**CPSC Staff Statement on University of Cincinnati Report
"Toxicity Review for 1,2-Cyclohexanedicarboxylic acid, dinonyl
ester, branched and linear (DINX)"[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 DINX 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

1,2-CYCLOHEXANEDICARBOXYLIC ACID,

DINONYL ESTER, BRANCHED AND LINEAR

(DINX)

Contract No. CPSC-D-17-0001
Task Order No. 003

Prepared by:
Risk Science Center
Department of Environmental Health
University of Cincinnati
160 Panzeca Way, Room G24
Cincinnati, OH 45267

Prepared for:
Kent R. Carlson, Ph.D.
U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814

August 8, 2018

UNIVERSITY OF
Cincinnati

This page intentionally left blank.

Table of Contents

1 Introduction

This report summarizes available data on the identity, physicochemical properties, manufacture, supply, use, toxicity, and exposure associated with 1,2-Cyclohexanedicarboxylic acid, dinonyl ester, branched and linear (DINCH®[1], DINX). 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. Authoritative reviews for general toxicity and physicochemical information were identified in the following databases using the CAS number for DINX 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)

---

[1] DINCH® is a registered trademark of BASF. Although DINCH® is the commonly used abbreviation, the alternate abbreviation DINX is used here to represent the generic chemical, even in studies that used the term DINCH.
.

- FDA (https://www.fda.gov/)
- Google
- 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/)

No new guideline-compliant DINX toxicology studies were identified in the literature searches. The searching of the primary literature identified only studies on toxicokinetics/biomonitoring, mechanism, and mechanistically-oriented studies of reproductive/developmental effects. All of the key toxicity studies were evaluated based on authoritative reviews. Secondary sources identified in the literature search also provided additional details on several of the studies reviewed by Versar (2010).
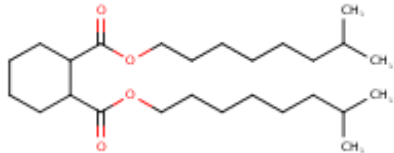
Several additional authoritative reviews (e.g., NICNAS, 2012; Danish EPA, 2014; ANSES, 2016a, SCENIHR, 2016), as well as an independent review (Bhat et al., 2014) have been published since the previous CPSC assessment (Versar, 2010). Several of these reviews had access to the primary studies and provided additional study details that were not previously available. The current assessment focused on the Bhat et al. (2014) review, since the authors had access to the primary studies and provided extensive study descriptions and data. This assessment also relied extensively on the two most recent agency reviews (ANSES, 2016a; SCENIHR, 2016), supplemented to a limited degree by other reviews.

The DINX toxicity section in the SCENIHR (2016) report contains an introductory statement indicating that all included studies were performed under Good Laboratory Practice (GLP) conditions according to Organisation of Economic Co-operation and Development (OECD) guidelines.

2 Physico-Chemical Characteristics

The manufacturer's Material Safety Data Sheet (MSDS) for DINX (BASF, 2006) provides the physical-chemical properties for this compound displayed in Table 1; additional characteristics are from the sources noted.

Table 1: Physicochemical Properties and Identification Information for 1,2-Cyclohexanedicarboxylic acid, dinonyl ester

Chemical Name	1,2-Cyclohexanedicarboxylic acid, dinonyl ester, branched and linear
Synonyms	Diisononyl hexahydrophthalate; Bis(isononyl)cyclohexane 1,2-dicarboxylate; Diisononyl cyclohexane-1,2-dicarboxylate; DINCH; Flocare 35138; Hexamoll; Hexamoll DINCH;
CAS Number	166412-78-8 or 474919-59-0
Structure	 (ChemID*plus*)
Chemical Formula	$C_{26}H_{48}O_4$
Molecular Weight	424.7 g/mol
Physical State	Liquid
Color	Colorless
Melting Point	-54º C (Bui et al., 2016)
Boiling Point	394º C
Vapor Pressure	9.75×10^{-7} mm Hg at 50ºC
Water Solubility	<0.02 mg/L at 25ºC
Log Kow	10
Flashpoint	N/A
Density	0.95 g/cm^3 at 20º C (Bui et al., 2016)
BCF	189
Source	ChemID*plus*, MSDS (BASF, 2006) unless otherwise stated

K_{ow} is the octanol-water partition coefficient. BCF is the bioconcentration factor. (Adapted from Remberger et al., 2005). See Appendix 2 for more detail.

DINX is a non-aromatic hydrogenated ester and appears under CAS number [474919-59-0] in the United States and Canada, and [166412-78-8] in the European Union (BASF, 2008a). DINX is the non-aromatic analog to the ring hydrogenated ester DINP (Figure 1); they contain the same alcohol component. DINX is produced by hydrogenation of DINP in the presence of a catalyst (Wilkes, 2005), and is said to be a suitable replacement to DINP due to its similar plasticizing performance. Additionally, mixtures of diisononyl esters of 1,2-cyclohexanedicarboxylic acid, whose isononyl radicals have a degree of branching from 1.2 to 2.0, are particularly suitable to replace DEHP in PVC applications (U.S. Patent Application 20080188601, 2008).

Figure 1. Conversion of DINP to DINX
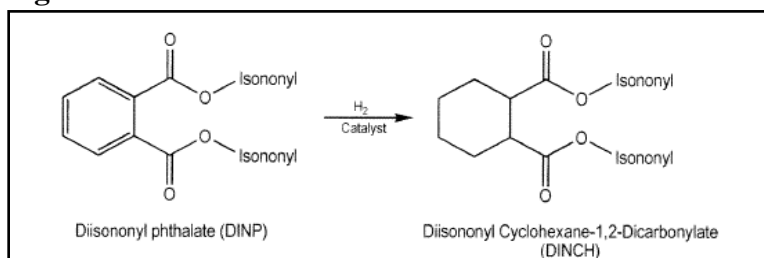
According to the manufacturer's technical leaflet (BASF, 2008a), DINX is a "colorless, clear and practically anhydrous liquid with a hardly noticeable odor." It is soluble in common organic solvents, is essentially insoluble in water, and is miscible and compatible with other monomeric plasticizers commonly used in PVC (BASF, 2008a). The DINX vapor pressure of 9.75×10^{-7} mm Hg (25°C) indicates that it can exist in the ambient atmosphere in both the gas and particle phases. Water solubility and log K_{ow} values for this compound indicate insolubility in water-based solutions. A BCF of 189 is considered moderate according to general EPA guidelines. The organic carbon normalized solid-water partition coefficient (K_{oc}) and Henry's Law Constant were not available for DINX as of December, 2008.

3   Manufacture, Supply, and Use

Manufacture and Supply

In 2013, DINX overall production was 200,000 tons/year (Bui et al., 2016), and thus DINX meets the definition of a HPV chemical. Production was more than 10,000 tons/year in the European Union (Bui et al., 2016).

Use

Hexamoll® DINX was developed by BASF (www.basf.com) for use as a PVC plasticizer and, specifically, to replace DEHP and DINP in products such as food contact applications, child care articles, and children's toys, such as modeling clay (Jobwerx, 2006; ANSES 2016a). DINX was reported in 2013 as being the alternative plasticizer most commonly used in children's toys (INERIS, 2013, as cited by ANSES, 2016a). Other application areas include medical articles (such as blood tubes and packaging for nutrient solutions) and shoes, as well as non-PVC applications such as adhesives, cosmetics, exercise mats, artificial leather, textile coatings, printing inks, and erasers (BASF, 2007; Bui et al., 2016; SCENIHR, 2016).

DINX has gained approval from the European Food Safety Authority (EFSA), the Japan Hygienic PVC Association (JHPA), and the German Institute for Risk Assessment (German BfR) for use as a food contact substance (BASF, 2008b). According to the petitioner (presumably BASF) to the EFSA, DINX is appropriately used as a plasticizer in PVC in concentrations up to 40%. It is used in PVC cling films for fresh meat packaging (10%), for aqueous food and fruits and vegetables (35%), artificial corks (35%), sealing gaskets for

beverage containers (35%), flexible tubes for beverages (40%), in other foods (12%), and on conveyor belts for fatty foods (12%) (EFSA, 2006).

4 Toxicokinetics

The toxicokinetics of DINX has been summarized by SCENIHR (2016) and Bhat et al. (2014). The animal toxicokinetic data were derived from two rat studies, one in which Wistar rats were administered a single gavage dose of 20 or 1000 mg/kg radiolabeled DINX (BASF AG, 2005a), and one in which male and female Wistar rats were administered a single gavage dose of radiolabeled DINX at 50, 300, or 1000 mg/kg (BASF AG, 2003a).

Absorption

DINX is rapidly absorbed in both humans and rats after oral administration, but absorption is saturable and incomplete. In a study in rats, absorption was estimated to account for 40-49% of the administered dose at the low dose tested (20 mg/kg), but only 5-6% at the high dose (1000 mg/kg), based on the amount of radioactivity in bile and urine (SCENIHR, 2016). No appreciable sex-related differences in absorption were noted (Bhat et al., 2014).

Distribution

Absorbed DINX is rapidly and widely distributed to organs and tissues, and there is no indication of bioaccumulation (SCENIHR, 2016).

Metabolism

DINX is metabolized initially through hydrolysis to the monoisononyl ester (MINX), which can be further metabolized in 2 ways, either by conjugation to glucuronic acid or by the hydrolysis of the mono ester to cyclohexane dicarboxylic acid (CHDA) (SCENIHR, 2016). CHDA is regarded as an unspecific metabolite of DINX (SCENIHR, 2016). The metabolic profiles in rats are similar to that in humans. The metabolic profile in the urine and feces of exposed rats was similar under conditions of single and repeated exposures DINX, and the rate or route of excretion was also similar (Bhat et al., 2014).

Elimination

Elimination of DINX is rapid. In humans, 75% to over 90% of an oral dose of DINX was excreted within 24 hours, with nearly complete excretion within 70 hours (Volkel et al., 2016; Koch et al., 2013). Absorbed DINX was eliminated in the bile (primarily as the glucuronic acid conjugate of the monoisononyl ester) and the urine (primarily as CHDA) (Bhat et al., 2014; Koch et al., 2013). Schutze et al. (2014, 2017) identified oxidized metabolites of the monoester as the main specific urinary metabolites (presumably after CHDA), followed by the unoxidized monoester (MINX). The CHDA metabolite cannot be used to estimate DINCX exposure, due to

its lack of specificity, but the specific DINX metabolites MINX, OH-MINX, oxoMINX, and cx-MINX can be used for biomonitoring. In humans dosed with 50 mg DINX (individual doses of 0.55 to 0.61 mg/kg), 39.2% of the oral DINX dose was excreted as metabolites in urine within 48 hours (23.7% as CHDA, 14.8% as monoesters with oxidative modifications and less than 1% as the simple, non-oxidised monoester) (Schutze et al., 2017; Koch et al., 2013).

SCENIHR (2016) reported that approximately 80% of the radioactivity is excreted 24 hours after dosing rats with radiolabeled DINX, and more than 90% after 48 hours. According to SCENIHR (2106), elimination of radioactivity from the plasma of rats was biphasic after oral administration, with the highest levels in plasma being observed 1 hour after administration. Fecal excretion primarily included unabsorbed DINX, and the percent fecal excretion increased with dose. Glucuronide metabolites in feces accounted for <10% of the dose in rats administered 20 mg/kg, and <0.5% of the dose at 1000 mg/kg.

Schutze et al. (2015) developed and calibrated a multi-compartment kinetic model of DINX in humans. Their results were consistent with urine metabolite from three male volunteers orally dosed with DINX, but the model was not able to replicate the ratios of urine metabolite in data from 24-hour general population samples. Possible explanations provided by the authors were differences in the exposure pattern, or that the controlled-dosing experiments did not fully described the long-term toxicokinetics of DINX.

Bhat et al. (2014) provided key toxicokinetic parameters in male and female rats administered a single dose of 50, 300, or 1000 mg/kg DINX (BASF AG, 2003a). Specifically, both Cmax and AUC (the area under the time-concentration curve) were provided, presumably for the parent compound. Both of these parameters showed a less than linear increase with dose, consistent with the observed induction of metabolic enzymes. The provided kinetic parameters can be used to refine the interspecies extrapolation to humans using the chemical-specific adjustment factor (CSAF) approach (IPCS, 2005) when similar human data become available.

5 Hazard Information[2]

5.1 Acute Single Dose Toxicity

5.1.1 Acute Oral Toxicity

Acute toxicity values were provided for DINX in the SCENIHR (2016) and Bhat et al. (2014) summaries and the BASF studies (1999). Acute LD_{50} values for DINX in rats were >5000 mg/kg for oral exposure (olive oil vehicle; BASF, 1999c, 1999d as cited in Bhat et al., 2014).

---

[2] Where available, this report provides significance level p values in all sections. However, source secondary references often report only that a change was significant without reporting the p level. If no p level is reported in

5.1.2 Acute Dermal Toxicity

The acute LD_{50} value for DINX was >2000 mg/kg following a semiocclusive 24 hour dermal exposure to 5 male and 5 female rats (BASF, 1999c, 1999d as cited in Bhat et al., 2014).

5.1.3 Acute Inhalation Toxicity

No data on acute inhalation (LC_{50}) were located in the published literature.

5.1.4 Irritation/Sensitization

Rabbits treated dermally with DINX for 14 days had moderate erythema immediately after the patch was removed, and mild to moderate erythema at 72 hours (ANSES, 2016a). ANSES (2016a) and NICNAS (2012) did not consider the results to be sufficiently severe to classify DINX as a dermal irritant. A previous CPSC review (Versar, 2010) reported that DINX was not a skin irritant in rabbits. DINX was not an eye irritant in rabbits or a skin sensitizer in a guinea pig maximization test (BASF, 1999a, 1999b, 2001a, 2002a, 2004b as cited in Bhat et al., 2014). No additional information was provided in the SCENIHR (2016) or Bhat et al. (2014) summaries of these studies.

5.2 Repeated Dose Toxicity

Induction of Hepatic Metabolic Enzymes

A discussion on the toxicological significance and adversity of metabolic enzyme induction is useful to inform identification of effect levels in this and longer-term studies. Hall et al. (2012) reviewed the state of the science related to liver enzyme induction and liver hypertrophy, with a goal of distinguishing adverse and non-adverse changes. They rationalized that pathologies related to increased gamma-glutamyltransferase (GGT) should be evaluated using a weight of evidence approach because increased GGT can be seen following enzyme induction and/or cholestasis (adverse partial obstruction of the intrahepatic bile ducts). The authors concluded that in the absence of biologically significant increases in transaminase activity (e.g. alanine aminotransferase (ALT) or aspartate aminotransferase (AST)) and liver histopathology, increased GGT was not considered adverse, even in the presence of increased liver weight or liver hypertrophy. Decreased bilirubin due to higher rates of conjugation and excretion in bile was also noted as a secondary consequence of liver enzyme induction. Increases in liver weight of 150% or less (in the absence of degenerative or necrotic liver changes at any dose or duration) were also noted as non-adverse for risk assessment purposes, although such increases could be used for setting the maximal tolerated dose (MTD) and could lead to liver tumors in rats and mice. The authors further concluded that "the use of the term non-adverse is only valid for the

---

this text, the p level was not available in the cited secondary reference, but the significance is presumed to be statistical.

dose and duration of exposure of that chemical as defined by the study in question" when adaptive responses might change to degenerative ones following continued or higher exposures (p. 986).

EPA science policy/guidance on evaluating the adversity of increased liver size/weight (U.S. EPA, 2002) is consistent with the Hall et al. (2012) conclusions. The EPA guidance states that such increases result from an increase in the size of liver parenchymal cells, and are usually an indication that something has changed in the cell, but the change may not be an adverse effect. It may indicate that xenobiotic exposures are causing an increased metabolic response, resulting in the induction of metabolic enzymes. Other liver endpoints, based on clinical chemistry and/or histopathology data, are used in a weight-of-evidence approach to evaluate the adversity of the effect. "In chronic studies, with no other liver toxicity, it is evident that these are not adverse events. Therefore, the dose with only hepatocellular hypertrophy and/or liver size/weight changes should be considered the study No-Observable-Adverse-Effect-Level (NOAEL). The Lowest-Observable-Adverse-Effect-Level (LOAEL) for the study should be the dose which elicits actual hepatotoxicity characterized by toxicologically significant changes in parameters such as clinical chemistry and/or histopathology. On the other hand, it is more difficult to use subchronic studies to determine whether hepatocellular hypertrophy and/or liver size/weight are associated with adverse events. However, a dose with only hepatocellular hypertrophy and/or liver size/weight should be considered the study NOAEL unless there is a known mode of action for toxicity and/or the other study data (e.g., clinical chemistry and histopathology) are not equivocal." The EPA guidance reviewed enzyme induction and its secondary effects on other organs such as the thyroid and concluded that "liver-mediated changes causing perturbations in circulating thyroid hormone levels and disruption of the thyroid-hypothalamus-pituitary axis can cause an adverse effect in the thyroid (e.g., hyperplasia). This cascade of events should be characterized based on available data. In this case, the LOAEL should be based on secondary (thyroid) effects or the liver effects, whichever occurs at a lower dose. (p. 6)"

Study Review

Two repeated-dose lab animal toxicity studies have been conducted for DINX.

In an OECD 407-compliant 28-day oral toxicity study, Wistar rats (5/sex/dose) were fed DINX (99.7% purity) in the diet at 0, 600, 3000 or 15,000 ppm (equivalent to 0, 64, 318, and 1585 mg/kg-day for males and 0, 66, 342, and 1670 mg/kg-day for females; BASF AG, 2000a, as cited in Bhat et al., 2014; ANSES, 2016a). After 28 days, these rats were sacrificed and toxicologically evaluated. Additional control and high-dose rats (5/sex/dose) were sacrificed and evaluated after a 14-day recovery period. Thyroid weights and serum thyroid hormones were not measured in this study, but weights of most other key organs, including the liver, were determined at study termination.

In this study, there were no effects on mortality, clinical signs, food and water consumption, or body weight at any dose level. A functional observational battery (FOB) performed after 28 days of treatment found no effect on open field observations, motor activity, or sensorimotor effects. An isolated increase in motor activity in high-dose females at one measurement interval, a significant decrease in rearing in high-dose males, and decreases in relative heart weight in some females of all treatment groups were considered incidental (BASF, 2000a, as cited in Bhat et al., 2014). The only treatment related effect reported was a significant increase in serum GGT activity (55%; p<0.002) and a significant decrease in serum bilirubin levels (20%; p≤0.02) in females at the high dose. GGT activity returned to normal following the 14 day recovery period. No histopathological lesions were observed in the liver or kidney of control and high-dose rats, but there was an increase in the number of degenerated epithelial cells in the urine of high dose males and significantly increased serum sodium in mid- and high-dose males.

The study author considered changes in GGT and total bilirubin to be treatment-induced and related to microsomal enzyme induction in the liver. Changes in sodium were not considered treatment-related. Increased degenerative epithelial cells were regarded as treatment-related and "suggestive of mild renal impairment despite the lack of associated renal or urinary-tract histopathology" (Bhat et al., 2014). All changes returned to normal following the recovery period, so the study author considered these changes "reversible" and the NOAEL to be 318 and 342 mg/kg-day for male and female rats, respectively. Although there were no histological lesions in the liver, Bhat et al. (2014) identified a NOAEL of 3000 ppm (equivalent to 318 mg/kg-d in males and 342 mg/kg-d in females), based on the increases in serum GGT and a decrease in serum bilirubin levels. Both SCENIHR (2016) and ANSES (2016a) identified the same NOAEL of 3000 ppm but determined that the critical effects in this study were increases in GGT serum level in females and degenerated epithelial cells in the urine in males. In a prior review based on fewer authoritative reviews (Versar, 2010), CPSC noted that the toxicological significance of the observed changes is uncertain. In its evaluation of the 90-day DINCH study, SCENIHR (2007) concluded that the increase in GGT and liver weight, accompanied by increased TSH and thyroid hypertrophy reflected enzyme induction and were not adverse.

Based on the interpretations of Hall et al. (2012) and the U.S. EPA (2005) report, this assessment does not consider the increased serum GGT or decreased bilirubin to be adverse, in the absence of increased liver weight or histopathology and in light of the mode of action discussed in Section 5.8. Thus, the high dose of 1670 mg/kg-day was a NOAEL for females. The high dose of 1585 mg/kg-day appears to have been a mild LOAEL for males, based on an increase in the number of degenerated epithelial cells in the urine and significantly increased serum sodium in the absence of kidney histopathology; the corresponding NOAEL was 318 mg/kg-day.

In an OECD guideline 408 compliant 90-day study, Wistar rats (20/sex/dose) were exposed through the diet to DINX (99.6% purity) at 0, 1500, 4500, or 15,000 ppm for 13 weeks (BASF AG, 2002b, and as cited in Bhat et al., 2014). The corresponding doses were reported by Bhat et

al. (2014) as 0, 107, 326, and 1103 mg/kg-day in males and 0, 128, 389, and 1312 mg/kg-day in females. Bhat et al. reported the corresponding respective human equivalent doses (HEDs) for specific durations (i.e., days 30, 62, and 91) (see Table 2). In the study, food consumption, body weights, clinical appearance, hematology, urinalysis, opthamology, gross pathology and histopathology were assessed for each rat. An FOB and motor activity assessment were also performed near the end of the study treatment.

Bhat et al. (2014) reported that there was no effect on mortality, clinical signs, hematology, body weight, or food consumption. Serum bilirubin levels were decreased in females and males (Bhat et al., 2014). In females, serum bilirubin level was significantly reduced at all doses on day 30 (37% at low dose, 21% at mid dose, and 33% at high dose). The respective reductions on day 62 were 11%, 1% and 12%, and 1%, 8%, and 13% on day 91. The changes were dose-dependent only on day 91[3]. In males, serum bilirubin levels were reportedly altered only at the terminal evaluation, where significant reductions of 13% and 16% were seen at the mid and high dose, respectively (Bhat et al., 2014). Aspartate aminotransferase (AST) activity was significantly *decreased* (an increase is indicative of liver damage) by 30% and 32% at the mid and high dose, respectively; no change in activity was reported in females. A significant increase in serum GGT activity (68%) was reported only in high-dose females. Kidneys, testes, liver and spleen weights in males and kidney weight in females were increased relative to controls, but the only increase of 10% or more was liver weight in high-dose females (12%) and kidney weight in high-dose males (10%). No histological lesions were reported in these organs. Several lines of evidence indicated kidney effects in the males. Erythrocytes and abnormal transitional epithelial cells were observed in the urine of treated males at the mid dose (days 29 and 86) and high dose (days 29, 61, and 86). Protein accumulation assessed using Mallory-Heidenhain staining was seen in the renal tubules at a high incidence in the controls, but the information provided by Bhat et al. (2014) suggested a dose-related increase in severity, although the provided information was not clear. Bhat et al. stated that immunohistochemistry for evaluating the presence of α2u-globulin was not included, but ANSES (2016a) and SCENIHR (2016) both identified the protein in the tubules as α2u-globulin. It is not clear if the latter assessments were based on an assumption regarding the identity of the protein, or if they were based on additional information not reported by or available to Bhat and colleagues. Bhat et al. (2014) noted the absence of other cellular alterations in proximal tubules (such as cell sloughing, apoptosis, pyknosis, or necrosis), and suggested protein accumulation may result from glomerular leakage, consistent with the presence of erythrocytes in urine. ANSES (2016a) and SCENIHR (2016) noted that effects related to α2u-globulin accumulation are not relevant to humans.

Thyroid weights were increased in females only at the low dose (14%), whereas increases of 12% and 20% were reported in males at the low and high dose, respectively. Despite the absence of an increase in thyroid weights in mid-dose males and in mid- and high-dose females, the

---

[3] Referred to as 90 days from here on, to avoid over-precision.

incidence of thyroid hypertrophy was increased in females at the high dose (see Table 2[4]). Thyroid hormones were assessed on days 30, 62 and 91. TSH levels were elevated in a dose-related manner across exposure durations in female rats (Table 2). In contrast, the TSH response in males and T_3/T_4 response in both males and females was less consistent. However, the results are limited by the absence of data for T3 (for which there was a more consistent decrease at 30 days) at the latter two time points; only T4 was measured at 62 and 90 days.

Based on their review of the study, Bhat et al. (2014) considered the decreases in serum bilirubin (males) and increase in thyroid gland weight and/or hypertrophy (both sexes) at all exposure doses to be adverse and therefore considered the low dose to be a LOAEL in both sexes. This is in contrast to the conclusions reached by the study authors, who assigned a NOAEL of 1500 ppm (107 mg/kg-day) for males based on abnormal urinalyses and of 4500 ppm (389 mg/kg-day) for females based on elevation in serum GGT activity at the high dose. The study authors attributed the thyroid effects to microsomal enzyme induction. SCENIHR (2016) agreed with the NOAELs of 1500 ppm (107.1 mg/kg-day) in male and 4500 ppm (389.4 mg/kg-day) in female identified by the study authors, with the exception that kidney effects were the critical effect in both sexes. In the previous CPSC assessment, Versar (2010) noted that induction of liver enzymes and an associated increase in liver weight by themselves would be considered an adaptive response to chemical exposure, but suggested that the point at which these changes become of sufficient magnitude to produce hyperplasia/hypertrophy of thyroid follicles could be considered a LOAEL.

The current assessment does not consider liver enzyme induction or decreased serum bilirubin to be adverse, as noted above. Erythrocytes and abnormal transitional epithelial cells in the urine are potentially adverse, meaning that the mid dose in males (326 mg/kg-day) is a LOAEL for kidney effects. SCENIHR (2016) and ANSES (2016a) stated that the protein that accumulated in the male rat kidney was α2u-globulin, but Bhat et al. (2014) said that the identity of the protein was not known. The reason for this discrepancy is not known, but it is noteworthy that Bhat et al. (2014) had access to the original unpublished studies. If the protein were α2u-globulin, associated effects would not be relevant to humans (U.S. EPA, 1991). However, there is no indication that α_2u-globulin accumulation results in erythrocytes or epithelial cells in the urine, and so those effects are still considered adverse and relevant to humans, resulting in a human-relevant kidney LOAEL of 326 mg/kg-day in males.

Finally, as described in Section 5.8, the weight of the evidence (WOE) supports the conclusion that the thyroid changes are secondary to the liver enzyme induction and increased clearance of T3 and T4 in the serum. As further described below, thyroid hypertrophy and downstream effects resulting from this mode of action (MOA) are not relevant to humans, but changes in T3 and T4 are relevant to humans, due to the potential for neurodevelopmental effects (Dellarco et

---

[4] ANSES (2016) reported increased hypertrophy at the mid and high doses in both sexes, but the basis for this statement is unclear.

al., 2006). Unfortunately, no clear data are available on the dose–time relationship for T3 or T4, presumably because of adaptive changes in the rat. Therefore, changes in TSH (the key event immediately following changes in T3 and T4) are used as a surrogate for changes in T3 and T4. This is a slightly non-conservative choice, since TSH follows T3 and T4 in the biological sequence. However, changes in all three hormones have been reported to occur at the same dose levels, within the precision of standard toxicology studies and NOAEL/LOAEL identification (Dellarco et al., 2006), and the data for this study provide no indication of changes in T3 and T4 occurring at lower doses. Indeed, decreases (the expected direction of change) in T3 and T4 were seen only at the 30-day time point, but this is likely due to adaptation at later time points. Even using TSH as a surrogate, identification of an effect level is challenging, due to the inconsistent dose-and time-response. However, based on the dose-related increases at all three timepoints, and the magnitude of the change at 90 days, the mid-dose (469 mg/kg-day at 30 days and 389 mg/kg-day at 90 days) is clearly an adverse effect level in females, the sex of concern for neurodevelopmental effects that may begin during pregnancy. In the rat, the finding of increased hypertrophy at the mid dose supports the conclusion that meaningful changes in TSH are occurring at the mid dose. The low dose (156 mg/kg-day at 30 days and 128 mg/kg-day at the 90 days) is a LOEL in females in light of the consistent dose-response but absence of a significant change at 90 days. No thyroid effect level was identified in male rats, based on the inconsistent TSH dose-response and absence of clear effects on the incidence of hypertrophy. In addition, effects in male rats (aside from those exposed in utero) are not relevant to humans.

TABLE 2. Serum Thyroid Hormone Levels After Dietary DINX Exposure up to 3 Months in Wistar Rats (BASF AG, 2002b, as cited in Bhat et al., 2014)

| | Rat dose (ppm and mg/kg-day) | | | | | |
| | Male rats | | | Female rats | | |
	1500 ppm	4500 ppm	15,000 ppm	1500 ppm	4500 ppm	15,000 ppm
Day 30: Rat dose (mg/kg-day)[a]	**141**	**430**	**1457**	**156**	**469**	**1566**
Human equivalent dose (mg/kg-day)[b]	36	110	371	36	108	358
Thyroid-stimulating hormone (TSH)	+3%	+21%	+3%	+19%*	+39%**	+71%**
Thyroxine (T4)	+2%	−4%	−5%	+10%	+17%	+20%
Triiodothyronine (T3)[c]	+2%	−14%	−6%	0	−8%	−8%
Day 62: Rat dose (mg/kg-day)	**117**	**358**	**1215**	**137**	**415**	**1390**
Human equivalent dose (mg/kg-day)	32	96	325	32	99	328
Thyroid-stimulating hormone (TSH)	+19%	+35%	+9%	+7%	+22%	+65%**
Thyroxine (T_4)	+6%	+7%	+3%	+10%	+14%	+14%

Day 91: Rat dose (mg/kg-day)	107	326	1103	128	389	1312
Human equivalent dose (mg/kg-day)	29	89	302	31	94	315
Thyroid-stimulating hormone (TSH)	+17%	+58% **	+29%	+22%	+52%	+114% **
Thyroxine (T_4)	+2%	-2%	-1%	+10%	+9%	+13%
Thyroid hypertrophy/hyperplasia[d]	14	11	16	1	3	15

Note. Significance indicated by $*p < .05$, $**p < .01$.

[a] Duration-specific mean consumed dose.

[b] Based on BW $^{3/4}$ allometric scaling (U.S. EPA, 2011b), 70 kg human body weight, and mean rat body weight at specified duration; conversion conducted by Bhat et al. (2014)

[c] T3 not measured at 62 or 91 day. All hormone levels relative to controls.

[d] Number of rats with thyroid hypertrophy/hyperplasia (sum of grade 1 and 2). All control and dose groups had 20 rats. Two control males and 1 control female had thyroid hypertrophy/hyperplasia.

5.3 Chronic Toxicity/Carcinogenicity

In an OECD Guideline 453-compliant combined chronic toxicity/carcinogenicity study, Wistar rats (50/sex/dose) were fed dietary DINX (99.6% purity) at concentrations in diet that were adjusted frequently to result in doses of 0, 40, 200 or 1000 mg/kg-day for two years (BASF, 2005a, as cited in Bhat et al., 2014; ANSES, 2016a; Danish EPA, 2014; SCENIHR, 2007; EFSA, 2006). Bhat et al. also reported the corresponding respective HEDs to be 0, 11.5, 58.2, and 286 mg/kg-day for males and 0, 9.87, 49.2, and 245 mg/kg-day for females. Ten additional rats per dose were included for blood and urine analysis at 3, 6, and 12 months and for interim sacrifice at 12 months. Endpoints evaluated included clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology. Thyroid hormones were not measured.

ANSES (2016a) noted slight but statistically significant decreases in hematological parameters, including mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in both low- and high-dose males after 6 and 12 months, and slight but statistically significant increased red blood cell counts in mid- and high-dose males after 12 months. Females exhibited higher platelet counts after 12 months. It is likely that these changes reflect natural variability, in light of the sporadic nature of the effects, and general lack of dose-response, although a definitive independent conclusion is not possible in the absence of the actual data.

Serum alkaline phosphatase (ALP) activity increased in high dose male rats at the 12-month exposure. ANSES (2016a) stated that the increase in ALP was possibly indicative of mild and adaptive impairment of liver function. GGT activity was increased and bilirubin was decreased. Liver weight was significantly (p<0.01) increased in high-dose females at the interim sacrifice and after 2 years. In males, absolute but not relative liver weight was significantly (p<0.05) increased at the high and mid-dose (<10%) after 2 years (ANSES, 2016a). No histopathological changes were seen in the liver.

Degenerated epithelial cells and granular cell casts were increased in the urine of males at 200 mg/kg-day at 3 months, but these changes were not seen at later time points or at the high dose at any time point. ANSES (2016a) considered kidney changes to be adaptive, as the effect was transitory and there were no increase in histological lesions in the kidneys at any dose or time point. Kidney weight was also elevated (more pronounced at 12 months) only at ≥ 200 mg/kg-day in male rats, but the increase was not dose-related.

Uterine weights were statistically significantly decreased at the mid (-70.1%) and high doses (-77.5%), with a smaller and not statistically significant decrease at the low dose. The authors attributed the decreased uterine weight to decreases in the number of uterine masses, and did not consider it to be a treatment-related effect (Bhat et al., 2014; NICNAS, 2012).

At terminal sacrifice, relative thyroid gland weights significantly increased, by 71.4% and 42.9% in males of the mid and high dose groups, respectively, and by 55.6% in females only in the high dose group (ANSES, 2016a). The thyroid weight increases in both sexes were accompanied by follicular cell hyperplasia. The current assessment did not consider the decreased[5] thyroid weight in the low-dose females at the 1-year sacrifice (18.5%) to be biologically meaningful, since the change was not dose-related and increases in thyroid weight are more consistent with the data and the general biological pattern. Bhat et al. (2014) also noted that EPL (2008) and Capen (2006) each conducted an independent re-examination of the mammary gland sections and thyroid-gland tissues slides, respectively, from the BASF (2005a) study (Table 3).

Based on the increased thyroid weight and follicular cell hyperplasia this assessment concludes that the thyroid NOAEL *in rats* is 40 mg/kg-day in males and 200 mg/kg-day in females; the corresponding LOAELs are 200 and 1000 mg/kg-day, respectively.[6] Benchmark dose (BMD) modeling conducted by Bhat et al. (2014) on the follicular cell hyperplasia data from the Capen (2006) reevaluation (Table 3) identified thyroid follicular hyperplasia in males as the most sensitive non-neoplastic effect, with a BMDL human equivalent dose (BMDL_{HED}) of 50 mg/kg-day. However, these changes to the thyroid are not relevant to humans, as discussed further in Section 5.8. As noted for the 90-day study, changes in T3 and T4 *are* relevant to humans, but

---

[5] Bhat et al. (2014) reported this as increased thyroid weight (additional information not provided), but did not show the relevant data, while ANSES (2016) presented the increases relative to control for all doses.

[6] This is the same as the previous CPSC conclusion (Versar, 2010), as well as that of the study authors. Other organizations also identified a NOAEL of 40 mg/kg-day, although there were some differences in what was considered a critical effect. EFSA (2006) based its NOAEL on thyroid effects, and identified a NOAEL of 200 mg/kg-day for other effects (based on increased platelet counts in females). The Danish EPA (2014) based its NOAEL on liver weight changes (both sexes) and kidney weight changes (males). NICNAS (2012) based its NOAEL on the renal effects. Considering both the original BASF AG (2005a) study and the reevaluation of the sections and tissues, Bhat et al. (2014) identified a LOAEL of 40 mg/kg-day for thyroid effects (presumably based on absolute thyroid weight decrease of 19% in females only at 40 mg/kg-day at the 1-year sacrifice; no NOAEL was identified.

thyroid hormones were not measured in this study. Uncertainty in extrapolating from the available data in the chronic study is discussed in Section 5.10.

The liver NOAEL was 1000 mg/kg-day, the highest dose tested, since enzyme induction is not adverse. The kidney effects are not considered adverse because they were transient, not dose-related, and because (unlike the subchronic study), they were not accompanied by erythrocytes in the urine. Therefore, this assessments considers the NOAEL for kidney effects is 1000 mg/kg-day.

Chronic dietary exposure to DINX resulted in mammary gland fibroadenomas and adenocarcinomas in female rats, and thyroid gland adenomas and adenocarcinomas in males and female rats. Table 3 shows the original incidence data and results of the reevaluations conducted by EPL (2008) for mammary gland lesions and by Capen (2006) for the follicular cell adenomas (as reported by Bhat et al., 2014). Several lesions considered to be mammary gland hyperplasia in the initial analyses were considered in the re-evaluation to be fibroadenomas. From the reevaluations, the incidence of fibroadenomas, but not adenocarcinomas, in female rats (EPL, 2008) increased in a dose-dependent manner, with statistical significance at $\geq$ 200 mg/kg-day compared to the concurrent control. The incidence of thyroid adenomas also increased in a dose-dependent manner in both males and females, but the incidence did not reach statistical significance at any dose level. Bhat et al. (2014) reported that the mammary fibroadenomas appeared to be incidental, age-related lesions based on the low incidence in the concurrent control group, and the historical control ranges, although it is noted that in general the concurrent control data are much more important than the historical control data. NICNAS (2012) and ANSES (2016a) agreed with this conclusion. However, Bhat et al. did conduct BMD modeling of the cancer data and estimated a $BMDL_{HED}$ of 58 mg/kg-day for the mammary fibroadenomas in females and of 74 mg/kg-day[7] for thyroid follicular adenomas in males. They determined that the cancer NOAEL was 40 mg/kg-day in males and of 200 mg/kg-day in females, based on the thyroid follicular adenomas. As noted below, the weight of the evidence is that the thyroid tumors occur via a MOA that is not relevant to humans.

Overall, this assessment concludes that the observed noncancer effects either were not adverse (liver, kidney), or not relevant to humans (thyroid). The available data were not sufficient to clearly extrapolate to a human-relevant thyroid effect level. The observed cancer endoints were either not relevant to humans (thyroid), or incidental (mammary). No human-relevant effect levels could be identified from this study.

---

[7] Danish EPA also noted that DINX caused an increase in the number of follicular adenomas in the thyroid glands of male rats administered $\geq$ 200 mg/kg-day and females at 1000 mg/kg-day, indicating a NOAEL of 40 mg/kg-day in males and of 200 mg/kg-day in females for follicular adenomas.

Table 3. Incidence of Thyroid Gland Hyperplasia, Adenomas and Adenocarcinomas and of Incidence of Mammary Gland Fibroadenomas and Adenocarcinomas in DINX-Treated Rats (BASF AG, 2005a, as cited in Bhat et al., 2014)

	Rat dose[a] (mg/kg-day)							
	Male rats				Female rats			
	0	40	200	1000	0	40	200	1000
Human equivalent dose (HED)[b] (mg/kg-day)	0	11.5	58.2	286	0	9.87	49.2	245
Thyroid Gland Hyperplasia, Adenomas and Adenocarcinomas[c]								
Follicular hyperplasia	7/50 (8/50)	6/50	11/50 (9/50)	17/50 (15/50*)	3/50	4/50	5/50	15/50 (14/50*)
Follicular adenoma	3/50	5/50	9/50 (11/50*)	11/50 (14/50*)	1/50	2/50 (3/50)	3/50	8/50 (9/50*)
Follicular adenocarcinoma	1/50 (2/50)	0/50	1/50	2/50 (1/50)	2/50	1/50 (0/50)	0/50	1/50
Mammary Gland Fibroadenomas and Adenocarcinomas[c]								
Mammary fibroadenoma					2/50 (1/50)	4/50 (2/50)	8/50* (5/50*)	11/50[d],* (9/50*)
Mammary adenocarcinoma					1/50 (2/50)	1/50	5/50	1/50

M, male; F, female.
[a] DINX concentration mixed in feed was standardized to deliver constant dose throughout study.
[b] Based on $BW^{3/4}$ allometric scaling (US EPA, 2011), 70 kg human body weight, and mean rat body weight at week 52.
[c] Where the results of the original assessment and the re-evaluation by Capen (2006) (for the thyroid) or EPL (2008, for the mammary gland) differed, the original analysis is shown in parentheses.
[d] 2/11 rats had multiple fibroadenomas.
* Significant difference at $p < .05$ compared to concurrent control.

5.4 Reproductive Toxicity

In the only identified human epidemiology study, Mínguez-Alarcón et al. (2016) evaluated the urinary concentration of OH-MINX (called cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester, MHiNCH) in a prospective cohort study of 113 women at a fertility center in Massachusetts.

Comparing the women with urinary concentrations below the detection limit with those above the detection limit, they reported no effect on mature oocyte yield and endometrial wall thickness. However, total oocyte yield was borderline significantly (p=0.05) reduced in women 37 and older, but not in the overall population (p=0.08). The authors reported that peak estradiol levels were also associated with higher metabolite levels, but the difference was not statistically

significant (p=0.09). Although this study suggests further study would be worthwhile, it is insufficient to show causality.

In a two-generation reproduction study (OECD Guideline 416), Wistar rats (25/sex/dose) were fed dietary DINX (99.6% purity) at 0, 100, 300 or 1000 mg/kg-day for 38 weeks (BASF AG, 2003b, as cited in Bhat et al., 2014, and ANSES, 2016a). The concentration in feed was adjusted regularly to maintain a constant dose based on body weight and feed intake.

Treatment did not affect survival of F0 parents and F1 animals in any dose group, or body weight or food consumption at any dose level. As in the systemic toxicity studies, increased organ weight and other changes consistent with metabolic enzyme induction were observed. Increases in serum GGT and decreases in total bilirubin levels were reported at ≥300 mg/kg-day in F0 parents and F1 generation females and at 1000 mg/kg-day in F1 generation males (ANSES, 2016a). DINX treatment also caused increased liver (4-15%), kidney (<10%), adrenals (<10%), and/or pituitary (17%) weights in F0 and F1 generation adults at ≥300 mg/kg-day (Bhat et al., 2014; ANSES, 2016a). ANSES (2016a) stated that thyroid gland weights increased in F0 parents at ≥300 mg/kg-day; the magnitude of the increase was not reported.

In the F1 generation (but not in F0 parents), renal tubular vacuolization (all grades combined) were observed in 9/25 and 25/25 males at 300 and 1000 mg/kg-day, respectively (Table 4). The severity in all affected rats was 1 or 2 out of 5, and there appeared to be a slight dose-related increase in severity (no statistical test performed). According to Bhat et al. (2014), the tubular vacuolization was not associated with degeneration, nuclear shrinkage, cell sloughing into the tubular lumen, necrosis, or inflammation. Based on this observation, Bhat et al. stated that the toxicological significance of this lesion is unknown. However, EFSA (2006) and ANSES (2016a) considered the renal changes adverse.

DINX treatment caused an increase in thyroid gland weights (11–16%) in F1 females at 1000 mg/kg-day and a dose-dependent increase in thyroid follicular epithelial hypertrophy/hyperplasia (reported collectively) (Table 4); dose-dependency of the thyroid weight increases was not reported. Thyroid gland histology was not conducted in F0 males or females or F1 males, since thyroid weights were not altered (Bhat et al., 2014). In addition, thyroid hormones were not measured in the study. ANSES (2016a) stated that the registrant considered the thyroid lesions to be a consequence of liver enzyme induction, as described in other studies, and thus is not considered to be an adverse effect of treatment. (See Section 5.8 for additional discussion of the mode of action.) As noted for the chronic study, the data are not sufficient to identify a human-relevant thyroid effect level, but that effect level would clearly be below the *rat* LOAEL of 1000 mg/kg-day.

Male and female pup body weights were increased (≤ 11%) when measured up to PND 21. However, Bhat et al. (2014) stated that the increases were inconsistent, not dose related, and considered to reflect usual biological variation in this strain.

There were no effects on fertility or reproductive performance in the F0 or F1 parental animals or developmental toxicity in the F1 or F2 pups. One high-dose male had reduced testicular and epididymal weights, tubular atrophy of the testis, and azoospermia in the corresponding epididymis, and failed to produce live pups with two fertile females. This was considered an incidental finding, since comparable effects on the reproductive organs were seen in one control male. Significant increases were observed in relative testes weight (5–12%) in F0 and F1 generation males at 300 mg/kg-day. However, the increases were not dose-related and were not associated with effects on sperm parameters (count, motility, morphology) or testes histology (ANSES, 2016a). Gross and histopathological evaluation did not reveal any treatment related adverse effects on reproductive performance or fertility in the F0 parents or F1 generation rats for all dose groups (NICNAS, 2012). NICNAS (2012) also stated that there were no substance-induced signs of developmental toxicity in the progeny of F0 and F1 generation animals. Viability and mortality of F2 pups were not affected by DINX treatment (NICNAS, 2012).

The highest dose of 1000 mg/kg-day was considered a NOAEL for general/systemic toxicity in F0 rats (ANSES, 2016a; Bhat et al., 2014; NICNAS, 2012) and for fertility and reproductive performance in F0 and F1 parental rats and developmental toxicity in F1 and F2 pups (ANSES, 2016a; SCENIHR, 2016; Bhat et al., 2014; NICNAS, 2012). However, EFSA (2006) considered the general/systemic NOAEL to be 100 mg/kg-day, based on renal toxicity findings (vacuolization of kidney tubular epithelia in males).

This assessment agrees that the high dose of 1000 mg/kg-day was a NOAEL for fertility and reproductive performance in F0 and F1 parental rats and developmental toxicity in F1, and F2 pups and for adverse liver effects. We agree with EFSA that there is a potential NOAEL of 100 mg/kg-day in males and a LOAEL of 300 mg/kg-day, based on renal tubular vacuolation. However, there is considerable uncertainty in this endpoint, since it was not seen in the subchronic or chronic toxicity studies in the same strain of rat. The high dose was a NOAEL for females. With regard to the thyroid effects, it is important to distinguish between adversity in the rat and human relevance. Based on the endpoints evaluated, the high dose was a NOAEL for thyroid effects in males. However, in females, the rat thyroid NOAEL was 100 mg/kg-day and the LOAEL was 300 mg/kg-day. The data are insufficient to identify a human-relevant thyroid effect level, but the NOAEL and LOAEL in rats are generally consistent with the data in the 90-day study.

Table 4. Incidence of Kidney and Thyroid Lesions in in F1-Generation Wistar Rats Exposed to DINX In Utero Through Adulthood (BASF AG, 2003b, as cited in Bhat et al., 2014)[a]

Parameter	Rat dose (mg/kg-day)							
	Male rats				Female rats			
	0	100	300	1000	0	100	300	1000
Renal tubular vacuolation in males (all severity grades combined)	1/25	1/25	9/25	25/25	NA	NA	NA	NA
Thyroid follicular hypertrophy/hyperplasia in females (all severity grades combined)	NA	NA	NA	NA	3/25	3/25	10/25	21/25

[a]After F0 and F1 in utero exposure, parameters were assessed after an additional 10 weeks of exposure in females and 17 weeks in males; thyroid lesion was reported and scored as "hypertrophy/hyperplasia" (Bhat et a., 2014).
NA, not assessed since thyroid weight was not statistically different from control males.

5.5   Prenatal, Perinatal, and Post-natal Toxicity

In a pre- and postnatal developmental study[8], pregnant Wistar rats (10/dose) orally[9] received 0, 750, or 1000 mg/kg-day on gestational day (GD) 3 until postnatal day (PND) 20 (BASF AG, 2002c, as cited in Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a). The offspring were exposed via the mothers during gestation and also via lactation until PND 20, and then all males and three females (not clear whether this was per dose or per dam) were raised to Days 100-105 post-partum and then evaluated. Anogenital distance (AGD) and anogenital index (AGI, AGD divided by pup weight) were measured at PND 1, and sexual maturation was determined (testes descendance, balanopreputial separation, penis evaluation/inspection, sperm evaluation and vaginal opening for females). Gross pathology was performed and histological examination was conducted on testes and epididymis. Bhat et al. (2014) stated that thyroid parameters were not assessed in this study. Only two doses were tested, but this did not impact interpretation of the study.

In the high dose (1,000 mg/kg-day) group, AGD ($p<0.05$) and AGI ($p<0.01$) were decreased in a statistically significant manner by 7% and 8%, respectively in male pups. These changes were not considered biologically significant by the study authors, since other associated endpoints (e.g., testes descent, balanopreputial separation, sperm parameters, organ weights, and histopathology) were not indicative of altered sexual development (Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016). In female pups, AGI was also significantly ($p<0.05$) decreased (8%

---

[8] Bhat et al. (2014) describes this study as OECD 414/415 compliant, but unlike a 415-compliant one-generation reproductive toxicity study, exposure did not begin before mating, and did not include both sexes, and unlike a 414-compliant study, the dams were not sacrificed prior to parturition. In addition, it is not clear whether use of half the dams/dose compared with the guidelines (10/dose instead of 20/dose) decreased the study sensitivity.
[9] The method of dosing is not clear from the available information, but is likely by gavage.

below control level), but there was no effect on vaginal opening. The similarity in decrease in AGI in males and females also supports the conclusion that DINX did not impair androgen-dependent development. However, ANSES (2016a) noted that there are questions about the potential biological significance of the effects on AGD in males. Citing a report by ToxServices (2013)[10], ANSES (2016a) noted that AGD was not affected in females, and that the statistically significant decrease in AGI in females may have simply reflected the slight increase in female pup body weight, coupled with a slight decrease in AGD. Thus, there is some outstanding uncertainty regarding the anti-androgenic potential of DINX. However, ANSES (2016a) noted that there were no male reproductive effects in the 2-generation study described above, and so ANSES concluded that, regardless of whether there was an effect on AGD in males, reproductive function is not affected.

In evaluating the changes in AGD, this assessment notes that normalizing AGD by the cube root of body weight is preferred over normalizing by body weight. Indeed, AGI decreases with increasing body weight in untreated rats (Gallavan et al., 1999). This means that it is not possible to determine whether the decreased AGI in female pups was attributable to the increased body weight or was a treatment-related effect. Independent calculation of the AGD normalized by the cube root of body weight was not possible, in the absence of the primary individual pup data. It is also noted that the other markers of sexual development noted by other assessments as being unaffected (e.g., testes descent, balanopreputial separation, sperm parameters, organ weights, and histopathology) may occur via different MOAs, and may not all occur in the same study. This decreases the impact on the weight of evidence of not seeing these other effects in the current study. Overall, in light of these considerations, this assessment identifies 750 mg/kg-day as a NOEL, and 1000 mg/kg-day as a LOEL, due to uncertainty about whether there was an adverse effect on AGD in male pups. No maternal effect level was identified, in the absence of information on maternal toxicity.

In an OECD 414-compliant developmental toxicity study, pregnant Wistar rats (25/dose) received DINX (99.7% purity in olive oil) via gavage to 0, 200, 600 or 1200 mg/kg-day on GD 6 – 19 (BASF AG, 2002d, as cited in Bhat et al., 2014; SCENHIR 2016; ANSES, 2016a). On day 20 post coitum all female rats were sacrificed and assessed for gross pathologies and reproductive organ weights, corpora lutea, implantation sites, and fetal soft, cartilage, and skeletal changes.

According to the review publications, no maternal toxicity was observed, although the study did not include assessment of thyroid parameters (Bhat et al., 2014). The current assessment, as well as the secondary sources (Bhat et al., 2014; SCENHIR 2016; ANSES, 2016a) conclude that there was no developmental toxicity, although there were some incidental increases in variations. The litter-based rate of uni- and bilateral dilation of the renal pelvis and/or ureter was elevated in all

---

[10] Full citation not provided by ANSES (2016).

treated groups (6-7% compared to 4% in controls). These effects were, however, within the historical range for this parameter (0-16%, with a mean of 7%) (Bhat et al., 2014). The incidence of incomplete or reduced ossification was significantly increased for the thoracic centrum (up to 9% at the low dose), and the parietal bone (7 and 8% at the mid and high dose, respectively). However, the incidence of litter-based skeletal malformations in treated groups (0-5%) was less than in controls (13%). The highest dose of 1200 mg/kg-day was identified as the NOAEL for maternal and fetal toxicity in this rat study.

Developmental toxicity was also assessed in an OECD guideline 414-compliant study in Himalayan rabbits (25/dose) which received mean doses of 0, 102, 311 or 1,029 mg/kg-day DINX (99.6% purity) via diet from Day 6 through 29 post insemination (PI) (BASF AG, 2004a, as cited in Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016). One rabbit each in the low- and mid-dose groups died but the cause of death was not apparent, and the mid-dose rabbit died prior to the beginning of exposure. Pregnancy rates were 96, 80, 84, and 96% in control, low-, mid-, and high-dose groups, respectively, but the reduced rate at the low- and mid-dose groups was not considered treatment related by Bhat et al. (2014), since treatment began after fertilization (on Day 6 PI). Treatment did not affect fetal or litter-based rates of soft tissue malformations or of fetal skeletal malformations. At the high dose, there was a significant increase in the litter-based rate of supernumerary thoracic vertebra, a skeletal variation. Because the increase was within the historical range, Bhat et al. (2014) considered this effect spontaneous; ANSES (2016a) and SCENIHR (2016) did not even note these variations. Based on the results, there was no maternal toxicity, no influence on gestation parameters, and no signs of developmental or teratogenic effects. Therefore, the highest dose tested of 1029 mg/kg-day was the NOAEL in rabbits for maternal toxicity and developmental toxicity.

In a screening assay conducted with phthalate esters and phthalate ester alternatives, pregnant Sprague-Dawley rats (3 or 4/chemical) were treated with 0 or 750 mg/kg-day DINX or GD 14-18 (Furr et al., 2014). There was no effect on fetal testosterone production, fetal viability or dam weight.

Two recent non-guideline compliant developmental toxicity studies were identified (Campioli et al., 2017; Nardelli et al., 2017). The Campioli et al. (2017) study evaluated the effects of DINX on male Sprague-Dawley rat progeny exposed *in utero* during the sensitive window for reproductive development and from the beginning of organogenesis. In this study, timed-pregnant rats (number per group not reported) were gavaged with DINX in corn oil at 0, 1, 10, or 100 mg/kg-day from GD 14 until parturition (corresponding to PND 0) or from GD 8 until parturition. Dosing apparently ended at parturition. Male offspring were euthanized on PND 3 or 60 and blood was collected from pregnant dams at GD 21 and from progeny on PNDs 3, 60 or 200. Body weight, AGD, fetal testosterone level and testes-specific gene expression were measured in PND 3 progeny. Various parameters associated with reproductive health and metabolism were also measured on PNDs 60 and 200.

Other evaluations conducted included measuring serum markers of liver dysfunction and kidney dysfunction, and levels of plasma testosterone, serum luteinizing hormone (LH) and testicular gene expression on PNDs 60 and 200, and levels of thyroid-associated hormones (T3, T4, and TSH) in the pregnant dams at GD 21. The study also evaluated the endocrine component of the testis, the Leydig cell. Campioli et al. (2017) did not report on survival, pregnancy rate, or fetal skeletal malformations.

No maternal toxicity was reported except for a significant decline in magnesium levels, a kidney marker, at GD 21, only at mid dose, 10 mg/kg-day compared to control. Thus, in utero exposure to DINX caused no adverse effects on the kidney in the pregnant dams. DINX also caused a significant decrease (about 15%) in magnesium level only in PND 60 progeny at the high dose compared to controls, with the levels in the PND 200 progeny being comparable to the corresponding levels in control progeny. In the SD dams or their PND 3 pups, levels of the major hormones regulating thyroid function were not significantly affected. Therefore, a NOAEL of 100 mg/kg-day, the highest dose tested, can be considered for thyroid effects in the Campioli et al. (2017) study.

There was no effect on pup weight, AGD, or fetal testosterone production on PND 3. However, changes in gene expression were seen. DINX caused a significant change in *Nes* (nestin), a sensitive marker for stem Leydig cells (SLCs), which are precursors to adult Leydig cells. However, the decrease in *Nes* was not correlated with a decrease in fetal testosterone production or change in AGD, and was not dose-related. DINX also caused a significant increase in the expression of the steroidogenic enzyme gene *Cyp11a1* on PND 3 at 100 mg/kg-day, although no change was seen at later time points. At PND 60, AGD was significantly (p<0.05) *increased* at the low dose. However, this was the opposite direction of the effect expected from an anti-androgen, and there was no significant effect at the mid- and high doses, suggesting that the low-dose change was not biologically meaningful. There was also a dose-related (p<0.01) decrease in relative seminal vesicle weight, which reached statistical significance (p<0.01) at the high dose, but there was no effect on relative testis weight. These effects had recovered by the PND 200 examination, with no dose differing significantly from controls in relative seminal vesicle or testis weight. Plasma testosterone was significantly (p<0.01) decreased at the low and high doses on PND 60. However, in the absence of an effect on the mid dose or a dose response, this assessment considers the testosterone changes to be normal variability. The study authors noted that there was no effect on testosterone in rats that were exposed on GD 8 through parturition, (i.e., beginning when organogenesis begins) and suggested that this indicates that the animals could compensate and adapt the developmental process. However, a simpler explanation is that the apparent effect on testosterone (when exposure began on GD14, but not when it began on GD8) reflected biological variability. No significant effect on testosterone was seen on PND 200. The authors reported enlarged interstitial space in the testes on PND 60 but not PND 200, and attributed the effect to an inflammatory reaction that was related to premature aging of the testis. However, no incidence data were provided, and the effect could not be independently

verified at the magnification of the micrographs in the publication. Following exposure to DINX, an increased presence of testicular atrophy was observed in 3/13 of the PND 200 progeny from animals exposed to 100 mg DINX/kg-day, compared to 1/13 in the corresponding control progeny. The authors concluded that *in utero* exposure to DINX affects Leydig cell function, causing premature aging of the testes, and resulting in decreased androgen production, altered AGD, and physical changes to the seminal vesicles and testicular atrophy. This assessment agrees that this study raises the question of whether *in utero* exposure can result in anti-androgenic effects that manifest after puberty. However, no definitive conclusion is possible, in light of the study limitations and incomplete reporting (e.g., absence of consistent dose-response, time-response or effects among related endpoints; absence of information on the number of dams or the number of independent litters that the sampled pups came from). In addition, it is noted that in the two-generation study (in which pups exposed in utero were mated and progeny evaluated), no effect on male reproductive capacity or any of the endpoints evaluated in both that study and by Campioli et al. (2017) were seen at doses up to 1000 mg/kg-day.

Nardelli et al. (2017) investigated the potential for DINX to cause reproductive effects by disrupting the endocrine system during *in utero* and lactational exposures. Timed-pregnant Sprague-Dawley rats (16-19/dose) were gavaged with corn oil vehicle or DINX at 0, 30 or 300 mg/kg-day from GD 8 until weaning (PND 21); treatment was not conducted on PND 0. Litters were culled to eight each on PND 3. The offspring were examined for effects on developmental and endocrine markers from PND 3 until PND 46.

One rat each died from the control group and low dose group and two from the high dose group. The deaths were deemed spontaneous and the cause was not determined. Organ weights were not affected by treatment. Treatment did not cause any changes in body weights, organ weights or serum enzymes reflecting damage to the liver (alanine transaminase, aspartate aminotransferase or alkaline phosphatase) and electrolytes (e.g., magnesium) in dams at PND 21. The lack of changes in the enzymes and magnesium levels, coupled with no organ changes, suggest DINX treatment in this study did not adversely affect the liver or the kidney. Therefore, the NOAEL for liver and kidney effects is 300 mg/kg-day. Treatment did not result in any significant effects on post-implantation loss, pup survival, or sex ratio, indicating that exposure did not affect pregnancy outcome. There were also no effect on pup weight, crown-rump length, or teratogenic effects (no effects on numbers of external malformations). Pup organ weights, including reproductive organs, at PND 21 and 46 were not affected.

In female offspring, treatment did not affect functional markers of reproductive development, AGI at PND 3, serum levels of LH or follicle stimulating hormone (FSH), expression of steroidogenic enzymes, or expression of genes important for reproductive function. Together these results indicate that DINX did not have adverse endocrine or reproductive effects on female offspring.

In male offspring, DINX treatment also caused no endocrine or reproductive effects. There was no effect on AGI, presence of retained nipples at PND 13, testosterone production, seminal vesicle weights on PND 21 and 46, or expression of testicular genes. The only adverse effect reported in this study was significant increases in the incidence of testicular hemorrhage in male offspring at PND 8 at both doses of DINX. However, at PND 21, hemorrhagic testis was not observed, and testicular histology did not show any major abnormalities in any treatment group. Therefore, this assessment considers the effect to be transient and a NOAEL of 300 mg/kg-day is estimated for endocrine, reproductive and developmental effects in this study.

5.6 Genotoxicity

The weight of evidence is that DINX is not genotoxic. As summarized by Bhat et al. (2014), DINX was negative *in vitro* in gene mutation assays and for chromosome aberrations, and negative *in vivo* for clastogenicity. Specifically, DINX was negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 uvrA in the presence or absence of S9, and in standard plate and pre-incubation assays (BASF AG, 2000b). It was also negative for gene mutation in mammalian cells in the forward gene mutation assay at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells with and without S9 (BASF AG, 2001b). DINX was negative for structural chromosome aberrations in V79 Chinese hamster lung cells +/- S9 (BASF AG, 2000c), and for micronucleus induction in the bone marrow of male mice given a single intraperitoneal injection at doses up to 2000 mg/kg. The highest dose caused reduced motor activity, indicating that the maximal tolerated dose was reached (BASF AG, 2001c).

5.7 Mechanistic Studies

Because the liver, kidneys and thyroid are target organs of DINX in the rat, most mechanistic studies have focused on these targets. Studies have been conducted to evaluate the effects of DINX on enzyme induction in the liver, its effect on S-phase cell proliferation in all three organs (since increased cell proliferation was observed in liver and thyroid glands, and to a lesser extent in kidneys), and the MOA of its effect on the thyroid gland (ANSES, 2016a).

The potential of DINX to induce hepatic Phase I and II enzymes was assessed in Wistar rats (5/sex) exposed to DINX in the diet to 15,000 ppm (equivalent to 1418 and 1568 mg/kg-day in males and females respectively) for 2 weeks (BASF AG, 2005b, as cited in Bhat et al., 2014; ANSES, 2016a). DINX treatment caused significant increases in total liver cytochrome P450 (CYP) (2.2-fold in both sexes). Increases were also seen in biomarkers used to assess CYP1A (ethoxyresorufin O-deethylase [EROD], 2.7- and 1.6-fold in males and females, respectively), CYP2B (pentoxyresorufin O-depentylase [PROD], 30- and 43-fold in males and females, respectively), and CYP3A (benzoxyresorufin O-debenzylase [BROD], 11- and 24-fold in males and females, respectively). Increased glucuronidation (3.3- and 2.4-fold in males and females, respectively), and 4-hydroxybiphenyl-glucoronyltransferase activity (7.2- and 2.7-fold in males

and females, respectively) were also seen. The increases in CYP level and the biomarkers were reflective of Phase I (oxidative) and II (glucuronyl transferase) metabolic enzyme induction in livers of treated rats (ANSES, 2016a; Bhat et al., 2014). Given the marked increases in PROD and BROD activities, Bhat et al. (2014) noted that activation of CAR/PXR is plausible; however, nuclear receptor activation associated with DINX exposure has not been directly assessed. In another study, hepatic peroxisome proliferation was measured via cyanide-insensitive palmitoyl-coenzyme A (CoA) oxidation in Wistar rats fed dietary DINX (99.7% purity) at up to 15,000 ppm (equivalent to 1585 mg/kg-d for males and 1674 mg/kg-d for females) for 4 weeks (BASF AG, 2000a, as cited in Bhat et al. (2014). There was no effect on peroxisome proliferation in treated rats.

In a cell proliferation study that assessed the effects on S-phase cell proliferation in the liver, thyroid and kidneys, Wistar rats were exposed in the diet to 0, 40, 200 or 1000 mg/kg-day of DINX for 1, 4 or 13 weeks (ANSES, 2016a). Increased cell proliferation was observed at all dose levels and in all three organs. Higher levels of proliferation were seen in the thyroid and liver than in the kidney, and kidney proliferation was seen only in males. The degree of proliferation decreased with time. The highest levels of proliferation were found after 1 week of treatment, with less proliferation after 4 weeks, and proliferation near control levels after 13 weeks of treatment. NICNAS (2012, as cited in ANSES, 2016a) also noted that there was evidence of thyroid follicular cell hypertrophy, mainly in the mid and high dose groups of both sexes, which progressively increased over the duration of the study.

BASF AG, 2005c (as cited in Bhat et al., 2014; ANSES, 2016a) conducted a study to determine if the effects of the DINX on the thyroid gland in male Wistar rats occur via a direct effect inhibiting the iodination in the thyroid gland or by indirect mechanisms (i.e., induction of liver metabolism). Groups of male Wistar rats were fed dietary DINX (99.7% purity) at 15,000 ppm (1301 mg/kg-day), propylthiouracil at 2000 ppm (133 mg/kg-day), or phenobarbital at 1000 ppm (86 mg/kg-day) for 4 weeks. On day 29, the rats were administered radiolabeled iodide, followed by either saline or perchlorate, in a perchlorate discharge assay. Perchlorate does not cause deiodination of thyroglobulins, but if iodide uptake is inhibited by perchlorate, diffusion of iodide out of the thyroid would result in decreased radioactive iodide in the thyroid.

Propylthiouracil and phenobarbital were selected as reference compounds. Propylthiouracil exerts direct antithyroid activity via inhibition of thyroglobulin iodination to form iodotyrosine, while phenobarbital exerts indirect thyroid toxicity by inducing hepatic microsomal enzymes that increase thyroid hormone clearance, resulting in a compensatory stimulation of the thyroid gland by increasing TSH production (Bhat et al., 2014). All three chemicals caused elevated thyroid weight. However, although propylthiouracil-fed rats had increased TSH and reduced T_4 and T_3, neither DINX nor phenobarbital caused any marked effects on T_4 and T_3 or significant increase in TSH levels (ANSES, 2016a; Bhat et al., 2014). As expected, propylthiouracil-fed rats had decreased radiolabeled iodide in the thyroid, and this decrease was enhanced by perchlorate. In

contrast, phenobarbital increased the level of radiolabeled iodide in the thyroid and increased the ratio of radiolabeled iodide in the thyroid versus the blood, both in the presence and absence of perchlorate. DINX increased both parameters in the presence of perchlorate, but not in the absence of perchlorate. These results indicate that DINX action on the thyroid in male rats is more similar to that of phenobarbital than propylthiouracil, acting indirectly by inducing hepatic metabolic enzyme activities that increase thyroid hormone turnover (ANSES, 2016a; Bhat et al., 2014). It is not clear why the increased radioactive iodide in the thyroid was seen only in the presence of perchlorate, but the results might be explained by a lower degree of metabolic enzyme induction. A more significant limitation is that no increase in TSH or decrease in T3 or T4 was seen in this study with DINX or with the positive control (phenobarbital), but such changes were seen in the 90-day study with DINX (BASF AG, 2002b). Thus, this assessment agrees with the conclusion by ANSES (2016a) that these results support the hypothesis that DINX causes thyroid toxicity indirectly by inducing hepatic metabolic enzyme activities. These results are not fully conclusive, however.

Several studies also investigated the potential effects of DINX on the male reproductive tract. In *in vitro* studies using mouse MA-10 Leydig and C18-4 spermatogonial cell lines, Boisvert et al. (2016) found no effect on viability of either cell line, or Leydig cell proliferation, but proliferation of the spermatagonial cell line was significantly decreased (p<0.05), with an IC_{50} of 19 μM. A biphasic response was reported for hCG-induced steroidogenesis (p<0.05), with an IC_{50} of 14 μM in the Leydig cell line. Testosterone levels were significantly (p<0.05) decreased at a similar concentration in perinatal organ cultures. Nardelli et al. (2015) also reported alterations of expression of genes involved in the ERK/MAPK signaling pathway (a pathway important in Leydig and germ cell function) in a TM4 Sertoli cell line at a similar concentration. Although these studies suggest the potential for DINX to interact with male reproductive cells, their toxicological significance is uncertain, in light of the very high concentrations tested.

5.8 Mode of Action

Liver Enzyme Induction MOA for Thyroid Toxicity

DINX consistently induces alterations in thyroid structure and function in subchronic and chronic animal studies. The MOA for these thyroid effects has been proposed to be related to the induction of liver enzymes (Danish EPA, 2014; Bhat et al., 2014; SCENIHR, 2016). Induction of liver enzymes with secondary changes to the thyroid is a well-studied MOA, and is also known as disruption of the thyroid-pituitary-hypothalamus axis. The key events in this MOA have been identified by Dellarco et al. (2006) as:

(1) induction of metabolic enzymes;

(2) increased hepatic clearance of T3 and T4;

(3) decreased serum levels of T3 and T4;

(4) negative feedback to the pituitary and hypothalamus resulting in compensatory increase in TSH;

(5) thyroid follicular hypertrophy;

(6) thyroid follicular hyperplasia; and

(7) thyroid tumors.

<u>Other MOAs for Thyroid Toxicity</u>

Other potential MOAs for thyroid effects and thyroid tumors are DNA mutation; inhibition of iodide uptake (as seen with perchlorate), and inhibition of thyroglobulin iodination (as seen with propylthiouracil). As discussed in Section 5.6, DINX does not cause point mutations or chromosome aberrations *in vitro* or *in vivo*. Furthermore, as discussed in Section 5.7, DINX does not act like perchlorate or propylthiouracil in mechanistic studies. ANSES (2016a) noted that other hypotheses on a possible mode of action have not been tested. However, evaluating MOA does not require that all possible MOAs be considered. Rather, a weight of evidence judgement is made based on a comparative weight of evaluation (Meek et al., 2014). Of the major MOAs for the observed thyroid effects, the one that is most consistent with the data is liver enzyme induction resulting in disruption of the thyroid-pituitary axis.

<u>Human Relevance of Animal Thyroid Tumors</u>

As described by Meek et al. (2003) and Boobis et al. (2006), the state of the science for evaluating the human relevance of a tumor (and later applied to noncancer endpoints) is using the International Programme on Chemical Safety (IPCS) MOA/human relevance framework. This framework includes three steps: (1) evaluate the MOA in the animal model, using the modified Hill criteria; (2) consider whether the human relevance can be excluded based on fundamental *qualitative* differences in key events between animals and humans, and (3) consider whether the human relevance can be excluded based on *quantitative* differences in kinetic or dynamic factors between animals and humans. Note that a critical aspect of the framework is separating the evaluation of the MOA in animals from the evaluation of human relevance. In addition, evaluation of adversity in the experimental animals (rats and mice) is separated from evaluating the MOA in animals and evaluation of human relevance. For this reason, the rat effect levels have been identified for thyroid effects in this document, even for endpoints not likely to be relevant to humans based on the MOA.

Bhat et al. (2014) reviewed the data on DINX and thyroid tumors in light of the modified Hill criteria. In brief:

- The data are generally consistent across studies, with studies of multiple durations showing liver enzyme induction and thyroid or thyroid hormone changes consistent with the MOA;
- The MOA is biologically plausible, based on the scientific understanding of the MOA;
- Reversibility asks whether the later key events and apical effect occur if the earlier key events are prevented. No study evaluated whether downstream events are prevented, but the 28-day study (BASF AG, 2000a, as cited in Bhat et al., 2014) found that serum GGT and bilirubin levels returned to pre-exposure levels following cessation of exposure, suggesting that the enzyme induction is reversible.
- Statistically significant changes of a biologically meaningful magnitude were observed (strength)
- The data are generally consistent regarding the expected dose-response and temporal relationships.

Table 5 further investigates the dose-response and temporal progression of the key events across studies. For ease of looking at the trends, similar dose levels are combined into ranges. As shown, the general pattern is as expected for the MOA, with earlier key events occurring at lower doses at earlier time points, and later key events occurring at higher doses and later time points.

Some exceptions to the expected pattern are noted. TSH was increased at 469 mg/kg-day at 30 days in female rats, but no clear increase in earlier key events was seen at this dose. The absence of liver enzyme induction may be because GGT was measured, rather than enzymes more specific to or indicative of the MOA, such as CYP2B1 or glucuronyltransferase. In addition, no clear dose-related decrease in T3 and T4 was observed (and the changes in males were even less consistent than those in females), and the initial decrease in T4 became an increase at later time points. The absence of a clear effect on T3 and T4 may be because the increase in TSH rapidly compensated for the increased T3 and T4 clearance, although this has not been shown for DINX. Another inconsistency was that increased thyroid weight was seen in F1 females, but not F0 females or F0 or F1 males in the two-generation study weeks (BASF AG, 2003b, as cited in Bhat et al., 2014, and ANSES, 2016a). Additional support for the MOA comes from the mechanistic study of thyroid effects (BASF AG, 2005b, as cited in Bhat et al., 2014; ANSES, 2016a), although the absence of an effect on T3 or T4 with phenobarbital, the positive control, limits the conclusions from that study.

In the second and third step of the human relevance framework, Dellarco et al. (2006) determined that the human relevancy of this MOA cannot be reasonably excluded based on qualitative differences, but can be reasonably excluded based on *quantitative* differences between rats and humans. Specifically, they noted that increases in TSH lead to goiter (hypertrophy), but not thyroid tumors in humans, due to the higher reserve capacity of T4 in humans compared to rats. Specifically, the half-life of T4 in rats is much shorter than that in humans (attributed to the absence of a high-affinity binding globulin for T4), and so rats have a

higher rate of T4 production and higher TSH levels. These higher TSH levels make rats more sensitive to agents that decrease T4 and increase TSH. Thus, Dellarco et al. (2006) concluded that thyroid tumors resulting from this MOA are not relevant to humans. The Dellarco paper represents the state of the science for interpreting thyroid tumors. The authors of the current DINX assessment view this paper as superceding the U.S. EPA (1998) monograph on thyroid tumors.

Unfortunately, none of the recent DINX authoritative reviews cited the Dellarco (2006) MOA evaluation, perhaps because it was not specific to DINX and so did not show up in a chemical-specific literature search. Thus, Versar et al. (2010) and Bhat et al. (2014) referred only to the U.S. EPA (1998) Risk Assessment Forum guidance, and not to the more recent Dellarco et al. (2006) paper in interpreting the human relevance of the thyroid tumors. According to U.S. EPA (1998), rodent noncancer thyroid effects resulting from disruption of the thyroid-pituitary axis are presumed to pose a noncancer health hazard to humans and that rodent cancer effects resulting from the same mechanism may pose a cancer health hazard to humans.   However, the scientific understanding of disruption of the thyroid-pituitary axis has advanced since 1998, as has the risk assessment science for evaluating human relevance.

Table 5. Dose- and Duration-Response for Key Events Hypothesized According to Dellarco et al. (2006)

Effect	Dose (mg/kg-day)			
	40	~100	300-500	~1000
Induction of metabolic enzymes	No effect	No effect	No effect	At 2 weeks (M, F – BASF AG, 2005b; 2000) At 90 days only in F (BASF AG, 2002b)
Increased hepatic clearance of T3 and T4	Not measured	Not measured	Not measured	Not measured
Decreased serum levels of T3 and T4	Not measured	No effect or increase	T3 decreased in M and F (not stat sig) at 30 days, not evaluated at later time points, T4 decr. only in males (not stat sig), inconsistent time and dose progression (BASF AG, 2002b)	T3 decreased in M and F (not stat sig) at 30 days, not evaluated at later time points, T4 decr only in males (not stat sig), inconsistent time and dose progression (BASF AG, 2002b)

Effect	Dose (mg/kg-day)			
	40	~100	300-500	~1000
Increased TSH	Not measured	At 30 days and later (F only – BASF AG, 2002b)	At 30 days and later (F only BASF AG, 2002b)	At 30 days and later (F only BASF AG, 2002b)
Thyroid follicular cell hypertrophy	No significant increase	No significant increase	No significant increase	At 13 weeks (F only BASF AG, 2002b)
Thyroid follicular cell hyperplasia	No significant increase	No significant increase	No significant increase	At 2 years (M, F BASF AG, 2005a)
Thyroid tumors	No significant increase	No significant increase	No significant increase	At 2 years (M, F BASF AG, 2005a)

5.9 Lowest Hazard Endpoints by Organ System and Exposure Duration

The overall database on repeated dose DINX studies is fairly complete, including most key study types. For most study types, however, only one study is available (e.g., one subchronic study, one chronic study, etc.). These studies have been performed primarily by the manufacturer. In addition, repeated dose data are available only in rats, with the exception of a developmental toxicity study in rabbits (BASF AG, 2004a). Data were available only for the oral route; no inhalation studies were located, and, with the exception of the acute toxicity studies, no dermal studies were located.

The available toxicity studies consistently show that the primary targets of DINX toxicity are the liver, kidney and thyroid (Table 6). Reproductive/developmental and endocrine-related effects may also occur secondary to the thyroid effects or other potential endocrine-related changes, particularly following prenatal or perinatal exposure. However, definitive data on such changes are currently lacking.

The liver is a target only for induction of metabolic enzymes and associated changes. Phase I and phase II enzymes (when measured) were consistently induced in 28-day, 90-day and chronic systemic toxicity studies. Specifically, increased GGT and decreased serum bilirubin were seen at the high dose (>1500 mg/kg-day for males and females) in the 28-day study (BASF AG, 2000a, as cited by Bhat et al., 2014), the high dose in the 90-day study (>1100 mg/kg-day for males and females) (BASF AG, 2002b, as cited by Bhat et al., 2014), and the high dose females (1000 mg/kg-day) in the 2-year study (Bhat et al., 2014; ANSES, 2016a). Liver weight was also increased by >10% in the high-dose females in the 90-day study. Increases in serum GGT and decreases in total bilirubin levels were also reported at ≥300 mg/kg-day in F0 parents and F1 generation females, and at 1000 mg/kg-day in F1 generation males in the two-generation study

(BASF AG 2003b, as cited in ANSES, 2016a); increased liver weight was also reported at these doses. BASF AG (2005b, as cited in Bhat et al., 2014; ANSES, 2016a) reported induction of phase I (cytochrome P450) and phase II metabolic enzymes (glucuronyl transferase) in rats given 1418 (males) or 1568 (female) mg/kg-day DINX in the diet for 2 weeks. The strongest induction was seen for CYP2B, which is also strongly induced by phenobarbital. There was no evidence of liver histopathology. Based on Hall et al. (2012) and U.S. EPA (2005), these changes were considered to reflect only enzyme induction and were not considered adverse, in the absence of any evidence of liver histopathology.

Kidney effects were reported in several studies in male rats (but not female rats), but the data are inconsistent regarding the specific effects and the dose at which effects occurred. In the 28-day study, there was no effect at 318 mg/kg-day, but males at the high dose (1585 mg/kg-day) had an increase in the number of degenerated epithelial cells in the urine and significantly increased serum sodium in males, but no kidney histopathology (BASF AG, 2000a, as cited by Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016). Similar urinary changes (erythrocytes and transitional epithelial cells in the urine) were seen in the 90-day study, with a NOAEL of 107 mg/kg-day and a LOAEL of 326 mg/kg-day (BASF AG, 2002b, as cited by Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a). Protein accumulation was reported at the same dose. The secondary sources were inconsistent with regard to whether the protein was $\alpha2u$-globulin. In the chronic study (BASF, 2005a, as cited in Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2007; NICNAS, 2012), degenerated epithelial cells and granular cell casts were increased in the urine of males at 200 mg/kg-day at 3 months, but these changes were not seen at later time points or at the high dose at any time point. Because these changes were transient, not dose-related, and because (unlike the subchronic study), they were not accompanied by erythrocytes in the urine, the kidney changes in the chronic study were not considered adverse for this assessment, consistent with the conclusions of ANSES (2016a), but not NICNAS (2012). Based on that conclusion, the high dose of 1000 mg/kg-day would be a NOAEL. Questionable kidney effects were also seen in the two-generation study (BASF AG, 2003b, as cited in Bhat et al., 2014, and ANSES, 2016a). Renal tubular vacuolization was observed at ≥300 mg/kg-day in the F1 males (but not in F0 parents). The secondary sources differed regarding the toxicological significance of this change, but this assessment considered it adverse in the absence of data to the contrary.

The progression of thyroid effects was discussed in detail in Section 5.8. As discussed in that section, the weight of evidence is that the thyroid tumors occur via a MOA (increased thyroid hormone metabolism leading to increased TSH and hyperplasia via disruption of the thyroid-pituitary axis) that is not relevant to humans (Dellarco et al., 2006), and so those effect levels are not further discussed here. In addition, the earlier thyroid effects, beginning with increased TSH levels and including hypertrophy, hyperplasia, and increased thyroid weight, all occur via the same MOA, which is not relevant to humans.

However, decreased T3 and T4 levels *are* relevant to humans, because they can result in neurodevelopmental effects. Clear effect levels for changes in T3 or T4 are not available. In the subchronic study where thyroid hormone levels were measured at 30, 62, and 90 days, decreases (in T3 and T4 in males and only T3 in females) were seen at 30 days, but the change was not dose-related (BASF AG, 2002b, as cited in Bhat et al., 2014). At the latter time points, no decrease was seen, but only T4 (and not T3) was measured. The absence of a clear decrease in T3 and T4 may reflect a homeostatic response to increased TSH levels. In a mechanistic study not conducted under GLP, no changes in thyroid-associated hormones (T3, T4, TSH) were reported on GD21 in pregnant dams exposed to doses up to 100 mg/kg-day on GD 8 through parturition (Campioli et al., 2017). The absence of an effect in this study may be related to the relatively low dose tested and the relative short exposure. No decrease in T3 or T4 or increase in TSH was seen in rats fed 1301 mg/kg-day for 2 weeks in a study evaluating the thyroid MOA (BASF AG, 2005c, as cited in Bhat et al., 2014; ANSES, 2016a). Although the absence of such changes at a high dose above the thyroid tumor effect level might argue against the hypothesized MOA, the positive control in this study (phenobarbital) also failed to induce changes in T3, T4, or TSH, even though phenobarbital is known to act via the hypothesized MOA. In the absence of a clear dose-response for T3 or T4, changes in TSH (the key event immediately following changes in T3 and T4) was used as a surrogate for changes in T3 and T4 and to protect from neurodevelopmental toxicity.

No effect on male or female reproductive function was seen in a two-generation reproductive toxicity study (BASF AG, 2003, as cited in Bhat et al., 2014, and ANSES, 2016a), although some of the perinatal effects might alter reproductive function of male pups exposed *in utero*.

No developmental toxicity was seen in a two-generation reproductive toxicity study (BASF AG, 2003, as cited in Bhat et al., 2014, and ANSES, 2016a), or in guideline-compliant developmental toxicity studies in rats (BASF AG, 2002d, as cited in Bhat et al., 2014; SCENHIR 2016; ANSES, 2016a) or rabbits (BASF AG, 2004a, as cited in Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016). However, in a pre- and postnatal developmental toxicity study (BASF AG, 2002, as cited in Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a) AGD and AGI were decreased in male pups at 1000 mg/kg-day, suggesting that DINX was acting to impair androgen-dependent development. However, it was not clear whether the change was biologically significant, because AGI (but not AGD) was also decreased in female pups. In light of this uncertainty, a NOEL of 750 mg/kg-day and a LOEL of 1000 mg/kg-day were identified. There was no effect on AGD in the male pups from dams exposed to doses up to 100 mg/kg-day (much lower than the NOEL in the perinatal study) on GD 8 through parturition (Campioli et al., 2017). In the Campioli study, AGD was significantly increased in male pups at PND 60, but the significance of an *increased* AGD in males is unclear.

The potential carcinogenicity of DINX has been investigated only in rats (BASF AG, 2005c, as cited in Bhat et al., 2014; ANSES, 2016a; and SCENIHR, 2016). Significantly increased

incidences of thyroid follicular cell adenomas, and of mammary gland fibroadenomas were reported. However, the weight of evidence supports the conclusion that the thyroid tumors occurred via a MOA that is not relevant to humans, and the mammary tumors appear to be incidental lesions, not causally related to DINX exposure. DINX does not cause point mutations or chromosome damage (BASF AG 2000b, 2001b, 2000c, 2001c, as cited in Bhat et al., 2014). Therefore, DINX is unlikely to be carcinogenic to humans. However, a definitive conclusion is not possible in the absence of a carcinogenicity study in a second species.

5.10 Uncertainties and Data Gaps

A number of uncertainties were identified in this assessment.

Database:

The overall database on DINX is fairly complete, including most key studies. For most study types, only one study is available. It also should be noted that these studies were perfomed by the manufacturer (e.g., one subchronic study, one chronic study, etc.). In addition, repeated dose data are available only in rats, with the exception of a developmental toxicity study in rabbits (BASF AG, 2004a). Data were available only for the oral route; no inhalation studies were located, and, with the exception of the acute toxicity studies, no dermal studies were located.

There were also several data gaps. Perhaps the most important data gap is the absence of a neurodevelopmental toxicity study, or of a study evaluating thyroid hormone levels in a pregnant rat. In light of the likely MOA for thyroid effects, neurodevelopmental toxicity is a potential concern, but no studies have directly evaluated this endpoint, or the immediate precursor of thyroid hormone changes in a pregnant animal. A repeated dose systemic toxicity study is also not available in as second species. In addition, most of the primary studies were not available, and the studies were evaluated based on secondary references.

Hazard:

Kidney: There were a number of inconsistencies in the kidney data, with each study reporting different histopathology (or no histopathology), complicating interpretation of the results. In particular, there were uncertainties in determining which sets of changes were adverse and which were not toxicologically significant. Perhaps in part due to this uncertainty, the NOAEL for kidney effects in the 2-year bioassay was higher than that in the 28-day and subchronic studies, contrary to normal expectations. In addition, secondary references were inconsistent regarding the nature of the protein that accumulated in the tubules in the 90-day study. Bhat et al. (2014) stated that immunohistochemistry for evaluating the presence of α2u-globulin was not included, but ANSES (2016a) and SCENIHR (2016) both identified the protein in the tubules as α2u-globulin. It is not clear if the latter assessments were based on an

33

assumption regarding the identity of the protein, or if they were based on additional information not reported by Bhat et al. (2014).

Developmental: The AGD decreased in male pups exposed pre- and postnatally, but it was unclear whether the observed decrease was biologically meaningful (BASF AG, 2002c, as cited in Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a). Similarly, the results of Campioli et al. (2017) raise the question of whether in utero exposure can result in anti-androgenic effects that manifest after puberty. However, several limitations and incomplete reporting (e.g., absence of consistent dose-response, time-response or effects among related endpoints, absence of information on the number of dams or the number of independent litters that the sampled pups came from) preclude definitive conclusions regarding the adversity of the findings. It would be useful to investigate this issue further in a carefully controlled study investigating the effects of prenatal, perinatal and postnatal exposure.

Thyroid: A key conclusion relates to the MOA for thyroid effects, but there are several uncertainties related to that conclusion, although the overall data support the relevant key events. In addition, although DINX acted similarly to phenobarbital (which acts via the hypothesized MOA), there were some key limitations. Specifically, DINX induced liver enzymes, particularly CYP2B1 (BASF AG 2005b, as cited in Bhat et al., 2014; ANSES, 2016a), and effects similar to phenobarbital were seen in an assay exploring the mechanism of thyroid toxicity (BASF AG, 2005c, as cited in Bhat et al., 2014; ANSES, 2016a), but the expected effects on T3, T4 and TSH were not observed with either DINX or the positive control (phenobarbital) in the mechanistic assay. In addition, as described in Section 5.8, some aspects of the expected dose-duration response for key events under the MOA were not observed.

Reproductive: No effect on male or female reproductive function was seen in a two-generation reproductive toxicity study (BASF AG, 2003, as cited in Bhat et al., 2014, and ANSES, 2016a). Suggestive evidence of potential effects on male reproductive targets were reported in an *in vitro* study at high doses (Boisvert et al., 2016; Nardelli et al., 2015), but the toxicological significance of these findings is unclear in light of the high concentrations tested and the absence of reported effects in the two-generation study. Similarly, the reported association of increased DINX urinary metabolite levels with a decreased number of retrieved oocytes among older women at a fertility clinic (Mínguez-Alarcón et al., 2016) is sufficient for hypothesis generation. However, caution should also be used in interpreting these results, in light of the absence of effects on related endpoints and the borderline statistical significance of the results.

Cancer: There are also uncertainties related to mammary tumors, which were considered incidental by Bhat et al. (2014); NICNAS (2012) and ANSES (2016a), but Bhat et al. (2014) also conducted a quantitative assessment based on these tumors.

Table 6. Summary of NOAELs/LOAELs Identified for DINX by Organ System

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
Wistar rat (M&F) OCED 407-compliant BASF AG, 2000a (as cited by Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016)[12]	28 days Diet 0, 600, 3000 or 15,000 ppm (equivalent to 0, 64, 318, and 1585 mg/kg-day for males and 0, 66, 342, and 1670 mg/kg-day for females) (5/sex/dose)	Liver	NOAEL = 1585 (M), 1670 (F) (No effect in males)	Increase in serum gamma-glutamyltransferase (GGT) activity (55%) and a decrease in serum bilirubin levels (20%) in females in the absence the absence of increased liver weight or histopathology not considered adverse.	Bhat et al. (2014), SCENIHR (2016) and ANSES (2016a) all identified the mid dose as the NOAEL and the high dose as the LOAEL. The LOAEL was based on increased serum GGT and decrease serum bilirubin levels (Bhat et al. (2014), or increased serum GGT in females and degenerated epithelial cells in the urine of males (SCENIHR, 2016 and ANSES, 2016a). The dose conversion was apparently in the original report, since all secondary sources reported the same doses.
	Recovery: additional 5/sex from control and high dose groups without treatment for 14 days	Kidney	NOAEL = 318 (M) LOAEL = 1585 (M) (No effect in females)	Increase in the number of degenerated epithelial cells in the urine and significantly increased serum sodium in males in the absence of kidney histopathology	

---

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

[12] SCENIHR, 2016 did not provide any citations, so the source of the data is unclear, but that assessment is clearly referring to the studies noted throughout this table.

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
Wistar rat (M&F) OECD 408-compliant BASF AG, 2002b (as cited by Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a)	90 days Diet 0, 1500, 4500, or 15,000 ppm (0, 107, 326, or 1103 mg/kg-day for males and 0, 128, 389, or 1312 mg/kg-day for females) (average at 90 days) Doses at 30 days were 0, 141, 430, or 1457 mg/kg-day for males and 0, 156, 469, or 1566 mg/kg-day for females (20/sex/dose)	Liver	NOAEL = 1103 mg/kg-day (M), 1312 mg/kg-day (F)	Increased serum GGT activity and decreased serum bilirubin in females not considered adverse	Bhat et al. (2014) considered the decreases in serum bilirubin (males) and increase in thyroid gland weight and/or hypertrophy (both sexes) at all exposure doses to be adverse and therefore considered the low dose to be a LOAEL in both sexes The study authors considered the mid dose to be a LOAEL for males based on urinalysis, and the high dose to be the female LOAEL, based on increased serum GGT. SCENIHR (2016) agreed on the effect levels but considered the kidney effects to be the critical effect in both sexes. ANSES (2016a) identified the same effect levels but did not identify the critical effects.
		Kidney	NOAEL = 107 (M) LOAEL = 326 (M) NOAEL = 1312 (F)	Erythrocytes and transitional epithelial cells in the urine of males, protein accumulation (perhaps α2u-globulin) in tubules of males. If the protein were α2u-globulin, associated effects would not be relevant to humans	
		Thyroid	No NOAEL (F) LOEL = 156 (F) at 30 days, 128 at 90 days LOAEL = 469 (F) at 30 days, 389 at 90 days No human relevant effect in males	Increased TSH in females, as a surrogate for decreased T3 and T4, for which the dose-response was inconsistent. Thyroid hypertrophy is not relevant to humans but neurodevelopmental effects secondary to decreased T3 and T4 are (Dellarco et al., 2006)	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
Wistar rat (M&F) OECD 453-compliant BASF AG, 2005c (as cited in Bhat et al., 2014; ANSES, 2016a; and SCENIHR, 2016)	24 months Diet 0, 40, 200 or 1000 mg/kg-day (corresponding HEDs were 0, 11.5, 58.2, and 286 mg/kg-day for males and 0, 9.87, 49.2, and 245 mg/kg-day for females) (50/sex/dose) Additional 10/dose for blood and urine analysis at 3, 6, and 12 months and for interim sacrifice at 12 months	Thyroid	*Rat* effect levels NOAEL = 40 (M) LOAEL = 200 (M) $BMDL_{HED}$ = 50 (M) NOAEL = 200 (F) LOAEL = 1000 (F)	Thyroid lesions (follicular cell hyperplasia) and increased thyroid weight Observed effects occur via a MOA not relevant to humans. Thyroid hormone levels were not evaluated.	Identification of effect levels considered updated incidence data from Capen (2006). All groups except Bhat et al. (2014) identified a NOAEL of 40. EFSA (2006) based its NOAEL on thyroid effects, and identified a NOAEL of 200 mg/kg-day for other effects (based on increased platelet counts in females). The Danish EPA (2014) based its NOAEL on liver weight changes (both sexes) and kidney weight changes (males). NICNAS (2012) based its NOAEL on the renal effects. Bhat et al. (2014) identified a LOAEL of 40 mg/kg-day for thyroid effects (presumably based on absolute thyroid weight decrease of 19% in females only at 40 mg/kg-day at the 1-year sacrifice). Thyroid follicular adenomas and adenocarcinomas observed in both sexes, but only adenomas was dose-dependent in both sexes without statistical significance at any dose level.
		Liver	NOAEL = 1000 (M, F)	Enzyme induction, decreased bilirubin, increased liver weight not considered adverse	
		Kidney	NOAEL = 1000 (M, F)	Degenerated epithelial cells and granular cell casts in the urine of males at 200 mg/kg-day at 3 months; increased kidney weight. Considered incidental because transient and not dose-related	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
		Cancer	NOAEL (M) = 40 LOAEL (M) = 200 BMDL$_{HED}$ (M) = 74 NOAEL (F) = 200 LOAEL (F) = 1000	Thyroid follicular adenomas Likely occur via MOA that is not relevant to humans	Mammary fibroadenomas were considered incidental, age-related lesions.
			Females: BMDL$_{HED}$ = 58	Mammary fibroadenomas Considered incidental	

Reproductive/Developmental Toxicity

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
Wistar rat Diet OECD 416-compliant BASF AG, 2003b (as cited in Bhat et al., 2014; ANSES, 2016a;	2 generations 0, 100, 300 or 1000 mg/kg-day (F0 and F1: 25/sex/dose) Actual doses were: F0: 0, 99-109, 283-334, and 968-1101;	Liver	NOAEL = 1000 (M, F) LOAEL = None	No adverse effects	ANSES (2016a), Bhat et al. (2014), NICNAS (2012) concluded there were no adverse effects on liver, kidney, or thyroid, but EFSA considered the kidney effects adverse.
		Kidney	NOAEL = 100 (M) LOAEL = 300 (M) NOAEL = 1000 (F)	Vacuolization of kidney tubular epithelia in F1 males. Toxicological significance unclear, not seen in subchronic or chronic studies or in F0 males.	
		Thyroid	NOAEL = 1000 M)	Increased thyroid weight and follicular cell hypertrophy/hyperplasia in F1 parental rats.	Thyroid gland histology was not conducted in F0 males or females or F1 males since thyroid weights were not altered.

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
SCENIHR, 2016)	F1: 0, 94-101, 271-301, and 942-1037 mg/kg-day (NICNAS, 2012)		*In rats:* NOAEL = 100 (F) LOAEL = 300 (F)	Data insufficient to identify human-relevant effect level.	
		Reproductive	F0 parents NOAEL = 1000 LOAEL = None F1 parents NOAEL = 1000 LOAEL = None	No reproductive effects in either generation.	Conclusions on reproductive effects reached by ANSES (2016a), SCENIHR (2016), Bhat et al. (2014), and NICNAS (2012).
		Developmental	F1 pups NOAEL = 1000 LOAEL = None F2 pups NOAEL = 1000 LOAEL = None	No developmental toxicity in either generation	Conclusions on developmental toxicity reached by ANSES (2016a), SCENIHR (2016), Bhat et al. (2014), and NICNAS (2012).
Wistar rats (pregnant) Pre- and postnatal developmental study) BASF AG, 2002c, as	Oral, presumably gavage 0, 750, or 1000 mg/kg-day Exposed GD 3 until PND 20; monitored until PND 100-105	Developmental	NOEL = 750 (M) LOEL = 1000 (M pups) NOAEL = 1000 (F pups)	AGD (p<0.05) and AGI (p<0.01) decreased statistically significantly by 7% and 8%, respectively, but it is unclear whether this change was biologically significant.	No information on maternal toxicity reported, except that thyroid parameters were not assessed. AGI decreased significantly (p<0.05) in F1 female pups (8%) at the high dose, but AGD was not and vaginal opening not affected No effect on testes descent, balanopreputial separation, vaginal openings, sperm parameters, organ weights, or histopathology. Furthermore, AGI was decreased to the same extent (8%) in females, suggesting not an anti-androgen

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
cited in Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a	(10 dams/dose), all male offspring, and 3 female pups (not clear whether per litter or dose)				effect. However, the decreased AGI in females could reflect the small decrease in AGD and small increase in pup body weight. The study authors and cited reviews concluded the effects are not biologically significant, but ANSES (2016a) noted an opposing view.
Wistar rats OECD 414-compliant developmental toxicity study BASF AG, 2002d, as cited in Bhat et al., 2014; SCENHIR, 2016; ANSES, 2016a)	Gavage (in olive oil) 0, 200, 600, 1200 mg/kg-day GD 6 – 19 (25/dose)	Maternal	NOAEL = 1000 LOAEL = None	No adverse effects	No maternal toxicity, but thyroid endpoints were not evaluated.
		Developmental	NOAEL = 1000 LOAEL = None	No adverse effects	Incidence of 6-7% of litter-based rate of uni- and bilateral dilation of the renal pelvis and/or ureter observed in all treated groups compared to 4% in controls and historical range of 0-16% (mean, 7%). The incidence of incomplete or reduced ossification was significantly increased for the thoracic centrum (up to 9% at the low dose), and the parietal bone (7 and 8% at the mid and high dose, respectively).Incidence of litter-based skeletal malformations in treated groups (0 – 5%) was less than in controls (13%). Changes were not considered adverse.
Himalayan rabbits OECD 414-compliant	Diet Mean doses of 0, 102, 311, or 1029 mg/kg-day	Maternal	NOAEL = 1029 LOAEL = None	No adverse effects	No maternal toxicity, but thyroid endpoints were not evaluated.
		Developmental	NOAEL = 1029 LOAEL = None	No adverse effects	At the high dose, significant increase was noted in litter-based rate of supernumerary thoracic vertebra, a skeletal variation; the

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
developmental toxicity study BASF AG, 2004a, as cited in Bhat et al., 2014; SCENHIR 2016; ANSES, 2016a	Day 6 through 29 post insemination (25/dose; 19–24 pregnant rabbits/ dose)				increase was within the historical range and the effect was not considered adverse.
Sprague-Dawley rat (time-pregnant dams) Non-guideline study Campioli et al., 2017	Gavage (in corn oil) 0, 1, 10, 100 mg/kg-day (13/dose) GD 14 to PND 0 or GD 8 to PND 0	Liver	NOAEL = 100 LOAEL = None	No adverse effects	Survival not affected; no effect on serum markers of liver or kidney dysfunction. No effect on thyroid-associated hormones (T3, T4, and TSH) evaluated in the pregnant dams at GD 21. No effect on limited maternal parameters examined: pregnancy rate; no fetal skeletal malformations Study raises the question of whether in utero exposure can result in anti-androgenic effects that manifest after puberty. However, several limitations and incomplete reporting (e.g., absence of consistent dose-response, time-response or effects among related endpoints, absence of information on the number of
		Kidney	NOAEL = 100 LOAEL = None		
		Thyroid	NOAEL = 100 LOAEL = None		
		Reproductive/ developmental	Maternal NOAEL = 100 LOAEL = None	No adverse effects	
			Developmental Effect levels cannot be determined	Testicular atrophy in 3/13 males in the PND 200 progeny from rats exposed in utero to 100 mg/kg-day compared to 1/13 animals	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
				in the corresponding control group. A variety of other endpoints related to male reproductive structure or function were observed sporadically, but the data were not sufficiently consistent to show causality or identify an effect level.	dams or the number of independent litters that the sampled pups came from) precludes definitive conclusion regarding adversity of the findings. This study was published after the latest authoritative review.
Sprague-Dawley rat (time-pregnant dams) Non-guideline study Nardelli et al., 2017	Gavage (in corn oil) 0, 30, 300 mg/kg-day (16-19/dose) Treated GD 8 to PND 21 Examined PND 3 through PND 46	Maternal	NOAEL = 300 LOAEL = none	No adverse effects	No effects on body weights, organ weights, serum magnesium, or serum enzymes reflecting damage to the liver; no effects on steroid hormone production No effect on pup body or organ weights, AGD/AGI, serum levels of LH or FSH in females, expression of genes important for reproductive function or steroidogenic enzymes or testosterone production; no increased incidence of external malformations.
		Reproductive/ developmental/ endocrine	NOAEL = 300 LOAEL = None	No effects in females. Increased incidence of testicular hemorrhage in male offspring at PND 8 at both doses, but not at PND 21. The significance of this transient increase is unclear.	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[11]	Toxicological Basis	Comments
Sprague-Dawley rat (pregnant dams) Non-guideline study Furr et al., 2014	Gavage (in corn oil) 0 or 750 mg/kg-day (3 dams/dose) GD 14-18	Endocrine	NOAEL = 750	No adverse effects	None

6 Exposure

The use of DINX in consumer products has been described in Section 3 of this report. No information could be found on leaching or migration of DINX from polymer resins. Information on total exposure is also not available (CPSC, 2014).

Oral

Oral exposure to DINX can occur when infants and children interact with child care articles and children's toys. CPSC (2014) estimated infant mean exposure to DINX from mouthing soft plastic objects (not including pacifiers). Based on the mean migration rate and mouthing duration, mean exposure was estimated at 1.4, 0.89, and 0.82 µg/kg-day for babies aged 3 - <12 months, 12 months - <24 months, and 24 - <36 months, respectively. The upper bound exposure was 5.4 µg/kg-day. CPSC (2014) further noted that DINX was present in about one-third of the toys and child care articles tested (Dreyfus, 2010, as cited by CPSC, 2014). In a similar analysis, ANSES (2016b) estimated the exposure of children <3 years to DINX from mouthing toys. The median daily dose was estimated at 1.64 µg/kg-day for children 0-12 months, and 0.50 µg/kg-day for children 13-24 months and for children 25-36 months. The 95th percentile for the youngest group was 25.2 µg/kg-day.

Oral exposure to DINX can also occur from food. In 2006, specific information on DINX migration was submitted to the European Food Safety Authority (EFSA) by its petitioner (presumably BASF). In this submission, migration of DINX from PVC cling film containing 10-17.8% of DINX into "food stimulants and foodstuffs" was determined by Gas Chromatography/Mass Spectrometry (GC/MS). Results showed that DINX migrated quantitatively into foods with high fat content; the migration rate of DINX from cling wrap was 29 mg/dm^2 for sunflower oil (6h at 10°C and 144h at 20°C) and 27.5 mg/dm^2 for high fat cheese (10d at 5°C). All other products tested (fresh meats and low fat cheeses) were below the 10 mg/dm^2 European legal limit (EFSA, 2006).

Migration of DINX from bottle closures containing a PVC sealing layer with 37% DINX was determined by the petitioner for carbonated mineral water, grape fruit juice, and orange lemonade. In all cases, migration into the aqueous beverages was low, in the range of 10-30 µg/kg. Results from a medical tubing migration study by Welle et al. (2005) determined that migration in a DINX feeding system was considerably lower than for DEHP systems. NICNAS (2012) estimated the dietary exposure of the general public to DINX as 0.081 mg/kg-day, with oils contributing 84% of the dose.

Bui et al. (2016) also reported that DINX was found in new infant crib mattress covers (53.2 mg/g material, 44.4% detection frequency) and in household dust at concentrations up to 110 mg/kg dust (Boor et al., 2015; Nagorka et al., 2011, as cited by Bui et al., 2016).

Dermal

Assuming 10% dermal and 100% inhalation absorption, and no oral exposure, NICNAS (2012) estimated that the typical exposure during occupational handling of DINX is 0.35-1.12 mg/kg-day.

Biomonitoring

Absorbed DINX is eliminated in the bile (primarily as the glucuronic acid conjugate of the monoisononyl ester) and the urine (primarily as CHDA) (Bhat et al., 2014; Koch et al., 2013). Schutze et al. (2014, 2017) identified oxidized metabolites of the monoester as the main specific urinary metabolites (presumably after CHDA), followed by the unoxidized monoester (MINX). The CHDA metabolite cannot be used to estimate DINCX exposure, due to its lack of specificity, but the specific DINX metabolites MINX, OH-MINX, oxoMINX, and cx-MINX can be used for estimating exposure from biomonitoring data.

Several recent studies have reported on biomonitoring data for DINX (e.g., Fromme et al., 2016; Gomez-Ramos et al., 2016; Giovanoulis et al., 2016; Correia-Sá et al., 2017). These data show increasing levels of DINX metabolites in urine, consistent with the increasing amounts of DINX being used as a phthalate substitute. For example, Calafat et al. (2015) reported that DINX metabolites were not detected in urine samples from Germany and the U.S. prior to 2002. In contrast, specific metabolites were detected in almost 20% of the urine from participants in the National Health and Nutrition Examination Survey (NHANES) in 2011-2012, and 98% of student samples from the German Environmental Specimen Bank (ESB) in 2012. Apel et al. (2017) reported on health-based human biomonitoring values developed by the German Biomonitoring Commission for DINX based on the urinary metabolites OH-MINX and cx-MINX. These biomonitoring values can be used to aid in interpreting the health implications of biomonitoring data.

Biomonitoring data for the urinary DINX metabolite, cyclohexane-1,2-dicarboxylate-mono-(7-hydoxy-4-methyl) octyl ester (OH-MINX, OH-MINCH), is currently available for both the 2011/2012 and 2013/2014 NHANES data cycles.

7   Discussion

7.1   Toxicity Under FHSA

Animal data were sufficient to support the conclusion that **DINX 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 or dermal exposures. Acute LD_{50} values for DINX in rats were >5000 mg/kg for oral exposure and >2000 mg/kg for dermal exposure (BASF, 1999c, 1999d as cited in Bhat et al., 2014. No data were available regarding acute inhalation toxicity. DINX caused mild to moderate skin irritation, but it was not an ocular

irritant or a skin sensitizer (BASF, 1999a, 1999b, 2001a, 2002a, 2004b as cited in Bhat et al., 2014).

Sufficient animal data exist to support the conclusion that DINX can be considered "toxic" under the FHSA due to its toxicity following short-term and subchronic exposures. DINX caused kidney toxicity in rats at durations ranging from 28 days to 90 (BASF AG, 2000a, as cited by Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016; and BASF AG, 2002b, as cited by Bhat et al., 2014; SCENIHR, 2016; ANSES, 2016a).

No toxicity was seen in standard reproductive or developmental toxicity studies (BASF AG, 2003, as cited in Bhat et al., 2014, and ANSES, 2016a; BASF AG, 2002d, as cited in Bhat et al., 2014; SCENHIR 2016; ANSES, 2016a; BASF AG, 2004a, as cited in Bhat et al., 2014; ANSES, 2016a; SCENIHR, 2016). However, there were some suggestions of antiandrogenic effects in male pups exposed in utero (BASF AG, 2002, as cited in Bhat et al., 2014; SCENHIR, 2016; ANSES, 2016a; Campioli et al., 2017) although data inconsistencies and questions about the biological significance of the observed changes precluded a definitive result.

There is sufficient evidence to support the conclusion that DINX is not a direct acting genotoxicant (Bhat et al., 2014).

DINX causes thyroid tumors in rats, but the most likely MOA is not relevant to humans.

The data are adequate to set exposure limits for a 4-week or subchronic oral exposure, although uncertainty factors would be needed to account for the absence of a neurodevelopmental toxicity study and uncertainty regarding doses affecting thyroid hormone levels, as well as the standard extrapolations. There is greater uncertainty for a chronic exposure limit, due to the absence of a clear adverse effect level relevant to humans in the chronic study. For the 28-day study, the effect level would be based on kidney effects in males. Following a 90-day exposure, the critical effects were urinary changes in males at 107 mg/kg-day, and increased TSH (as a surrogate for decreased T3 and T4) in females.

8 References

Albert O, Nardelli T, Lalancette C, Hales BF & Robaire B (2016) In The 9th Symposium of the Réseau Québécois en reproduction. 83-83. [Cited in Campioli et al., 2017].

ANSES (French Agency for Food, Environmental and Occupational Health & Safety). (2016a) Analysis of the most appropriate risk management option (RMOA). 1,2-Cyclohexanedicarboxylic Acid, Diisononyl Ester (DINCH®). Available at: https://echa.europa.eu/documents/10162/fc77bffd-e7ec-4846-b080-11de2564e582

ANSES (French Agency for Food, Environmental and Occupational Health & Safety). (2016b) Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on "plastic toys and children's equipment intended for children under three years of age." Request No 2013-SA-0176.

Apel P, Angerer J, Wilhelm M, Kolossa-Gehring M (2016) New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int. J. Hyg. Environ. Health. 220(2 Pt A): 152-166.

BASF AG (1999a) 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester—Acute eye irritation in rabbits. Laboratory project identification: 11H0223∕992126. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (1999b) 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester—Acute dermal irritation∕corrosion in rabbits. Laboratory project identification: 18H0223∕992125. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (1999c) Acute dermal irritation∕corrosion in rabbits. Laboratory project identification: 18H0745∕002210. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (1999d) 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester—Maximization test in guinea pigs. Laboratory project identification: 30H0223∕992127. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2000a) 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester—Repeated dose oral toxicity study in Wistar rats, Administration in the diet for 4 weeks and recovery period of 2 weeks. Final report. Project no. 37S0223∕99062. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2000b) *Salmonella typhimurium/Eschericia coli* reverse mutation assay (standard plate test and preincubation test) with 1,2-cyclohexane dicarboxylic acid, di (isononyl) ester.

Final report. Project no. 40MO223/994088. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Germany. [Cited in Bhat et al., 2014].

BASF AG (2000c) *In vitro* chromosome aberration assay in Chinese hamster V79 cells with 1,2-cyclohexane dicarboxylic acid, di(isononyl) ester. Unpublished study conducted by RCC Cytotest Cell Research. RCC-CCR project 651400. ZHIT-Project no. 32MO2231999040. Sponsored by BASF Aktiengesellschaft, Germany. [Cited in Bhat et al., 2014].

BASF AG (2001a) Acute dermal irritation/corrosion in rabbits. Laboratory project identification: 18H0745/002210. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2001b) In vitro gene mutation test with 1,2-cyclohexane dicarboxylic acid, di(isononyl) ester. Project no. 50M0107/014031. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG. 2001c. Micronucleus assay in bone marrow cells of the mouse with 1,2-cyclohexane dicarboxylic acid, di(isononyl) ester. Unpublished study conducted by RCC Cytotest Cell Research. RCC-CCR project 698101. Sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2002a) Plastisol Rezeptur (auf BasisHexamoll DINCH®) Acute dermal irritation/corrosion in rabbits. Study no. 18H0084/022004. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2002b) 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester—Subchronic oral toxicity study in Wistar rats Administration in the diet for 3 months. Project no.: 50S0107/01009. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2002c) 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester—Pre-/postnatal developmental toxicity study in Wistar rats oral administration gavage. Laboratory project identification: 60R0223/99095. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2002d) 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester—Prenatel developmental toxicity study in Wistar rats oral administration. Project no. 30R0223/99124. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2003a) 14C-1,2-cyclohexanedicarboxylic acid, diisononyl ester—Study of the biokinetics in rats. Project no. 02B0608/016021. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2003b) 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester—Two generation reproduction toxicity study in Wistar rats continuous dietary administration. Laboratory project identification: 70 R01 07/01021. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2004a) 1,2-cyclohexane dicarboxylic acid, di(isononyl) ester—Prenatal developmental toxicity study in Himalayan rabbits administration in the diet. BASF project no. 42R0107/01135. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2004b) Hexamoll DINCH®—Acute dermal irritation/corrosion in rabbits. Project no. 18H0010/042033. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG. (2005a) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester—Combined chronic toxicity/carcinogenicity study in CrlGlxBrlHan: WI-rats; Administration in the diet up to 24 months. Project no. 82S0107/01094. Unpublished study conducted and sponsored by BASF. Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2005b) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester—Liver enzyme induction study in Wistar rats Administration in the diet over 2 weeks. Final report. Project no. 48C0107/01181. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF AG (2005c) 1,2-cyclohexane dicarboxylic acid, di(isononyl) ester—Thyroid function study in male Wistar rats using perchlorate discharge as a diagnostic test—Administration in the diet over 4 weeks. Laboratory project identification: 48C0107/01180. Unpublished study conducted and sponsored by BASF Aktiengesellschaft, Ludwigshafen, Germany. [Cited in Bhat et al., 2014].

BASF. 2006. Safety Data Sheet: Hexamoll® DINCH. November 5, 2006. [Cited in Versar, 2010].

BASF. 2007. Product information and applications. Hexamoll® DINCH. Available: http://www.hexamoll.com/icms/basf_6/en/dt.jsp?setCursor=1_216770. [Cited in Versar, 2010].

BASF. 2008a. Hexamoll ® DINCH. Technical Leaflet. BASF. January, 2008. [Cited in Versar, 2010].

BASF. 2008b. Hexamoll ® DINCH: The plasticizer for sensitive applications. Leaflet. BASF. 2008-09. [Cited in Versar, 2010].

Bhat VS, Durham JL, Ball GL and English JC (2014) Derivation of an Oral Reference Dose (RfD) for the Nonphthalate Alternative Plasticizer 1,2-Cyclohexane Dicarboxylic Acid, Di-

Isononyl Ester (DINCH). Journal of Toxicology and Environmental Health, Part B: Critical Reviews. 2:63-94.

Boisvert A, Jones A, Issop L, Erythropel HC, Papadopoulos V, Culty M (2016). In vitro functional screening as a means to identify new plasticizers devoid of reproductive toxicity. Environ Res 150:496-512.

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology 36:781-792

Boor BE, Liang YR, Crain NE, Jarnstrom H, Novoselac A, Xu Y (2015) Identification of phthalate and alternative plasticizers, flame retardants, and unreacted isocyanates in infant crib mattress covers and foam. Environ. Sci. Technol. Lett. 2:89–94.

Bui TT, Giovanoulis G, Cousins AP, Magnér J, Cousins IT, deWit CA (2016). Human exposure, hazard and risk of alternative plasticizers to phthalate esters. Sci Total Environ. 541:451-467.

Calafat AM, Valentin-Blasini L, Ye X (2015) Trends in exposure to chemicals in personal care and consumer products. Curr. Envir. Health. Rpt. 2:348–355

Campioli E, Lee S, Lau M, Marques L and Papadopoulos V. (2017) Effect of prenatal DINCH plasticizer exposure on rat offspring testicular function and metabolism. Sci Rep. 7(1):11072.

Capen, C.C. (2006) Expert opinion on proliferative lesions of the thyroid follicular cells from Wistar rats administered Hexamoll DINCH® in the diet for two years (Carcinogenicity study no. 01094). Unpublished report submitted to NSF International by BASF Corporation, Wyandotte, MI. [Cited in Bhat et al., 2014].

Correia-Sá L, Schütze A, Norberto S, Conceição C, Domingues VF, Koch HM (2017) Exposure of Portuguese children to the novel non-phthalate plasticizer di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH). Environ. Int. 102 :79–86.

CSPC (U.S. Consumer Product Safety Commission). (2014) Chronic hazard advisory panel on phthalates and phthalate alternatives. Directorate for Health Sciences Bethesda, MD 20814.

Danish EPA (Danish Environmental Protection Agency) (2014) Alternatives to classified phthalates in medical devices, Environmental Project No. 1557, 2014. Available at: https://www2.mst.dk/Udgiv/publications/2014/03/978-87-93178-27-4.pdf

Dellarco VL, McGregor D, Berry C, Cohen SM. Boobis AR (2006) Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. Critical Reviews in Toxicology. 36:793–801

Dreyfus M. (2010) Phthalates and Phthalate Substitutes in Children's Toys. U.S. Consumer Product Safety Commission, Bethesda, MD. Available at: http://www.cpsc.gov/PageFiles/126545/phthallab.pdf. [Cited in CPSC, 2014].

EFSA (2006) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 12th list of substances for food contact materials. EFSA/AFC/FCM/293-Rev.IB/45705 of June 2006. Ref 45705 EFSA Journal 395 to 401, 1-21. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/428.pdf

EPL (Experimental Pathology Laboratories, Inc.) (2008) Pathology peer review of mammary glands from a chronic feeding study in rats with 1,2-cyclohexanedicarboxylic acid, diisononyl ester-combined chronic toxicity/carcinogenicity study in CrlGlxBrlHan: Wl-Rats:Administration in the diet up to 24 months. EPL project no. 717-008. Unpublished report submitted to NSF International by BASF Corporation, Wyandotte, MI. [Cited in Bhat et al., 2014].

Fromme H, Schütze A, Lahrz T, Kraft M, Fembacher L, Siewering S, Burkardt R, Dietrich S, Koch HM, Völkel W (2016) Non-phthalate plasticizers in German daycare centers and humanbiomonitoring of DINCH metabolites in children attending the centers(LUPE 3). Int. J. Hyg. Environ. Health 219:33–39.

Furr JR, Lambright CS, Wilson VS, Foster PM, Gray LE Jr (2014) A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxicol Sci. 140(2):403-24.

Gallavan RH Jr., Holson JF, Stump DG, Knapp JF, Reynolds VL (1999) Interpreting the toxicologic significance of alterations in anogenital distance: potential for confounding effects of progeny body weights. Reproductive Toxicology 13 (1999) 383–390

Giovanoulis G, Alves A, Papadopoulou E, Cousins AP, Schütze A, Koch HM, Haug LS, Covaci A, Magnér J, Voorspoels S (2016) Evaluation of exposure to phthalate esters and DINCH in urine and nails from a Norwegian study population. Environ. Res. 151:80–90

Gomez Ramos MJ, Heffernan AL, Toms LML, Calafat AM, Ye X, Hobson P, Broomhall S, Mueller JF (2016) Concentrations of phthalates and DINCH metabolites in pooled urine from Queensland, Australia. Environ. Int. 88:179–186

Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A, Küttler K,Malarkey DE, Maronpot RR, Nishikawa A, Nolte T, Schulte A, Strauss V, York MJ (2012) Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol. 40(7):971-94.

INERIS (2013) Données technico-économiques sur les substances chimiques en France : SUBSTITUTS DU DEHP. DRC-12-126866-13682A, 51 p. (http://rsde.ineris.fr/ or http://www.ineris.fr/substances/fr/) [Cited in ANSES, 2016].

IPCS(International Programme on Chemical Safety) (2005) Chemical-Specific Adjustment Factors for Interspecies Differences and Human Variability: Guidance Document for use of Data in Dose/Concentration–Response Assessment. Harmonization Project Document No. 2. World Health Organization, Geneva.

Jobwerx. 2006. Plastics solutions for soft PVC toys and medical devices. Jobwerx Manufacturing Network. Available: http://www.jobwerx.com/news/basf_plasticizers_biz-id=948491_881.html. [Cited in Versar, 2010].

Koch HM, Schutze A, Palmke, C, Angerer J, Bruning T (2013) Metabolism of the plasticizer and phthalate substitute diisononyl-cyclohexane-1,2-dicarboxylate (DINCH®) in humans after single oral doses. Arch Tox 87:799-806.

Meek M, Bucher J, Cohen S et al. (2003) A framework for human relevance analysis of information on carcinogenic modes of action. Critical Reviews in Toxicology 33:581-653.

Meek ME, Palermo CM, Bachman AN, North CM, Lewis JR (2014) Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. J Appl Toxicol. 34(6):595–606.

Mínguez-Alarcóna L, Soutere I, Chiu Y-H, Williams PL, Ford JB, Ye X, Calafat AM, Hauser R (2016) Urinary concentrations of cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester, a metabolite of the non-phthalate plasticizer di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), and markers of ovarian response among women attending a fertility center. Environ Res. 151:595–600.

Nagorka R, Conrad A, Scheller C, Sussenbach B, Moriske HJ (2011) Diisononyl 1,2-cyclohexanedicarboxylic acid (DINCH) and Di(2-ethylhexyl) terephthalate (DEHT) in indoor dust samples: concentration and analytical problems. Int. J. Hyg. Environ. Health 214:28–37.0

Nardelli TC, Erythropel HC, Robaire B (2015) Toxicogenomic Screening of Replacements for Di(2-Ethylhexyl) Phthalate (DEHP) Using the Immortalized TM4 Sertoli Cell Line. PLoS One. 10:(10): e0138421. doi:10.1371/journal.pone.0138421

Nardelli TC, Albert O, Lalancette C, Culty M, Hales BF, Robaire B (2017) In Utero and Lactational Exposure Study in Rats to Identify Replacements for Di(2-ethylhexyl) Phthalate. Sci Rep. 7(1):3862.

NICNAS (The Australian National Industrial Chemicals Notification and Assessment Scheme) (2012) Public report on 1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester ('Hexamoll DINCH'). File No: EX/170 (STD/1259). Available at: https://www.nicnas.gov.au/search?query=DINCH&collection=nicnas-meta

Remberger M, Andersson J, Cousins AP, Kaj L, Ekheden Y, Dusan B, Brorstrom-Lunden E, Cato I. 2005. Results from the Swedish National Screening Programme 2004. Subreport 1: Adipates. Swedish Environmental Research Institute. [Cited in Versar, 2010].

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks) (2007) Preliminary Report on The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk. Available at: https://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_008.pdf

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks) (2016) Opinion on The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015 update). Available at: https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_047.pdf

Schutze A, Kolossa-Gehring M, Apel P, Bruning T, Koch HM (2014) Entering markets and bodies: Increasing levels of the novel plasticizer Hexamoll® DINCH® in 24h urine samples from the German Environmental Specimen Bank. Int. J. Hyg. Environ. Health. 217:421-426.

Schutze A, Lorber M, Gawrych K, Kolossa-Gehring M, Apel P, Brüning T, Koch HM (2015) Development of a multi-compartment pharmacokinetic model to characterize the exposure to Hexamoll(R) DINCH(R). Chemosphere 128:216-224.

Schutze A, Otter R, Modick H, Langsch A, Bruning T, Koch HM (2017) Additional oxidized and alkyl chain breakdown metabolites of the plasticizer DINCH in urine after oral dosage to human volunteers. Arch Toxicol 91:179–188. DOI 10.1007/s00204-016-1688-9.

US EPA (United States Environmental Protection Agency) (1991) Alpha2µ-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. Risk Assessment Forum, Washington, DC. EPA/625/3-91-019F.

US EPA (United States Environmental Protection Agency) (1998). Assessment of Thyroid Follicular Cell Tumors. Risk Assessment Forum, Washington, DC. EPA/630/R-97/002.

U.S. EPA (U.S. Environmental Protection Agency). 2002. Hepatocellular Hypertrophy. HED Guidance Document #G2002.01. The HED TOXicology Science Advisory Council, Health Effects Division, Office of Pesticide Programs, Washington, DC. October 21, 2002.

US EPA (United States Environmental Protection Agency) (2011) Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/100/R11/0001. Available at: https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf

US EPA (United States Environmental Protection Agency) (2013a) Health effects testing guidelines. U.S. Code of Federal Regulations, Title 40, Part 798. U.S. Environmental Protection Agency, Washington, DC. [Cited in Bhat et al., 2014].

US Patent Application 20080188601 (2008) Mixture of Diisononyl Esters of 1,2-Cyclohexanedicarboxylic Acid, Method For the Production Thereof and Use of These Mixtures. Available: http://www.freepatentsonline.com/y2008/0188601.html. [Cited in Versar, 2010].

Versar MA (2010) Review of Exposure and Toxicity Data for Phthalate Substitutes. U.S. Consumer Product Safety Commission. Contract No. CPSC-D-06-0006, Task Order 004. Bethesda, MD.

Volkel W, Kiranoglu M, Dettbarn G, John A, Jessel S, Seidel A, Fromme H (2016) Urinary toxicokinetics of di-(isononyl)-cyclohexane-1,2-dicarboxylate (DINCH) in humans following single oral administration. Toxicol Letters 248:16-24.

Wilkes CE, Summers JW, Daniels CA. 2005. PVC Handbook. Hanser Gardner Publications. Cincinnati, Ohio. p. 182. [Cited in Versar, 2010].

APPENDIX 1

Search Terms Used

"Diisononyl hexahydrophthalate" OR "1,2-Cyclohexanedicarboxylic acid, diisononyl ester" OR "Bis(isononyl)cyclohexane 1,2-dicarboxylate" OR "Diisononyl cyclohexane-1,2-dicarboxylate" OR "DINCH" OR "Flocare 35138" OR "Hexamoll" OR "Hexamoll DINCH" OR (474919-59-0) OR (166412-78-8)

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).