

Explanation of Physico-chemical Parameters

The organic carbon normalized solid-water partition coefficient (K_{oc}), also known as the organic carbon adsorption coefficient, is defined as the ratio of the chemical's concentration in a state of sorption (i.e. adhered to soil particles) and the solution phase (i.e. dissolved in the soil water). K_{oc} is crucial for estimating a chemical compound's mobility in soil and the prevalence of its leaching from soil. For a given amount of chemical, the smaller the K_{oc} value, the greater the concentration of the chemical in solution. Thus, chemicals with a small K_{oc} value are more likely to leach into groundwater than those with a large K_{oc} value (http://www.acdlabs.com/products/phys_chem_lab/logd/koc.html).

Henry's law, one of the gas laws formulated by William Henry, states that "at a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid (http://en.wikipedia.org/wiki/Henry's_law)." Henry's Law Constants characterize the equilibrium distribution of dilute concentrations of volatile, soluble chemicals between gas and liquid phases (http://www.epa.gov/athens/learn2model/part-two/onsite/esthenry.htm).

The octanol/water partition coefficient (K_{ow}) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. In recent years, this coefficient has become a key parameter in studies of the environmental fate of organic chemicals. It has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Because of its increasing use in the estimation of these other properties, K_{ow} is considered a required property in studies of new or problematic chemicals (http://www.pirika.com/chem/TCPEE/LOGKOW/ourlogKow.htm).

The bioconcentration factor (BCF) is the concentration of a particular chemical in a tissue per concentration of chemical in water (reported as L/kg). This property characterizes the accumulation of pollutants through chemical partitioning from the aqueous phase into an organic phase, such as the gill of a fish. The scale used to determine if a BCF value is high, moderate or low will depend on the organism under investigation. The U.S. EPA generally defines a high potential BCF as being greater than 5,000; a BCF of moderate potential as between 5,000 and 100; a low potential BCF as less than 100 (http://en.wikipedia.org/wiki/Bioconcentration_factor; http://sitem.herts.ac.uk/aeru/footprint/en/Quest/ecotox.htm).