GreenScreen™ Assessment for Diisononyl phthalate (DINP) (CAS #28553-12-0)

GreenScreen™ Version 1.2 Draft Assessment
Note: Validation Has Not Been Performed on this GreenScreen™ Assessment

Chemical Name: Diisononyl phthalate (DINP) (CAS #28553-12-0)

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Date: December 1, 2011
Revised: February 10, 2012

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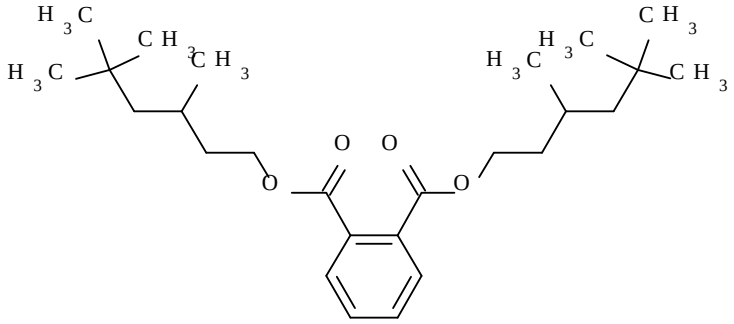
Date: February 13, 2012

Confirm application of the *de minimus* rule[1]: yes

Chemical Name (CAS #): Diisononyl phthalate (DINP) (CAS #28553-12-0; 68515-48-0)

Also Called: 1,2-Benzenedicarboxylic acid, diisononyl ester (CAS #28553-12-0), and 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9 rich (CAS #68515-48-0)

Chemical Structure(s):



Identify Applications/Functional Uses:
(e.g. Cleaning product, TV casing)
1. Plasticizer

[1] Every chemical in a material or formulation should be assessed if it is:
1. intentionally added and/or
2. present at greater than or equal to 100 ppm (0.01%).

GreenScreen™ Rating[2]: DINP was assigned a GreenScreen™ Benchmark Score of 1 based on High Endocrine Activity (E), Reproductive (R) toxicity, and Developmental (D) toxicity. This corresponds to GreenScreen™ benchmark classification 1e in CPA 2011a. Data gaps (dg) exist for Neurotoxicity (N) (not listed, but not tested) and Carcinogenicity (C) (relevance of tumors unknown). As outlined in CPA (2011c) Section III(1)(Benchmarking Chemicals With Data Gaps), DINP meets requirements for a GreenScreen™ Benchmark Score of 1, despite the hazard data gaps. In a worst-case scenario, if DINP were assigned a High score for C, it would remain a GreenScreen™ Benchmark 1 chemical.

GreenScreen™ Hazard Ratings: Diisononyl Phthalate (DINP)																			
Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeat*	single	repeat*										
dg	L	H	H	H	L	dg	M	dg	dg	L	*L*	L	M	L	L	vL	vL	L	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values and lower confidence. Hazard levels in **BOLD** font reflect values based on test data (See Guidance).
Note: Please see Appendix A for a glossary of hazard acronyms.

Transformation Products and Ratings[3]:
Identify relevant fate and transformation products (i.e., dissociation products, transformation products, valence states) **and/or moieties of concern**[4]

Functional Use	Life Cycle Stage	Transformation Pathway	Transformation Products	CAS #	On CPA Red List[5]?	GreenScreen™ Rating[6]
n/a	**End**	Combustion	Carbon Monoxide	**630-08-0**	**Y**	**n/a**
n/a	**End**	Combustion	Carbon Dioxide	**124-38-9**	**N**	**n/a**

Introduction

DINP is a mixture (even in a "pure" form) of mainly C9 branched isomers with an average chemical formula of $C_{26}H_{42}O_4$. Several different formulations of DINP are used in industry. DINP is produced by esterification of phthalic anhydride with isononyl alcohol. DINP is primarily used as a plasticizer in PVC and non-PVC applications (EU RAR 2003). DINP is manufactured by the either the n-butene process (CAS #28553-12-0) or the "Polygas" process (CAS 68515-48-0) (EU RAR 2003). The latter compound is the technical grade substance (ChemIDplus 2011). A third, discontinued DINP production process is n- and iso-butane based (also CAS #28553-12-0).

[2] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[3] Products that contain phthalates or phthalate alternatives are often plastics. Plastics are often disposed of via incineration. Therefore, health and environmental effects associated with combustion byproducts are of particular concern.
[4] A moiety is a discrete chemical entity that is a constituent part or component of a substance. A moiety of concern is often the parent substance itself for organic compounds. For inorganic compounds, the moiety of concern is typically a dissociated component of the substance or a transformation product.
[5] The CPA "Red List" refers to chemicals: 1) flagged as Benchmark 1 using the GreenScreen™ List Translator , or 2) flagged as Benchmark 1 or 2 using the GreenScreen™ List Translator and further assessed and assigned as Benchmark 1. The most recent version of the GreenScreen™ List Translator should be used (CPA 2011b).
[6] GreenScreen reviews of transformation products depend on the GreenScreen Benchmark Score of the parent chemical (See Guidance in CPA 2011c).

Hazard Classification Summary Section:
Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M or L): dg
DINP was assigned a data gap for carcinogenicity. The carcinogenicity of phthalates is controversial. While the EU RAR considered available cancer data to be of unknown relevance to humans, DINP caused multiple types of cancers in multiple species and the mechanisms are not all fully understood. Therefore, DINP is not classifiable as a Low hazard under GreenScreen™ criteria.

- EU RAR 2003-
  - Results from chronic toxicity/carcinogenicity assays (study design and methodology are reported in the chronic toxicity section, below) indicate that DINP (CAS# 68515-48-0)induced neoplastic and non-neoplastic lesions in rats and mice. In one study (GLP- compliant with OECD and EU guidelines), DINP induced non-neoplastic liver lesions in F344 rats after 18 months and 24 months of treatment, but no treatment related preneoplastic or neoplastic liver lesions were observed after oral administration (Exxon Biomedical Sciences 1986; Hazleton 1986; Lington et al. 1997). The primary malignancy was an statistically significant increase in mononuclear cell leukemia (MNCL) in the mid and high dose groups of Fisher rats (52% and 55% in males, 29% and 41% in females compared to 34% and 23% in controls, in males and females respectively) that was the most common cause of mortality followed by pituitary tumors. Kidney neoplasms were also observed in F344 rats at the mid and high dose groups but were not statistically significant. Primary brain tumors were observed in 2 high-dose male and 2 mid-dose female F344 rats and no statistically significant excess of testicular tumors were observed (Exxon Biomedical Sciences, 1986; Hazleton 1986; Lington et al. 1997). A NOAEL of 15 mg/kg and 18 mg/kg in males and females respectively and a LOAEL of 152 mg/kg and 184 mg/kg in males and females respectively, based on increased incidence of MNCL were reported.
  - In a second chronic study in SD rats (GLP status unknown), higher incidence of neoplastic liver nodules were observed that were within historic range for the strain and hepatocellular carcinomas in mid- and high-dose groups exceeding historic range (no incidence data provided) (CAS 71549-78-5) (Bio/Dynamics 1986). In addition, an increase of testicular cell hyperplasia in high-dose males was found (data not shown). A slight increase of pancreatic islet cell tumors and parathyroid gland hyperplasia in high-dose males, an increase of endometrial hyperplasia in high-dose females, and a slightly higher incidence of interstitial cell tumors were of uncertain significance according to the RAR authors. A NOAEL of 27 mg/kg and 33 mg/kg, for males and females respectively, was determined for this study by the RAR authors. The LOAEL of 271 mg/kg and 333 mg/kg, in males and females respectively, was based on increased incidence of hepatocellular carcinomas.
  - A third, GLP-compliant, chronic study in F344 rats (CAS# 68515-48-0) found PPARa-related increases in hepatocyte proliferation in the high-dose groups of both sexes, reversible treatment-related hepatocellular enlargement (14/32 males, severity = 0.9, and 27/37 females, severity = 1.5) and histopathological changes, and late onset of increased incidence of hepatocellular neoplasia in high dose groups of both sexes but higher in males than females (17/80 in males compared to 5/80 in controls; 8/80 in females compared to 1/80 in control) (Aristech 1994 and 1995: Butala et al. 1996; Covance, 1998). Malignant kidney lesions (tubule cell carcinomas) were only observed in the high dose males (2/60 in high dose group and 4/50 in the high-dose recovery group) but not in females. MNCL was increased in excess of historic range for the strain in the highest two dose groups in both sexes (32/65 and 30/65 in males and 29/65 and 30/65 in females) and correlated with statistically significant increases of mean absolute and relative spleen weights (not shown). A NOAEL of 88 mg/kg and 109 mg/kg, in males and females respectively, and a LOAEL of 359 mg/kg and 442 mg/kg, in males and females respectively, based on increased incidence of MNCL were determined by the RAR authors for carcinogenic potential of DINP in rodents.
  - A chronic, GLP-compliant study in mice found significantly increased incidence of hepatocellular neoplasms in high-recovery-dose females (37% compared to 7% in controls) and in mid-high-dose males (47% compared to 23% in controls) after oral DINP administration (CAS# 68515-48-0, > 99% purity) (Aristech Chemical Corporation 1995c; Butala et al. 1997). Significantly increased liver adenoma were seen only in high dose and recovery females (18/70 and 8/50, respectively). Hepatocellular carcinoma was significantly elevated in mid-high and high-dose males (17/60 and

20/60, respectively) and in mid-high-, high-, and recovery high-dose females (7/60, 18/60, and 13/50, respectively). Lung masses were also observed primarily in males in all dose groups. A NOAEL of 112 mg/kg in females and 275 in males, and a LOAEL of 335 in females and 742 in males, were determined.

- With regards to the mechanisms of carcinogenesis in rodents, the RAR authors concurred with the conclusions of the authors of these studies. Specifically, it was demonstrated that liver tumors were a result of peroxisome proliferation and PPARα induction. Peroxisome proliferation has been regarded as not relevant to humans and IARC concluded that DEHP carcinogenicity through peroxisome proliferation and PPAR induction was not relevant. In addition, studies in monkeys showed no signs of cancer or peroxisome proliferation to further support this indication that these may be rodent specific effects. Kidney tumors were determined to be related to α2u-globulin mechanism (Caldwell 1999) which is considered not relevant for humans. In addition, MNCL is common if F344 rats and IARC has categorized MNCL as "an unclassified leukemia with no known human counterpart." Based on the available data, the EU RAR considered the carcinogenicity of DINP to be of an unknown relevance and not classifiable to human beings.
- While tumors identified for DINP have been categorized as irrelevant or of unknown relevance to humans, there is still debate as to its overall relevance and mechanism of action. Given carcinogenicity data for phthalates is still in question, this endpoint is assigned a data gap score for insufficient data available to assess the potential carcinogenicity to humans.

Mutagenicity/Genotoxicity (M) Score (H, M or L): L

DINP was assigned a score of Low for mutagenicity based on testing negative for both mutagenicity and clastogenicity following *in vitro* and *in vivo* assays in prokaryotic and eukaryotic cells.

- EU RAR 2003 –
  - DINP (both CAS# 28553-12-0 and CAS# 68515-48-0) has been tested in several Ames bacterial mutagenicity assays utilizing *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations ranging from 0.5 to 5,000 µg/plate with and without metabolic activation (EG&G Mason Research Institute 1980; NTP 1983; Zeiger et al. 1985; BASF 1986; BASF 1995b; Exxon Biomedical Sciences 1996a). DINP did not produce a positive response in any study either in the presence or absence of metabolic activation in any strain tested, and was consistently reported as negative for mutagenicity. Two studies were GLP-compliant (Exxon Biomedical Sciences 1996a and EG&G Mason Research Institute 1980), while the GLP-status of the others was not specified.
  - Two GLP-compliant mouse lymphoma assays were identified utilizing L5178Y TK +/- mouse lymphoma cells in the presence and absence of metabolic activation at DINP (CAS# 28553-12-0 and CAS# 68515-48-0) concentrations ranging from 0.5 to 100 µl/ml (EG&G Mason Research Institute 1981; Hazleton 1986b). DINP did not produce any significant increases in forward mutations and was reported to be negative for mutagenicity under all tested conditions.
  - An *in vitro* chromosomal aberration assay was conducted utilizing Chinese Hamster Ovary (CHO) cells with DINP (CAS# 68515-48-0) at concentrations 0, 5, 10, 20, 40, 80, and 160 µg/ml (in acetone) in the presence and absence of metabolic activation (Exxon Biomedical Sciences 1996b). An increase in aberrant cells was reported in the high-dose group but was within historical controls of the laboratory. The test substance was reported as negative for clastogenicity by the study authors. This study was GLP-compliant.
  - An *in vivo* cytogenetic assay was conducted using male and female F344 rats (number not reported) (Microbiological Associates 1981a). Rats were administered DINP (CAS# 28553-12-0) doses of 0, 0.5, 1.7, and 5 mg/kg/day, diluted in corn oil for 5 days. Samples of femoral bone marrow were analyzed for chromosomal aberrations. There were no changes in mitotic index at any tested dose, and no significant increases in aberrant cells were reported. The study was conducted using a small number of cells, which may have affected the power of the study to detect chromosomal aberrations. The GLP status of this study is not specified.

Reproductive Toxicity (R) Score (H, M, or L): H

DINP was assigned a score of High for reproductive toxicity based on body weight changes, and decreased sperm parameters in offspring and parental animals following reproductive and developmental toxicity studies.

- EU RAR 2003-
  - A (GLP compliant) two-generation reproductive toxicity study was conducted using male and female Crl:CDBR, VAF Plus rats (30/sex/group) (Exxon Biomedical Sciences 1996c). Rats were administered DINP (CAS 68515-48-0) in the diet at doses equivalent to 0,118, 236 and 477 mg/kg in females, and 0, 215, 426, 852 mg/kg in males (P1 generation) from ten weeks prior to mating and during the mating period, and until weaning of offspring in female rats. Clinical in-life observations, body weights, and food consumption were recorded; reproductive parameters and gross necropsy were also performed. There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices in the P1 generation. Significantly lower body weights in the high dose group of the P2 generation were attributed to lower body weights at the start of the P2 generation (13% in males and 10% in females). A slight but not statistically significant decrease in male mating, male fertility, female fertility, female fecundity indices were observed in the P2 generation, treated with the same range of doses (calculated actual doses vary slightly). Biologically significant increases in the mean absolute and/or mean relative kidney (males only) and liver (both sexes) weights were observed in both P1 and P2 the mid- and high-dose (data not shown). A statistically significant decrease in the mean left ovary weight was also found in high-dose P1 females but was inconsistent with other findings (data not shown). In high-dose P2 males, there was a statistically significant increase of relative right and left epididymis weights with a concurrent but not statistically significant increase of absolute epididymis weight (data not shown). No treatment-related clinical findings and no biologically significant differences in the F1 or F2 offspring survival indices were observed between the treated and control offspring. No adverse effects on post-mortem findings were reported. A dose-dependent decrease in body weights was reported for all dose groups in the F1 and F2 offspring but was attributed to decreased quality and quantity of milk consumption that in turn was due to decreased food consumption of the dams. A NOAEL could not be determined either for parental systemic toxicity, due to microscopic liver changes observed at the lowest dose, or for offspring toxicity, due to reduced body weights. A LOAEL of 159 mg/kg for both parental and offspring effects was estimated taking into account food consumption differences.
  - A prior one generation pilot study under similar test guidelines was conducted (GLP status unknown), in which the doses of the two generation study were selected (Exxon Biomedical Sciences 1996d). Rats were administered DINP (CAS 68515-48-0) in the diet at doses in the ranges of 0, 363, 734 and 1,114 mg/kg in females, and 0, 301, 622, 966 mg/kg in males (P1 generation) from ten weeks prior to mating and during the mating period, and until weaning of offspring in female rats. A range of dose estimates was given for each dietary concentration that also varied depending on the treatment period. The one-generation also reported decreased offspring body weights in all dose groups (up to 11%, 27%, and 46% in males and up to 10%, 26%, and 47% in females, in low, mid, and high dose respectively) (LOAEL 301 mg/kg), as well as decreased offspring survival indices at the highest dose (LOAEL 966 mg/kg). No NOAEL could be determined for the offspring, based on decreased body weight from the lowest dose (a NOAEL of 622 mg/kg was determined for decreased live birth and survival indices). Parental toxicity pertained primarily to statistically significant increases in liver and kidney weights observed at all dose levels (data not shown). In addition, statistically significant lower mean food consumption was observed primarily in the mid and high-dose animals (data not shown). A statistically significant decrease in the mean absolute and relative right ovarian and mean absolute left ovarian weights was seen in high-dose females (data not shown). Although, microscopic evaluation was not performed on any organs in either sex at any dose to determine if any structural changes occurred in reproductive organs, no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were observed. No NOAEL could be determined for parental toxicity, based on increased liver and kidney weights from the lowest dose.

- Boberg et al. 2010 –
    - A GLP-compliant developmental toxicity study was conducted (method not reported) using pregnant female Wistar rats (n=80). Rats were administered doses of 0, 300, 600, 750, and 900 mg/kg of DINP via oral gavage on days 7 to 17 of gestation. Rats were examined for fetal testosterone production, testicular histopathology, postnatal development, reproductive organs, semen quality, and behavior. A dose-dependent increase (0%, 25%, 60%, 100%, 100%) in the number of animals with histological changes in the testis were identified, these effects were found to be significant at 600 mg/kg and above. In male pups a dose-dependent decrease in anogenital distance was reported, reaching statistical significance in the high-dose group. In addition, a dose-dependent increase in nipple retention was reported for male pups, reaching significance in the top two dose groups. Furthermore, a dose-dependent decrease in sperm motility was reported reaching significant in the 600 mg/kg and above groups. Study authors concluded that the reported effects (nipple-retention, reduced anogenital distance, and disruption of semen quality), parameters not examined in waterman et al, clearly support that DINP is anti-androgenic, and a reproductive toxicant. A NOAEL of 300 and LOAEL of 600 mg/kg were reported by study authors.
- Kwack et al. (2009) –
    - Male Sprague-Dawley rats administered 500 mg/kg of DINP orally for four weeks showed a significant decrease in sperm parameters (sperm count and sperm motility). Abstract only – further details not available.
- The EU RAR reported that the LOAEL of 159 mg/kg was considered for risk characterization classification and that no classification was required. However, based on reductions in semen quality identified in Boberg et al. (2010), and also decreased sperm parameters identified by Kwack et al. (2009), in addition to reduced body weights reported in the EU RAR study, ToxServices has determined that sufficient evidence is available for classification as a GHS Category 1B reproductive toxicant (GHS 2011) as clear evidence of animal toxicity with potential relevance to human toxicity has been established.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M or L): H

DINP was assigned a score of High for developmental toxicity based on skeletal malformation, developmental toxicity, and variations in offspring following developmental toxicity studies.

- EU RAR 2003 –
    - A range finding study was conducted in Crl:CDBR mated female rats treated with DINP (CAS 68515-48-0) at doses of 0, 40, 200, 500 and 1,000 mg/kg/d by oral gavage on gestation day 6 through 15 (Nikiforov et al. 1994). No overt signs of maternal toxicity were apparent at any dose level, and no adverse effects for fetal observations or body weight were seen at any dose level. A NOAEL of 1,000 mg/kg was determined, and no LOAEL could be determined. The GLP status of this study is unknown.
    - A GLP compliant developmental toxicity study (method not reported) was conducted using female Sprague-Dawley rats (25/dose) (Exxon Biomedical Science 1994, Waterman et al., 1999). Rats were administered doses of 0, 100, 500, and 1,000 mg/kg of DINP (CAS# 68515-48-0) via gavage on days 6-15 of gestation. Statistically significant, decreases of maternal body weight gain and mean food consumption were observed at 1,000 mg/kg/day A dose-related increase in total fetuses with visceral variations on a per fetus and per litter basis were reported. However, these variations were only significantly increased at 1,000 mg/kg (6/24 vs. 0/24 in controls) on a per litter basis, which is the preferred method of analysis for developmental toxicity studies. Skeletal variations were statistically significant on a per litter basis only at the highest dose (22/24 vs. 15/24 in controls). Based on visceral variations, a NOAEL of LOAEL of 500 and 1,000 mg/kg were assigned for developmental toxicity.
    - A GLP compliant study evaluating the toxicity of three DINP formulations (DNP1, CAS# 68515-48-0; DNP2 and DNP3, CAS# 28553-12-0) in Wistar rats at doses of 0, 40, 200, and 1,000 mg/kg/d by gavage on days 6-15 post-coitum (BASF 1995a; Hellwig et al. 1997). Fetal skeletal variations were observed in all three formulations at 1,000 mg/kg. DNP1 caused rudimentary cervical in 11 fetuses and accessory 14^{th} ribs in 37 fetuses vs. 0 of each in controls (group size not shown); DNP2 caused accessory 14^{th} rib in 5/10 vs. 0/10 in controls,

and DNP3 caused rudimentary cervical in 78% fetuses and accessory 14th ribs in 89% fetuses vs. 0 of each in controls. DNP3 was the only formulation that caused soft tissue variations; hydroureter in 89% of fetuses vs. 33% in controls on a per litter basis, and dilated renal pelvis in 20/57 fetuses vs. 12/65 in controls. This may indicate a substance dependence (this formulation is no longer produced). A NOAEL and LOAEL of 200 and 1,000 mg/kg respectively, were established based on skeletal variations.

- No treatment-related effects were reported in one other GLP-compliant study (Hazleton, 1981b). Slightly higher incidence of resorptions and higher incidence of visceral variations at 1,000 mg/kg were not statistically significant. A NOAEL of 1,000 mg/kg was assigned.

- Boberg et al. 2010 –
  - A GLP-compliant developmental toxicity study was conducted (method not reported) using pregnant female Wistar rats (n=80). Rats were administered doses of 0, 300, 600, 750, and 900 mg/kg of DINP via oral gavage on days 7 to 17 of gestation. Rats were examined for fetal testosterone production, testicular histopathology, postnatal development, reproductive organs, semen quality, and behavior. A dose-dependent increase (0%, 25%, 60%, 100%, 100%) in the number of animals with histological changes in the testis were identified, these effects were found to be significant at 600 mg/kg and above. In male pups a dose-dependent decrease in anogenital distance was reported, reaching statistical significance in the high-dose group. In addition, a dose-dependent increase in nipple retention was reported for male pups, reaching significance in the top two dose groups. Furthermore, a dose-dependent decrease in sperm motility was reported reaching significant in the 600 mg/kg and above groups. Study authors concluded that the reported effects (nipple-retention, reduced anogenital distance, and disruption of semen quality), parameters not examined in waterman et al, clearly support that DINP is anti-androgenic, and a reproductive toxicant. A NOAEL of 300 and LOAEL of 600 mg/kg were reported by study authors.
- Hannas et al. 2011 –
  - DINP caused a decrease in fetal testosterone production. This decrease was 2.3-fold less potent than DEHP, but still present. Doses administered were not available in the abstract. Abstract only – further details not available.
- Gray et al. 2000 –
  - DINP was administered orally to dams from gestation day 14 to post natal day 3. DINP induced a significant increase in the incidence of reproductive malformations and an increase in nipple retention. Abstract only – further details not available.
- The EU RAR reports that a NOAEL of 500 mg/kg is appropriate for use in risk characterization classification and that no classification was required. However, further data supporting fetal malformation, and developmental toxicity were identified in Boberg et al (2010), Hannas et al. (2011) and Gray et al. (2000). Therefore, based on GHS criteria, ToxServices categorizes DINP as a GHS Category 1B reproductive toxicant (GHS 2011) as clear evidence of animal toxicity with potential relevance to human toxicity has been established.

Endocrine Activity (E) Score (H, M or L): H

DINP was assigned a score of High for endocrine activity based on some evidence of anti-androgenic activity in human infants and evidence of anti-androgenic activity in male rats.

- EU RAR 2003 -
  - DINP shows no activity in the *in vitro* assays designed to test the ability of binding to rodent or human estrogen receptors or to induce estrogen receptors-mediated gene expression (Harris et al. 1997; Zacharewski et al. 1998). In one study (Harris et al. 1997), DINP gave non-reproducible results in the recombinant yeast screen at concentrations ranging from 10^{-3} M to $5x10^{-7}$ M, and induced proliferation in the human breast cancer cell proliferation assay at concentrations of 10^{-5}, 10^{-6} and 10^{-7} M to a significantly greater extent than the control, which is in contrast to the findings for this chemical using the yeast screen. In the second study DINP was negative in the estrogen receptor competitive binding assay (1-1,000 µM), the MCF-7 and HeLa cells gene expression assays (at 0.1-1 µM and 10 µM, respectively), and the ER-mediated yeast growth assay (10 µM).
  - *In vivo* studies were also considered negative. DINP was tested in the uterotrophic assay and vaginal epithelial cell cornification assay at concentrations 20, 200, and 2,000 mg/kg in ovariectomised Sprague-Dawley rats (10 females/dose, two experiments) (Zacharewski et al., 1998). Results of both assays were negative, although the high variability of responses made the value of the test questionable. Nonetheless, DINP exhibited an absence of estrogen receptor-mediated estrogenic activity (NOAEL=2,000 mg/kg). The GLP status of this study is not reported. In a recent reproductive study DINP was tested at a single dose (750 mg/kg) for 14 days and showed malformations of testis, epididymis, accessory reproductive organs and external genitalia (Earl Gray et al., 2000). The GLP status of this study is not reported. In light of this evidence that DINP might have anti-androgenic potency, the NTP has proposed further testing that addresses landmarks of sexual maturation.
- U.S. CPSC 2010 –
  - A Danish-Finish cohort study examined the effects of monoisononyl phthalate (MINP), the primary metabolite of DINP (Main et al. 2006). Some effects on reproductive hormones were observed. A 10-fold increase in the level of MINP was correlated to a doubling of serum luteinizing hormone (LH) ($p = 0.019$). The CPSC indicated that increasing LH is an indicator of anti-androgenic effects. MINP was also correlated with non-significant increase in sex-hormone binding globulin, LH:free testosterone, and total testosterone. Increases in LH, SHGB, and LH:testosterone are also indirect indicators of anti-androgenic effects. While effects seen were not of statistical significance, the study does indicate that there is some evidence of anti-androgenic effects in human male infants.
- Boberg et al. 2010 –
  - A GLP-compliant developmental toxicity study was conducted (method not reported) using pregnant female Wistar rats (n=80). Rats were administered doses of 0, 300, 600, 750, and 900 mg/kg of DINP via oral gavage on days 7 to 17 of gestation. Rats were examined for fetal testosterone production, testicular histopathology, postnatal development, reproductive organs, semen quality, and behavior. A dose-dependent increase (0%, 25%, 60%, 100%, 100%) in the number of animals with histological changes in the testis were identified, these effects were found to be significant at 600 mg/kg and above. In male pups a dose-dependent decrease in anogenital distance was reported, reaching statistical significance in the high-dose group. In addition, a dose-dependent increase in nipple retention was reported for male pups, reaching significance in the top two dose groups. Furthermore, a dose-dependent decrease in sperm motility was reported reaching significant in the 600 mg/kg and above groups. Study authors concluded that the reported effects (nipple-retention, reduced anogenital distance, and disruption of semen quality), parameters not examined in waterman et al, clearly support that DINP is anti-androgenic, and a reproductive toxicant. A NOAEL of 300 and LOAEL of 600 mg/kg were reported by study authors.
- The NTP *in vivo* rat study shows a clear dose-related anti-androgenic effect in male rats. Although the EU Risk Assessment lists several assays that showed negative results, these studies all focused on estrogenic activity. No evidence of estrogenic activity has been found for phthalates and is not a concern. Evidence of anti-androgenic activity needs further testing as recommended by the NTP.

Several studies have been identified since the NTP study. First, Boberg et al. (2010) concluded that DINP is a clear reproductive toxicant with anti-androgenic effects. Furthermore, more current studies have been identified which identify that DINP has effects on fetal testosterone production, decreased sperm parameters, and produces areolas and reproductive malformations (Kwack et al. 2009; Hannas et al. 2011; Gray et al. 2000). While DINP has been reported as "less toxic" than DEHP or other "active" phthalates, the GreenScreen™ is a hazard based assessment, and any evidence of endocrine activity must be considered. Furthermore, evidence of direct and indirect anti-androgenic effects in human male infants of MINP, the primary metabolite of DINP were reported in the 2010 U.S. CPSC assessment. Based on the anti-androgenic effects in male rats, and some evidence of human anti-androgenic effects in male infants, a high score is assigned for endocrine activity. Furthermore, the two-generation reproductive toxicity study conducted by waterman et al. (2000) did not sufficiently characterize the potential male reproductive toxicity or endocrine activity of DINP. A critical review of this study can be found in Appendix B.

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.2 Benchmark system. For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints and test data for single or repeated exposures may be used. If data exist for single OR repeated exposures, then the endpoint is not considered a data gap. If data are available for both single and repeated exposures, then the more conservative value is used.

Acute Mammalian Toxicity (AT) Group II Score (vH, H, M or L): L

DINP was assigned a score of Low for acute mammalian toxicity based on oral, dermal and inhalation LD/C_{50} values above the 2,000 mg/kg or 5 mg/L cutoffs (CPA 2011).

- EU RAR 2003 –
  - Oral LD_{50} (rat) > 9,800 to > 50,000 mg/kg (BASF 1961; BASF 1981d; Hazleton 1968c; Hazleton 1980b; Hüls 1985a; Midwest Research Institute 1981b)
  - Dermal LD_{50} (rat) > 3,160 mg/kg (Hazleton 1968a)
  - Inhalation LC_{50} (rat) > 0.067 mg/L to > 4.4 mg/L (Industrial Bio-test Laboratories 1975a, b, c; BASF 1981a; Hazleton 1980a)

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)

Group II Score (single dose: vH, H, M or L): dg

- No relevant data were identified.

Group II* Score (repeated dose: H, M, L): M

DINP was assigned a score of Moderate for systemic toxicity/organ effects based on some evidence of liver and kidney toxicity in rodents following multiple repeated dose studies.

- EU RAR 2003 –
  - A GLP-compliant 2-year chronic toxicity/carcinogenicity study (method not reported; OECD and EU guidelines compliant) was conducted using male and female F344 rats (110/sex, due to interim sacrifices) (Exxon Biomedical Sciences 1986; Hazleton 1986; Lington et al. 1997). Rats were administered doses of 0, 18, 184, and 375 mg/kg in females and 0, 15, 152, and 307 mg/kg in males of DINP (CAS# 68515-48-0) daily in the diet for 2 years. Subgroups of 10/sex/dose were scheduled for interim sacrifice after 6, 12, and 18 months. Observations included body weights and food consumption, clinical chemistry, urinalysis, hematology, gross necropsy morphological examination of the liver (2/sex/group), and histopathology on all major organs.

    No statistically significant clinical in-life observations were reported for the 24-month test period. Statistically significant decreases in food consumption occurred in high-dose males during the last 12 months of the study, and significant increases occurred in mid- and high-dose females during the first 12 months (data not shown). Compared to controls high-dose males had a significantly decreased body weight after 12 months until termination (4-7%).

    Males and female rats in the mid- and high-dose groups exhibited statistically significant, dose-related increase in relative weights of the kidney (8% and 9% for males and females, respectively) and liver (20% and 29%, respectively) throughout the study. Statistically significant increases in

absolute and relative spleen weights occurred in mid- and high-dose males (2.53 g and 2.4 g vs. 1.68 g in controls; 74% and 74% vs. 46% in controls) and increased relative spleen weights in high dose females (66% vs. 42% in controls). Relative adrenal weights were significantly increased in both sexes in the mid- and high-dose group (2.3% and 2.0% vs. 1.7% in males, 2.6% vs. 2.4% in females). A statistically significant increase in relative testes weights (13%) was measured in the high dose group.

At study termination, dose-related increases in white blood cell (WBC) counts were measured, although not statistically significant when compared to controls. In addition, mid-dose female rats exhibited a statistically significant increase in mean WBC counts as compared to controls (data not shown). Red Blood Cell (RBC) count, hemoglobin and hematocrit values were also slightly lower in mid- and high-dose males, only reaching statistical significance in the high dose (data not shown). An increase in nucleated RBC, polychromatophilic red cells, and reticulocytes were also measured in high-dose males and females (data not shown).

Serum chemistry revealed some statistically increased parameters associated with liver function, including, alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Although these values were statistically increased in mid- and high-dose males at all study intervals, there was a high rate of individual variation at study termination and no corresponding effects in female rats (data not shown). Study authors were unsure of the biological relevance of these effects or of their relevance to treatment.

Statistically significant changes in potassium and glucose levels were measured in mid and high-dose males throughout the study but not at termination, and high-dose males had increased urine volumes throughout the study (data not shown).

At study termination, mid- and high-dose males and high-dose females exhibited an increased incidence of splenic enlargement (data not shown). Examination of the liver did not reveal any signs of peroxisome proliferation. Lesions were observed in the liver and kidney of high-dose rats including slight centrilobular to midzonal hepatocellular enlargement and a minimal increase in tubular cell pigment of the renal tubular epithelium. An increased incidence of spongiosis hepatitis was noted in mid- and high-dose males (51/80 and 62/80 vs. 24/81 in controls) and increased hepatocellular enlargement in both sexes at the high dose (9/80 and 11/80 in males and females vs. 1/81 in both controls). Focal necrosis was also observed in both sexes and was significantly increased in high-dose males (26/80 vs. 10/81 in controls). Hepatopathy associated with leukemia was also increased in mid and high-dose groups (24/80 and 33/80 in males, 24/80 and 33/80 in females, vs. 22/81 and 16/81 in respective controls). In the kidneys, an increased severity of tubular cell pigmentation was observed in animals with advanced leukemia (data not shown). A retrospective tissues evaluation determined that kidney lesions in male rats were due to the α2u-globulin mechanism of male rat specific nephropathy and therefore, not relevant to humans.

Based on slight decreased survival data in females, liver findings in males, increase of absolute and relative liver and kidney weights in both sexes, increases in relative and absolute spleen weights, a NOAEL of 15 and18 mg/kg for males and females, respectively and a LOAEL of 152 and184 mg/kg for males and females, respectively were established by the EU RAR authors.

- A GLP-compliant, 104-week carcinogenicity study (in accordance to EPA guidelines, 40 CFR Part 798.330) was conducted using male and female B6C3F1 mice (70/sex/dose) (Aristech Chemical Corporation 1995c; Butala *et al.* 1997). Mice were administered dietary DINP (CAS not specified > 99% purity) at doses of 0, 90, 275, 741, and 1,560 mg/kg in males and 0, 112, 335, 910, and 1,887 mg/kg (> 99% purity) in females daily in the diet for 2 years. Observations included mortality and clinical signs of toxicity, body weights and food consumption, clinical chemistry, urinalysis, hematology, and histopathology on all major organs.

Survival rates of male mice were significantly decreased in the high-dose group (calculated values were 87, 87, 76, 79, 63 and 81% for males and 81, 79, 81, 62, 77 and 75% for females from the control, low, mid-low, mid-high, high and recovery high-dose groups, respectively). Clinical observations included hunched posture, hypo-activity, decreased fecal output, urine stains, and swelling in the ventral-abdominal region. Swelling occurred primarily in mid to high-dose males and high-dose females and appeared to correspond to animals with liver masses at necropsy. Food consumption was slightly but significantly increased in males in the top two dose levels (not shown). Final body weights of male mice were also significantly decreased in the top two dose groups (10% and 17%, respectively).

Clinical findings include decreased leukocytes, lymphocytes and segmented neutrophil count in high dose group of both sexes. Increased total protein, albumin, globulins, ALAT, and AST were measured in high-dose males. Higher urine volumes with lower osmolarity were also measured among high-dose animals of both sexes.

In male mice, absolute kidney weights were significantly decreased in the top three dose groups (11.4, 24.3 and 27%, respectively) and relative kidney weights decreased in the top two dose groups. In addition, relative and absolute liver weights were significantly increased in both mid- and high-dose males (13.2% and 32%, respectively). In females, absolute liver weights were also increased in the top three dose groups (23.4%, 18%, and 35%), although differences in relative liver weights were neither dose-dependent, nor statistically significant. In the top two dose groups, relative and absolute testis weights were decreased in male rats.

At study termination, substantial lung masses were observed in all groups, and liver masses in the top two dose groups. In female mice, enlarged spleens (all dose groups), granular pitted/rough kidneys (top two dose groups), and distended urinary bladder (top two dose groups) were observed.
Peroxisome proliferation findings indicated that in the top dose group DINP caused a high level of peroxisome proliferation (1.90% vs. 0.59% in males and 1.44% vs. 0.41% in females).

Based on available data, including deceased kidney weights, increased incidence of liver masses, increased liver weights, and decreased body weight gains, a NOAEL of 90 mg/kg and 112 mg/kg in males and females, respectively, and a LOAEL of 275 mg/kg and 335 mg/kg in males and females, respectively were established.

- A (GLP status not reported) 90-day toxicity study (method not reported) was conducted using male and female Sprague-Dawley rats (10/sex/group) (Hazleton 1981a). Rats were administered DINP (CAS not specified) at doses of 0, 60, 180, and 600 mg/kg in the diet for 90-days. Observations included mortality and clinical signs of toxicity, body weights and food consumption, clinical chemistry, urinalysis, hematology, and histopathology on all major organs.

  Slight decreases in hematocrit (HCT), hemoglobin (HGB) and red blood cells (RBC) were observed in mid- and high-dose males and high-dose females. In addition, a slight decrease in globulin was measured in mid- and high-dose females, and a decrease of total bilirubin and an increase in blood urea nitrogen in high-dose males were measured.

  Increases in relative liver weights were observed in mid-and high-dose females, and increases in relative and absolute liver weights were observed in high-dose males and females (data not shown). Absolute and relative kidney weights were also increased in high-dose males and females (relative weights only) (data not shown).

  Histopathological lesions were found in the kidneys only. An increased incidence of focal mononuclear cell infiltration and mineralization was observed in low dose males, in the mid-dose an increased incidence of regenerative epithelium was observed in addition to the prior effects, and in the high-dose group an increase of proteinaceous casts was found along with both prior

effects (data not shown). . Based on kidney effects, a LOAEL of 60 mg/kg was reported for this study, but NOAEL cannot be determined.

- A GLP compliant, 90-day toxicity study (EPA Guidelines) was conducted using male and female B6C3F1 mice (10/sex/group) (Hazleton 1992). Mice were administered DINP (CAS# 28553-12-0) at doses of 0, 365, 972, 2,600 and 5,770 mg/kg daily in the diet for 90days. Criteria evaluated included mortality, body weights, food consumption, ophthalmology, clinical pathology, organ weight, gross pathology and microscopic pathology.

  Dose-dependent, decreases in body weight and body weight gain were measured in the high-dose group (both males and females) (data not shown). Food consumption was significantly increased in males in the top two dose groups, and in females in the second highest dose group only (data not shown). No ophthalmology or hematological changes were reported from the main study. Increased transaminases (ALT and AST) were reported in high-dose males. Urinary evaluation showed statistically significant decreases in the urinary values of sodium, chlorides, and creatinine, along with a slight increase in urine volume and a lower specific gravity in high-dose animals of both sexes.

  Enlarged livers were measured at 972 mg/kg and above in males and 2,600 mg/kg and above in females (data not shown). Granular kidneys were also reported in high-dose animals of both sexes (data not shown). Absolute and relative weight of the uterus was decreased in high-dose females (data not shown). Absolute and relative liver weights in both sexes were increased at 972 mg/kg and above and absolute relative and absolute kidney weights in males only in the same dose groups (data not shown).

  Moderate to moderately severe diffuse hepatocellular enlargement, pigments in Kupffer cells, and bile canaliculi, and liver degeneration/necrosis were observed in all high-dose animals. In addition, tubular nephrosis in the kidneys, immature/abnormal sperm forms, lymphoid depletion in spleen and thymus, hypoplasia in the uterus and absence of corpora lutea in the ovaries were observed in high-dose animals.

  EU RAR authors assigned a NOAEL and LOAEL of 365 mg/kg, and 972 mg/kg, respectively based on liver effects.

- Additional chronic and subchronic toxicity studies were identified in the EU RAR report, and the results are summarized in the table below.

Additional DINP Chronic and Subchronic Toxicity Studies (EU RAR 2003)

Study	Species	Doses	NOAEL	LOAEL
2-Year Study (reported above) Exxon Biomedical Sciences 1986; Hazleton 1986; Lington et al. 1997	F344 Rats	0, 18, 184, and 375 mg/kg in females and 0, 15, 152, and 307 mg/kg in males	15 mg/kg (M),; 18 mg/kg (F)	152 mg/kg (M),184 mg/kg (F) Increased liver and kidney weights and increased incidence of non-neoplastic changes.
2-Year Study (reported above) Aristech Chemical Corporation 1995; Butala et al. 1997	B6C3F1 mice	0, 90, 275, 741, and 1,560 mg/kg in males and 0, 112, 335, 910, and 1,887 in females	90 mg/kg (M),112 mg/kg (F)	275 mg/kg (M),335 mg/kg (F) Based on deceased kidney weights, increased incidence of liver masses and increased liver weights, and decreased body weight gains
2-Year Study Aristech 1994,	F344 Rat	0, 29, 88, 358, and 733 mg/kg in males	88 mg/kg (M),103 mg/kg	358 mg/kg (M),442 mg/kg (F)

1995b; Butala et al. 1996; Covance 1998		and 0, 36, 108, 442, 885 mg/kg in females	(F)	Increased kidney weights, kidney toxicity, liver toxicity, liver weight and histopathology.
2-Year Study Bio/dynamics 1986	SD Rats	0, 27, 271, and 553 mg/kg in males and 0, 33, 331, and 672 mg/kg in females	-	27 mg/kg (M),33 mg/kg (F) Minimal to slight focal hepatocellular necrosis.
13-Week Study (reported above) Hazleton 1981a	SD Rats	0, 60, 180, and 600 mg/kg	-	60 mg/kg Kidney toxicity including increased incidence of mononuclear cell infiltration and mineralization of the kidneys.
13-Week Study (reported above) Hazleton 1992	B6C3F1 mice	0, 365, 972, 2,600 and 5,770 mg/kg	365 mg/kg	972 mg/kg Enlarged liver, increased and absolute liver weights.
13-Week Study Hazleton 1971b	Rats	0, 50, 150, 500 mg/kg	150 mg/kg	500 mg/kg Increased kidney and liver weights with hepatocytic hypertrophy
13-Week Study Bio/dynamics 1982a	F344 Rats	0, 77, 227, 460, 767, and 1,554 mg/kg	77 mg/kg	227 mg/kg Increased kidney, liver weights and decreased cholesterol levels.
13-Week Study Bio/dynamics 1982b	SD Rats	0, 201, and 690 mg/kg in males and 0, 251, and 880 mg/kg in females	-	201 mg/kg (M), 251 mg/kg (F) Increased kidney and liver weights. Decreased triglycerides and urine chemistry changes.
13-Week Study BASF 1987b	Wistar rats	0, 152, 512, and 1,543 mg/kg in males and 0, 200, 666 and 2,046 mg/kg in females.	-	152 mg/kg (M), 200 mg/kg (F) Decreased triglyceride levels and decreased alimentary peripheral fat deposits in hepatocytes.
13-Week Study Hazleton 1991a	F344 rats	0, 176, 354, 719 and 1,545 mg/kg in males and 0, 218, 438, 823, and 1,687 mg/kg in females.	-	176 mg/kg (M), 218 mg/kg (F) Increased liver and kidney weights.
13-Week Study Hazleton 1971a	Beagle Dogs	0, 37, 160, 2,000 mg/kg	-	37 mg/kg Increased liver weight, and increased ALT.
13-Week Study Huntington Life	Marmoset Monkeys	0, 100, 500, and 2,500 mg/kg	500 mg/kg	2,500 mg/kg

Sciences 1998				Decreased body weight and decreased body weight gain.

Additional 2 to 4-week studies were identified in the published literature, but are not reported in the written GreenScreen™ due to a sufficient body of evidence from 90-day and 2-year carcinogenicity studies in a wide range of species and strains.

- The EU RAR based their risk characterization of DINP on a NOAEL of 88 mg/kg in rats and 276 mg/kg from mice in well conducted 2-year chronic toxicity/carcinogenicity studies.

Neurotoxicity (N)
Group II Score (single dose: vH, H, M or L): dg
DINP has been assigned a data gap for neurotoxicity. Although it is not a known neurotoxicant, neurotoxicity testing has not performed on the chemical.

- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011b).
- No relevant data were identified for DINP

Group II* Score (repeated dose: H, M, L): dg
DINP has been assigned a data gap for neurotoxicity. Although it is not a known neurotoxicant, neurotoxicity testing has not performed on the chemical.

- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006).
- Not listed as a potential neurotoxicant on the Red List of Chemicals (CPA 2011b).
- No relevant data were identified for DINP.

Skin Sensitization (SnS) Group II* Score (H, M or L): L
DINP was assigned a score of Low for skin sensitization based on no evidence of sensitization following studies in humans or animals.

- EU RAR 2003 –
  - A (GLP status not reported) Human Repeat Insult Patch Test (method not reported) was conducted using 76 human volunteers (Hill Top Research, 1995b). Induction applications of 0.2 ml of undiluted DINP (CAS# 68515-48-0) were three times a week for three weeks with a challenge application after a 10-17 day rest period. No evidence of clinical sensitization or irritation was observed.
  - A (GLP status not reported) Buehler test conducted using female Hartley guinea pigs (n=40). Inductions applications of 0.4 ml were applied on day 0, 7 and 14 for 6 hours each day (Exxon Biomedical Sciences 1992). Challenge doses of 5% DINP (CAS# 68515-48-0) in peanut oil were made on days 28 and 35. No responses were following the first challenge dose on day 28. Following the re-challenge dose 10 treated animals at day 36 and 4 treated animals on day 37 showed positive reactions for erythema. However, 4 of 10 control animals also showed positive skin reactions on day 37. Authors concluded that some evidence of sensitization may be present due to a higher score (2 in treated/ 1 in controls) for erythema.
  - A GLP-compliant Buhler test was conducted using female guinea pigs (n=40) (Huntingdon Research Centre, 1994). Induction applications of 0.5 ml DINP (CAS# 68515-48-0) were applied at day 1, 8 and 15 under occlusive conditions and then a challenge dose was applied at day 29. No evidence of skin sensitization was observed in any of the treated animals.
  - The EU RAR determined that following EU criteria does not justify a classification for sensitization of DINP.
- Based on available data, the weight of evidence suggests that DINP is not sensitizing.

Respiratory Sensitization (SnR) Group II* Score (H, M or L): *L*
DINP was assigned a score of Low for respiratory sensitization based on data for structurally similar phthalates.

- EU RAR 2003 –
  - Pulmonary and sensitizing properties have not been demonstrated with any of the phthalates. Therefore a low potential of respiratory sensitization can be expected.

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M or L): L
DINP was assigned a score of Low for skin irritation/corrosivity based on minimal to slight transient irritation in animals and no evidence of irritation in humans.

- EU RAR 2003 –
    - DINP (CAS# 68515-48-0) was applied undiluted (0.2 ml) for 24 hours to the skin of human volunteers (14F/1M) under occluded conditions to the paraspinal region of the back (Hill Top Research, 1995a). No responses were observed following a 24 hour observation period after patch removal.
    - A GLP compliant skin irritation/corrosion study (OECD 404) was conducted using male New Zealand white rabbits (n=6) (Exxon Biomedical Sciences 1996e). DINP (CAS# 68515-48-0) was applied undiluted (0.5 ml) for 4-hours to the clipped intact skin of rabbits. Slight erythema was observed in one animal after an hour and one animal after 24 hours. No other signs of irritation were identified in the study.
    - A (GLP status not reported) skin irritation/corrosion study (Draize method) was conducted using male and female White Vienna rabbits (5F/1M) (BASF 1981b). DINP (CAS# 28553-12-0, DINP2) was applied undiluted (0.5) ml for 24 hours under occlusive conditions to intact and abraded skin followed by an 8 day observation period. Slight erythema was observed at 48 and 72 hours on intact skin and abraded skin, and slight edema was observed at 48 hours on intact and abraded skin. All effects were reversible by day 8 and highest mean scores were 1.7 and 0.8 for erythema on intact and abraded skin, respectively, and 0.5 and 1.0 for edema on intact and abraded skin, respectively.
    - A (GLP status not reported) skin irritation/corrosion study (OECD 404) was conducted using male and female White Russian rabbits (3/sex/group) (Hüls 1985b). Rabbits were exposed to the test substance (CAS 28553-12-0, DINP 2) for 4 hours under protective gauze covered with a polyethylene film. The skin reactions were evaluated 1, 24, 48, and 72 hours after removal and a mean erythema score of 0.39 was reported.
    - According the EU RAR authors DINP may be considered a slight skin irritant with symptoms being reversible in a short amount of time and no classification is required under EU criteria.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M or L): M
DINP was assigned a score of Moderate for eye irritation/corrosivity based on data indication DINP is mildly irritating with ocular effects reversing in short periods of time.

- EU RAR 2003 –
    - A (GLP status not reported) eye irritation study (method not reported) was conducted using male and female New Zealand white rabbits (6/sex) (Hazleton 1968b). Single applications of 0.1 ml of undiluted DINP (CAS# 68515-48-0) were administered into the conjunctival sac of the left eye of each rabbit. Redness and discharged occurred within the first four hours, with slight to moderate irritation at 24 hours and no irritation at 48 or 72 hours.
    - A (GLP status not reported) eye irritation study (Draize test) was conducted using male and female White Vienna rabbits (2M/4F) (BASF 1981c). Single applications of 0.1 ml of undiluted DINP (CAS# 28553-12-0, DINP2) were administered into the conjunctival sac of the left eye of each rabbit. Slight redness occurred at 24 hours only, and slight corneal opacity at 72 hours only. The Iris was unaffected by treatment.
    - A (GLP status not reported) eye irritation study (OECD 405) was conducted using male and female White Russian rabbits (3/sex) (Hüls 1985c). Single applications of 0.1 ml of undiluted DINP (CAS# 28553-12-0, DINP 2) were administered into the conjunctival sac of the right eye of each rabbit. No effects on the cornea or iris were observed. Redness and discharge were observed in the conjunctiva at 1 and 24 hours, at 48 hours all effects had been reversed.
    - According the EU RAR authors DINP may be considered a slight eye irritant with symptoms being reversible in a short amount of time and no classification is required under EU criteria.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M or L): L
DINP was assigned a score of Low for acute aquatic toxicity based on L/EC_{50} values above the 100 mg/L cutoff for low classification (CPA 2011a).

- EU RAR 2003 –
    - DINP has reported L/EC_{50} values of ≥ 500 mg/L (fish, 96-hr) (DINP2, BASF 1982), ≥ 500 mg/L (daphnid, 48-hr) (BASF 1988), and ≥ 500 mg/L (algae, 72-hr) (BASF 1988).

Chronic Aquatic Toxicity (CA) Score (vH, H, M or L): L
DINP was assigned a score of Low for chronic aquatic toxicity based on no effects being predicted at saturation.

- EU RAR 2003 –
    - Chronic aquatic toxicity studies in fish (Birge et al. 1978), daphnia (CMA 1984a; Rhodes et al. 1995; Croudace et al. 1995; Brown and Williams 1994), and aquatic plants (CMA 1984b; Hüls et al. 1995a) indicate that a NOEC is not attainable at saturation. No chronic aquatic toxicity is expected from DINP.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL
European Union in a Final Risk Assessment Report.

- EU RAR 2003 –

Several biodegradation studies have been completed in standard test systems. DINP1 met the 10-day readily biodegradable window following OECD 301F and a modified Strum Test (Exxon Biomedical Sciences 1996f). Further tests indicated that DINP either met the 10-day window or achieved biodegradation near the 10-day window criterion (Exxon Biomedical Sciences 1995; Hüls 1995b; O'Grady et al. 1985; Sugatt et al. 1984). The EU RAR concluded that because DINP is an isomeric mixture, and some components may be resistant to biodegradation. However, the majority of isomers in DINP were able to meet the 10-day window in standard biodegradation tests. Based on these studies, the EU RAR concluded DINP is expected to be readily biodegradable

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL
DINP was assigned a score of Very Low for bioaccumulation based on a BCF lower than 100, which is the GreenScreen™ cutoff for Very Low/Low classification.

- ECHA 2012 –
    - DINP has a reported BCF of < 3 in *Oncorhynchus mykiss*.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M or L): L
DINP was assigned a score of Low for reactivity based on not being classified as reactive by the EU in a final Risk Assessment Report.

- EU RAR 2003 –
    - DINP is not explosive and was not classified as a reactive substance under EU criteria by the EU RAR.

Flammability (F) Score (vH, H, M or L): L
DINP was assigned a score of Low for flammability based on not being classified as flammable by the EU in a final Risk Assessment Report EU RAR 2003 –

- DINP has flash points of > 200°C and is not considered flammable (BASF 1983; BASF 1987a).

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APPENDIX A: Hazard Benchmark Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(Cr) Corrosion/ Irritation (Skin/ Eye)

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: ToxServices Review of Waterman et al. (2000)

In response to client questions regarding the GreenScreen™ classification of their product as "moderate" for Endocrine Activity, ToxServices obtained the manuscript cited by the client (Waterman et al. 2000). We critically evaluated data presented in the manuscript to determine the appropriate Endocrine Disruption classification. We specifically evaluated the endpoints evaluated in the paper, adequacy of experimental methods as described in the paper, data reported in the paper, and the authors' interpretation of the data (including, for example, assignment of NOAELs). We also compared the experimental procedures to OECD Test Guideline 416 , which is an internationally-accepted method for the conduct of two-generation reproductive toxicity studies that is routinely submitted to various regulatory agencies. Comments and questions on specific sections of the manuscript are included below, followed by general findings.

Section 2.4.1 specifies that the rats were mated 1:1 for the 2-generation study. However, it is not clear if re-pairings were permitted. This is critical because an adverse effect on mating success/fertility/pregnancy can be masked by allowing re-pairings.

In the discussion of allocation of F1 pups (Section 2.4.2), pups sacrificed on PNDs 4 and 21 received limited post-life evaluations, such as organ weights and histopathological examination of reproductive tissues. This represents a data gap because the adverse effects of phthalates on the male reproductive system may be most evident in neonatal or juvenile animals. Specifically, testicular damage was more pronounced in prepubertal rats than in adult rats exposed to mono-butyl phthalate . To address this possibility, OECD recommends that histopathological examination be conducted on a subset of weanlings not selected for mating.

In the two-generation study, litter evaluations consisted of mortality, general appearance, number, weight, sex, and external appearance. However, anogenital distance was not evaluated. This represents a data gap because AGD is a critical parameter that can be used as an indication of endocrine disruption and is known to be affected by exposure to various phthalates. Similarly, because AGD is sexually dimorphic, and some phthalates affect AGD, the pups should have been sexed internally at sacrifice to confirm that sex assignment was correct. Additionally, the manuscript is unclear regarding the process for selection of F1 animals for mating. Were F1 animals randomly selected or were the healthiest animals selected, thus excluding any F1 animals that would have shown compromised fertility?

Statistical procedures for evaluation of the reproductive/developmental data were somewhat unclear (Section 2.7); data should be evaluated using the dam as the experimental unit. For example, both litter size and pup weight are inter-dependent (as they do point out), and they both depend on maternal factors. Their description of the relationship between litter size and pup weight was unclear, and may warrant evaluation by a statistician.
In Section 2.8, the body weights were used in the daily dose calculations were not specified. This is important as body weights differ between control and treated and pregnant and non-pregnant animals. Similarly, it was unclear whether the food consumption values used in the calculations were from control or treated animals. There was an effect on body weight, so it's possible that food consumption in treated animals was reduced. It's important to use the most relevant body weight and food consumption data so that daily intake values can be accurately calculated. Relative organ weight data for the parents, number of litters delivered per group, and standard deviation of all reproductive endpoints were not found in the paper. It would have been valuable to include historical data on these endpoints to facilitate interpretation. Absolute organ weight data are difficult to interpret, and relative organ weights were not provided for any of the organs, and were not discussed for the male reproductive organs in particular. Relative organ weights are necessary for correct interpretation of the data, particularly since there was a treatment-related effect on body weight. It should be noted that OECD recommends weighing the prostate, seminal vesicles, and coagulating glands together as a unit. Additionally, in the text the authors conclude that "there were no remarkable effects on accessory male reproductive organs." However, these organs were not histologically examined, and organ weights were only obtained from parental animals. The conclusion that no adverse effects on the male reproductive system were noted is not substantiated by the presented data.

In their discussion in Section 4.1, the authors assign a NOAEL of ~1000 mg/kg-day for reproductive effects, based on their conclusion that no adverse effects on the evaluated reproductive parameters were observed in the 1.5% group in the one-generation study. However, this is problematic because the complete suite of relevant reproductive endpoints was not evaluated/reported in the one-generation study, specifically anogenital distance, relative organ

weights, and male reproductive organ histology. Thus, while it may be appropriate to conclude that the NOAEL for gestational index is ~1000 mg/kg-day, it is not correct to extrapolate this to reproductive toxicity in general. Similarly, it is problematic to base a NOAEL on absolute organ weight rather than relative organ weight, as was done in Section 4.2, particularly in this case, because this compound caused changes in body weight.

In Section 4.3, the authors discuss the observed decrease in pup body weights at birth. Lower pup body weights were associated with increased number of pups. The authors characterized litter size as a confounding factor on body weight. However, earlier in the paper the authors cited a publication in which litter size was characterized as a covariate to fetal weight. Covariation and confounding are different statistical concepts, so it may not be appropriate to attribute the reduced pup body weights to a confounding effect of litter size. Nonetheless, the authors did conclude that the toxicological significance of reduced pup weights at the 1.5% exposure in the one-generation study was a statistical anomaly.

Overall, this paper presents an incomplete evaluation of reproductive/developmental toxicity. There was little or no data on some of the most critical endpoints for this group of chemicals: relative organ weights, sperm parameters, pup sexual development, extent of the histological examination (i.e., if all stages of spermatogenesis were evaluated), and testicular histology in young animals. Given these data gaps, the authors' conclusion that "the data provide no evidence that DINP treatment influences endocrine-dependent processes" is not fully supported. Numerous evaluations of the adverse effects of phthalate esters, including DINP, on the male reproductive system are available in the peer-reviewed literature. Additionally, DINP has been assessed by the National Toxicology Program . Many of the available papers are more recent than the Waterman (2000) paper; a reference list is included at the end of this evaluation. ToxServices recommends that these papers be evaluated to ensure that the GreenScreen™ is conducted according to the weight-of-evidence of the scientific database for DINP. Some of the studies cited below suggest that DINP exposure may result in histopathological effects in fetal testes, nipple retention, reduced anogenital distance, and adverse effects on sperm parameters. Kwack et al. (2009) show that DINP significantly lowered sperm counts and sperm motility without an effect on testis weight; it is unknown if effects on testicular histology occurred.

In conclusion, the Waterman et al. (2000) paper is not a complete examination of the reproductive and developmental toxicity potential of DINP. In the absence of data on the critical endpoints discussed in this evaluation, DINP is most appropriately classified as "moderate" or "high" for endocrine disruption potential. Its ultimate classification should be based on the collective weight of evidence.

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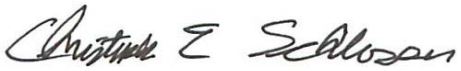
Diisononyl Phthalate (DINP) GreenScreen™ Evaluation Prepared By:



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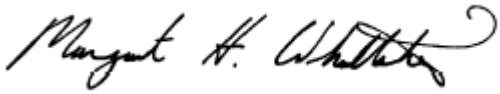

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