ATTACHMENT 1a

ENDPOINT SUMMARIES FOR MAMMALIAN AND ENVIRONMENTAL TOXICITY SUBMITTED IN THE REACH REGISTRATION DOSSIER FOR DIISONONYL PHTHALATE (DINP)

AND

TABLE OF CONTENTS FOR DINP DOSSIER INFORMATION ON THE ECHA WEBSITE AND PROVIDED TO CPSC ON A DVD

Contents

Explanation of Contents

ExxonMobil Chemical Company is submitting the attached information to assist Consumer Product Safety Commission's Chronic Hazard Advisory Panel in its evaluation of diisononyl phthalate (DINP). This information consists of extracts from the dossier submitted to the European Chemicals Agency (ECHA) to support registration of DINP under REACH.[1] The data in that dossier was prepared in accordance with REACH requirements and ECHA guidance.[2]

Our package – consisting of this document and pdfs provided via DVD (Attachment 1b) – includes key information from the registration dossier that has been posted to the Internet by ECHA.[3] This information can be accessed by the public; however, to make it more easily accessible to you, we have printed out each webpage of the DINP entries.[4] In addition, we are providing endpoint summaries which supplement the toxicity data contained in Sections 5, 6, and 7. The website and our package do not provide the full dossier for DINP and do not include certain production and analysis information that was claimed as Confidential Business Information in accordance with European Union law and REACH guidance.[5] These sections have been included in Attachment 1b as labeled blank pages just as they are shown on the ECHA website.

The dossier for DINP has been accepted by ECHA and the substance is registered as of February 17, 2010. Registration indicates that ECHA has undertaken a completeness check and has ascertained that all elements required under REACH have been provided. The completeness check does not include an assessment of the quality or adequacy of the submitted information.[6] It is ExxonMobil's belief, however, that the enclosed data is accurate and provides a useful summary of the database for this product.

Please let us know if you have any questions about the enclosed materials or about their significance within the REACH context. You can contact Angela Rollins, Oxo Americas Regulatory Affairs Advisor, at angela.rollins@exxonmobil.com.

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1 Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), O.J. L 396, 30.12.2006, pp. 1-849.

2 *See* ECHA, Guidance Documents, http://guidance.echa.europa.eu/guidance_en.htm .

3 ECHA, Search for information on registered substances, http://apps.echa.europa.eu/registered/registered-sub.aspx. To access the information, search on the CAS registry number (DINP - 68515-48-0: DIDP - 68515-49-1).

4 For DINP, we have provided that information submitted under CAS registration number 68515-48-0, which is the number for ExxonMobil's product.

5 *See* ECHA (2007), Guidance on data sharing, Chapter 11: Confidential Business Information (CBI), European Chemicals Agency, Guidance for the Implementation of REACH, http://guidance.echa.europa.eu/docs/guidance_document/data_sharing_en.pdf

6 *See* REACH, Article 20.

Table of Contents for DINP Dossier Information on the ECHA Website

Robust summaries of the studies on which the following endpoint summaries are based are available on the ECHA website. The following is the table of contents for the information on the website.

The website can be accessed by the public at http://apps.echa.europa.eu/registered/registered-sub.aspx (search on CAS registry number 68515-48-0). However, use of the site requires lots of clicking; therefore, ExxonMobil is providing via DVD a series of pdfs scanned from a printout of the web pages. This table of contents can be used to navigate those pdfs.

Because of the large database for DINP and the formatting of the ECHA summaries, the hardcopy printout is very large (626 pages). Therefore, we are providing only the pdf form, but would be pleased to provide hard copy upon request.

Endpoint Summaries of Mammalian Toxicity Data
Submitted in the REACH Registration for DINP

Toxicokinetics, Metabolism and Distribution – Section 7.1

Absorption
Dermal absorption of 14C-DINP was studied in male Fischer 344 rats in both conditioned (pretreatment with non-labeled DINP) and non-conditioned skin (ExxonMobil, 1983a; McKee et al., 2002). Following exposure, the dosed area was occluded. Under all conditions, the amount of DINP absorbed after 7 days ranged from 2 to 4% with approximately 93−99% of the administered radioactivity recovered at the site of application. Radioactivity in feces and gut of the exposed rats suggested some excretion occurred via the biliary route. These results are in agreement with the work published by Elsisi et al (1989) which demonstrated that dermal absorption decreases as carbon chain length increases.

Absorption of DINP via the gastrointestinal tract decreases as dose increases (49% at the low dose of 50 mg/kg compared to 39% at the high dose of 500 mg/kg; eliminated in urine) leading to an estimated absorption of approximately 50%. In addition, absorption of DINP seems to be of a saturable process. Increasing the dose results in an increased amount of unabsorbed compound being eliminated (fecal radioactivity associated with parent compound increased from 8% to 41% from a single low dose to the high dose).

Distribution
In male and female Fischer 344 rats receiving single or repeated oral doses of 14C-DINP, radioactivity cleared from the tissues rapidly, but analysis of tissues within 1 hour after the exposure indicated that the highest levels were in liver (4.7% of administered dose), kidneys (0.31%), and blood (1.62 %). Fat and testes contained small amounts of metabolites. No bioaccumulation occurred over 72 hours post-dosing.

Metabolism
Once absorbed, DINP is de-esterified to the monoester and then further metabolized by side-chain oxidation, in both the gut and liver, of the ester group or by hydrolysis to phthalic acid. Most of the 14C collected in the urine of rats following a single oral dose of 14C-DINP was in the form of phthalic acid or side-chain oxidation products of the monoester (MINP). The relative amount of phthalic acid in the urine decreased at the high dose. The monoester itself, as well as the diester, was present in only trace amounts. In feces, 8 and 41% of the radioactivity was associated with the diester following administration of a low (50 mg/kg) or a high (500 mg/kg) oral dose of 14C-DINP. This indicates saturation of metabolism at the high dose. The remainder of the fecal radioactivity was associated with the monoester or its side-chain oxidation products. Major metabolites in the liver were the monoester and its side-chain oxidation products. The same metabolites and phthalic acid were in testes. The fat compartments contained the monoester and its oxidation products. Repeated exposures revealed similar metabolites in the tissues. Repeated dosing did not result in accumulation of DINP and/or its metabolites in blood and tissue, but rather in increased formation and elimination of the monoester-oxidation products. In summary, in the rat, DINP was de-esterified to the monoester, which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. Formation of oxidation products appeared to increase following the high dose or repeated dosing, while the hydrolysis to phthalic acid decreased.

Urinary metabolites of DINP have also been quantified in several human studies with the hopes of using them as biomarkers of exposure. In a single subject human metabolism study of DINP (Koch and Angerer., 2007), it was observed that metabolites included the urinary excretion of the simple monoester, mono-iso-nonylphthalate (MINP), and oxidized isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups. Within 48 h, 43.6% of the applied dose in urine was recovered as the above DINP metabolites: 20.2% as OH-MINP, 10.7% as carboxy-MINP, 10.6% as oxo-MINP and 2.2% as MINP. Elimination followed a multi-phase pattern; elimination half-lives in the second phase (beginning 24 h post-dose) can only roughly be estimated to be 12 h for the OH- and oxo-MINP-metabolites and 18 h for carboxy-MINP metabolites. After 24 h, the carboxy-MINP metabolites replaced the OH-MINP metabolites as the major urinary metabolites. With regard to ambient exposure to DINP, studies that examined urinary metabolites in the general population identified MINP and oxidative metabolites (Silva et al., 2004, 2006b), in agreement with the metabolism study of Koch and Angerer (2007). Thus, in humans, as in animals, approximately half the ingested DINP is absorbed and then rapidly metabolized and excreted in urine and feces.

Excretion
DINP is rapidly excreted; the majority of orally administered material excreted in urine and feces within 24-48 hours, and less than 0.1% of radioactivity was recovered in tissues after 72 hours. The major routes of excretion for orally administered DINP in rats were urine and feces, with about equal amounts excreted by either route at low doses, but more excreted in feces at high doses. The biological half-life is approximately 7 hours. Repeated dosing did not cause accumulation of DINP or its metabolites in blood or tissue, but rather increased formation and elimination of the monoester side-chain oxidation products.

In humans, within 48 h of administration, 43.6% of the applied dose in urine was recovered as DINP metabolites: 20.2% as OH-MINP, 10.7% as carboxy-MINP, 10.6% as oxo-MINP and 2.2% as MINP (Koch and Angerer., 2007). Elimination followed a multi-phase pattern; elimination half-lives in the second phase (beginning 24 h post-dose) can only roughly be estimated to be 12 h for the OH- and oxo-MINP-metabolites and 18 h for carboxy-MINP metabolites. After 24 h, the carboxy-MINP metabolites replaced the OH-MINP metabolites as the major urinary metabolites.

In summary, studies in both laboratory animals and humans demonstrate that DINP is rapidly absorbed from an oral route of exposure and quickly metabolized into the mono-ester (MINP) which can then be further transformed into oxidative metabolites.

Acute Toxicity – Section 7.2

Like other high molecular weight phthalate esters, DINP was observed to have a low order of toxicity by the inhalation, dermal, oral routes of exposure in acute toxicity animal studies with LD50/LC50 values exceeding the limit dose to test animals (ExxonMobil., 1968a, b; BASF, 1981a, b). For example, in oral studies, no significant signs of toxicity are reported, even in studies using doses well above the limit dose recommended by current regulatory guidelines. In dermal studies, limited reversible irritation is the only effect associated with treatment.

Due to their low vapor pressures and the technical difficulties of generating a vapor at ambient temperatures, few studies by the inhalation route of exposure are available, most of which are relatively old and conducted before the development of testing guidelines or the implementation of good laboratory practice procedures. Although poorly documented, there were no reports of body weight changes, gross lesions or microscopic alterations of lungs; only slight tearing of the eye and

slight clear nasal discharge when animals were exposed to DINP at the saturated vapor concentration. As reported in the European Union Risk Assessment for DINP, the LC50 for an aerosol is greater than 4.4 mg/l (European Commission).

Irritation – Section 7.3

Acute skin irritation studies with DINP have been conducted in both humans and rabbits (ExxonMobil, 1995b; 1996b). In laboratory animals, DINP is slightly irritating to the skin and eyes (BASF AG, 1981d; ExxonMobil, 1968c); and the effects are fully reversible in a relatively short period of time. In human studies, there are no indications DINP produces skin irritation. These results are similar to the results observed with other high molecular weight phthalate esters. Respiratory tract irritation has not been reported in humans. Based on these observations, DINP is not considered irritating or corrosive.

Sensitization – Sections 7.4 and 7.10

Two sensitization tests were conducted in guinea pigs using the method of Buehler. One of them gave a positive response after re-challenge whereas the other produced negative results. However, the positive response obtained is inconsistent with other information and suggestive of a technical abnormality during the study. Specifically, a strong irritant effect during induction phase was observed in this assay which is in contradiction to the minimal evidence for irritancy observed in several other studies. Additionally, DINP was administered in a solution of peanut oil leading to the possibility of an allergic response to the vehicle.

Due to conflicting information, tests were conducted in humans. No positive reactions were reported in a repeated insult patch test conducted with DINP in humans (ExxonMobil, 1995). Accordingly, it is concluded that DINP is not a sensitizer in humans and is not classified.

Respiratory sensitisation has not been reported for DINP or any other high molecular weight phthalates. In a study that examined the cytokine profile associated with bronchial asthma, no response was mediated by DINP (Butala et al. 2004), suggesting little or no sensitizing potential.

Repeated Dose Toxicity – Section 7.5

The primary findings in the repeated dose studies are effects observed in the liver and kidney in two separate two-year chronic toxicity studies.

The key study for which the NOAEL of 88 mg/kg bw/day is derived for effects observed from repeated dose administration, is the well documented study reported by Moore (1998, Covance). Based on dose spacing, this study has been given preference for regulatory purposes. The study published by Lington et al. identified a NOAEL of 15 mg/kg bw/day for males and 18 mg/kg bw/day with a LOAEL of 152 mg/kg bw/day for females based on liver and kidney weight increases. The dose spacing of the Moore study (29, 88, 358, or 733 mg/kg/day) covers the range between the NOAEL and the LOAEL in the Lington Study. Therefore the 88 mg/kg bw/day is regarded to be a better representation of to the NOAEL.

In this study (Moore, 1998) DINP was administered daily to rats in the diet for at least 104 weeks at dietary concentrations of 0, 500, 1500, 6000, or 12000 ppm (29, 88, 358, 733 mg/kg/day). Rats in the recovery group were administered DINP at a dietary concentration of 12000 ppm for 78 weeks, followed by a 26-week recovery period during which they were administered the basal diet alone.

Administration of DINP for at least 104 weeks at levels of 358 and 733 mg/kg/day resulted in compound- related histomorphologic alterations in the liver and kidneys. Liver changes consisting of increased cytoplasmic eosinophilia and hepatocellular enlargement were observed only in the animals of the 733 mg/kg/day group. An increased incidence of hepatocellular neoplasia was observed in rats of both sexes of the 733 mg/kg/day group, but was not present in the high-dose recovery group.

Kidney changes at 104 weeks consisted of mineralization of the renal papilla and increased pigment in tubule cells at 358 and 733 mg/kg/day. Increased mineralization was noted in the renal papilla of the males of the 358, 733 mg/kg/day and recovery groups but was not present in the females.

Mononuclear cell leukemia occurred with increased frequency in rats of the 6000, 12000 ppm and recovery groups, and renal tubule cell carcinomas were noted in two and four males of the 12000 ppm and recovery groups. No evidence for sustained cell proliferation associated with the peroxisome proliferation induced by DINP was observed.

Based on the test results, the NOAEL for systemic toxicity was found to be 1500 ppm (88.3 and 108.6 mg/kg bw/d for males and females, respectively).

In the second two-year chronic study, DINP was administered to Fischer 344 rats (110/sex) at dietary concentrations of 0, 0.03, 0.3, and 0.6% (w/w) for 2 years (Lington et al., 1997). The mean daily intakes over the 2 years were 15, 152, and 307 mg/kg/day for male rats and 18, 184 and 375 mg/kg/day for female rats, corresponding to the 0.03, 0.3 and 0.6% dose levels, respectively.

High dose males exhibited a statistically significant, dose-related decrease in body weight beginning at 12 months of treatment and persisting until termination. This was not noted for the females. Males and females from the mid and high-dose groups exhibited a statistically significant, dose related increase in relative kidney and liver weights throughout most of the treatment period; the absolute liver and kidney weights demonstrated a similar trend. Statistically significant changes in organ weights consisted of dose-related increased absolute and relative spleen weights of the high-dose males, increased relative spleen weights of the high-dose females and a relative increased adrenal weight in both sexes as well as relative increased testes weights in high-dose males.

At 18 and 24 months, non-neoplastic lesions were observed in the liver and kidney of high-dose rats. Ultrastructural examination of liver specimens from representative rats of each sex from the four groups did not reveal any treatment-related peroxisome proliferation. An increased incidence of spongiosis hepatis, a degenerative change, was noted in males receiving 0.3 and 0.6% DINP in the diet, and of hepatocellular enlargement in both sexes at the high dose. Focal necrosis was increased in both sexes from 0.3%, but was only significant in males of the high-dose group. Hepatic pathology was significantly increased only in males from 0.3% and at 0.6% in females.

Statistically significant increased incidence of mononuclear cell leukemia (MNCL), a spontaneous, age-related tumor in F344 rats, was observed in the mid and high-dose groups (both sexes) and with a significantly increasing trend over time. The MNCL was associated with a variety of hepatic alterations (non-neoplastic lesions), however scientific consensus indicates a low human relevance to the observation of MNCL in Fischer 344 rats (Haseman et al, 1988).

Chronic progressive nephropathy, a normal aging lesion in rodents, was seen in most of the rats, and not related to treatment of severity grade. Renal neoplasms were seen in 3 mid-dose and 2 high-dose

male rats. The renal tumors were not statistically elevated when compared to controls and there was no evidence of any treatment related pre-neoplastic renal lesions. The underlying mechanism of the kidney tumors observed with the high-dose in male rats was determined to be an α2u-globulin mechanism of tumorigenesis which is not regarded as relevant to humans (EPA, IARC).

The effects observed in the liver in both of these studies, besides some minor and probably adaptive effects, are indicative of peroxisomal proliferation and include increased PCoA, liver weights, and liver hypertrophy and are not relevant for humans. Indeed, it has been shown that these effects are mediated through the peroxisome proliferation-activated receptor alpha (PPARα) and the levels of PPARα are higher in rodents than humans and the phthalate monoesters are more avid receptor agonists in rats than in humans.

It is accepted that peroxisome proliferation is specific to rodents. Peroxisome proliferators exhibit their pleiotropic effects due to activation of PPARα. PPARα is expressed only at low level in humans, explaining the absence of a significant response in humans to peroxisome proliferators. In studies conducted in non-human primates, the data obtained following oral administration of DINP for up to 13 weeks provides no evidence that the compound caused induction of peroxisome proliferation (Hall et al, 1999; Pugh et al., 2000). The NOAEL of 500 mg/kg/d from the marmoset and cynomolgus monkey studies clearly indicates that non-human primates and by read-across humans, are far less sensitive than rodents to peroxisome proliferation and its relative liver effects.

For the observed kidney effects, a NOAEL of 88 mg/kg/d is also derived from the Moore (1998) and based on increased kidney weights in both sexes. Effects on the rat kidneys were described in the majority of the rat studies as slight to moderate changes in the kidney weight, sometimes with modifications of physiological parameters often more marked in males (increases of blood urea and/or blood creatinine concentrations, proteins in urine and decrease of the specific gravity). Histologically, there was an increase in frequency/severity of chronic progressive nephropathy at low doses, specifically in males. Histological features are consistent with the specific male rat nephropathy irrelevant to humans, namely alpha 2u globulin nephropathy. It is assumed that the accumulation of protein droplets from continued chemical treatment results in progressive histological changes in male rats: papillary mineralization and atypical hyperplasia, leading to renal adenomas or carcinomas on prolonged exposure. Exposure to DINP results in a dose-dependent alpha 2u-globulin accumulation in male rat kidneys (ExxonMobil, 1986) and is likely the mechanism for kidney tumors seen only in male rats administered high dietary levels (1.2%) of DINP (Moore, 1998; Caldwell et al.,1999, Schoonhoven et al.2001).

In mice, progressive nephropathy is also observed at higher doses: tubular nephrosis at 20,000 ppm (5,700 mg/kg/d) in a 13-week study and granular pitted/rough kidneys in female mice at 8,000 ppm (1,900 mg/kg/d) in a chronic/carcinogenicity study (Moore, 1998b). Progressive renal nephrosis is an age-related lesion in rodents. In dogs, renal effects were observed at the high dose of 2% (2,000 mg/kg/d), and consisted of hypertrophy of kidney tubular epithelial cells in few animals in the 13-week study (ExxonMobil, 1971). No kidney effects were reported in monkeys up to 2,500 mg/kg/d in a 13-week study (Huntington life Sciences, 1998).

Concerning effects on reproductive organs, in the 2-year study with Fischer 344 rats (Lington et al, 1997) there was a statistically significant increase in relative testis weights at the high dose of 0.6% (307 mg/kg/d in males) associated with a slight, but not statistically significant, increase (13%) of absolute testis weight. In some sub-acute and sub-chronic studies with Fischer 344 rats, relative testis weights were statistically significantly increased with or without concurrent increase of absolute testis

weights and decrease of body weights at quite high doses (about 1,500 mg/kg/d in one week study, about 700 mg/kg/d in 13-week studies).

In mice, a NOAEL of 1,500 ppm (276 mg/kg/d) can be derived from a 104-week study (Moore, 1998b) based on testicular weight decrease observed from 4,000 ppm (742 mg/kg/d) and is used for the risk characterization. In addition, in a 4-week and a 13-week repeated-dose mouse studies, slight decreases of testis weight were observed accompanied by the presence of abnormal / immature sperm forms in the epididymis at doses of 6,500 mg/kg/d and 5,700 mg/kg/d, respectively (25,000 and 20,000 ppm). In those mouse studies (4-week and 13-week) effects were noted in uterus (hypoplasia and absence of endometrial glands) and in ovaries (absence of corpora lutea suggesting an arrest of ovulation) at doses of 20,000 ppm and 25,000 ppm.

It should be noted that in the 13-week study in monkeys, no changes were reported in testis weight and testis microscopic examination. In addition, there were no treatment-related changes in estradiol and testosterone concentrations assessed (Hall et al, 1999).

In a 6-week dermal exposure study in beagle dogs, the highest dose tested only produced a localized response of slight to moderate erythema. No systemic toxicity was observed (ExxonMobil, 1969).

In conclusion, the most sensitive endpoints, which are also not relevant to humans, are for effects on the liver and kidneys observed in two well-documented studies. A NOAEL of 88 mg/kg/d is determined in rats regarding results found in a chronic / carcinogenic study (Moore, 1998a).

Mutagenicity/Genotoxicity – Section 7.6

DINP is not mutagenic in vitro in bacterial mutation assays or mammalian gene mutation assay (with and without metabolic activation) and was not clastogenic in a cytogenetic assay in vitro in CHO cells or in an in vivo assay in bone marrow of Fisher 344 rats (BASF, 1986; 1995a; McKee et al., 2000). As reported in the European Union Risk Assessment Report for DINP, negative results were obtained in an in vivo study. This data suggests that DINP is not genotoxic in vivo or in vitro.

Carcinogenicity – Section 7.7

The key study for which the NOAEL of 88 mg/kg bw/day is derived for effects observed from repeated dose administration, is the well documented study reported by Moore (1998a, Covance). Based on dose spacing, this study has been given preference for regulatory purposes. The study published by Lington et al. identified a NOAEL of 15 mg/kg bw/day for males and 18 mg/kg bw/day with a LOAEL of 152 mg/kg bw/day for females based on liver and kidney weight increases. Therefore the 88 mg/kg bw/day is regarded to be a better representation of the NOAEL.
The mouse is less sensitive than the rat. Therefore, for risk characterization, the resulting NOAEL of the rat studies i. e. 88 mg/kg bw/day is used.

In this study, DINP was administered daily to rats in the diet for at least 104 weeks at dietary concentrations of 0, 500, 1500, 6000, and 12000 ppm (the average daily consumed doses of DINP were 29.9, 88.3, 358.7 or 733.2 mg/kg bw/day in males and 36.4, 108.6, 442.2 or 885.4 mg/kg bw/day in females). Rats in the recovery group were administered DINP at a dietary concentration of 12000 ppm (637.3 or 773.6 mg/kg bw/day for males or females) for 78 weeks, followed by a 26 -week recovery period during which they were administered the basal diet alone.

Administration of DINP for at least 104 weeks at levels of 6000 and 12000 ppm resulted in compound-related histomorphologic alterations in the liver and kidneys. Liver changes consisting of increased cytoplasmic eosinophilia and hepatocellular enlargement were observed only in the animals of the 12000 ppm group. An increased incidence of hepatocellular neoplasia was observed in rats of both sexes of the 12000 ppm group, but was not present in the high-dose recovery group.

Kidney changes at 104 weeks consisted of mineralization of the renal papilla and increased pigment in tubule cells at 6000 and 12000 ppm. Increased mineralization was noted in the renal papilla of the males of the 6000, 12000 ppm and recovery groups but was not present in the females.

Mononuclear cell leukemia occurred with increased frequency in rats of the 6000, 12000 ppm and recovery groups, and renal tubule cell carcinomas were noted in two and four males of the 12000 ppm and recovery groups. No evidence for sustained cell proliferation associated with the peroxisome proliferation induced by DINP was observed.

Based on the test results, the NOAEL for systemic toxicity was found to be 1500 ppm (88.3 and 108.6 mg/kg bw/d for males and females, respectively).

In the second two-year chronic study, DINP was administered to Fischer 344 rats (110/sex) at dietary concentrations of 0, 0.03, 0.3, and 0.6% (w/w) for 2 years (Lington et al., 1997). The mean daily intakes over the 2 years were 15, 152, and 307 mg/kg/day for male rats and 18, 184 and 375 mg/kg/day for female rats, corresponding to the 0.03, 0.3 and 0.6% dose levels, respectively.

High dose males exhibited a statistically significant, dose-related decrease in body weight beginning at 12 months of treatment and persisting until termination. This was not noted for the females. Males and females from the mid and high-dose groups exhibited a statistically significant, dose related increase in relative kidney and liver weights throughout most of the treatment period; the absolute liver and kidney weights demonstrated a similar trend. Statistically significant changes in organ weights consisted of dose-related increased absolute and relative spleen weights of the high-dose males, increased relative spleen weights of the high-dose females and a relative increased adrenal weight in both sexes as well as relative increased testes weights in high-dose males.

At 18 and 24 months, non-neoplastic lesions were observed in the liver and kidney of high-dose rats. Ultrastructural examination of liver specimens from representative rats of each sex from the four groups did not reveal any treatment-related peroxisome proliferation. An increased incidence of spongiosis hepatis, a degenerative change, was noted in males receiving 0.3 and 0.6% DINP in the diet, and of hepatocellular enlargement in both sexes at the high dose. Focal necrosis was increased in both sexes from 0.3%, but was only significant in males of the high-dose group. Hepatic pathology was significantly increased only in males from 0.3% and at 0.6% in females.

Statistically significant increased incidence of mononuclear cell leukemia (MNCL), a spontaneous, age-related tumor in F344 rats, was observed in the mid and high-dose groups (both sexes) and with a significantly increasing trend over time. The MNCL was associated with a variety of hepatic alterations (non-neoplastic lesions), however scientific consensus indicates a low human relevance to the observation of MNCL in Fischer 344 rats.

A clear increased incidence of mononuclear cell leukemia (MNCL) is observed in the two studies conducted with Fisher rats (outside the historical range of spontaneous leukemia), along with shortening of the onset of MNCL. However, MNCL is a spontaneous tumor which occurs frequently in

the F-344 rat and is the most common cause of spontaneous death in that strain and species (e. g., Haseman et al., 1998). National Toxicology Program (NTP) historical control data show that MNCL occurs in 14 to 74 percent of control animals (Haseman et al., 1998). Background incidence is seen to be highly variable and has more than doubled during the two decades since the Haseman et al. report in 1985. (Thomas et al., 2007). MNCL is found at much lower incidence in other rat strains (Iatropoulos, 1983) and has not been reported in mice (e. g., Harleman et al., 1994). There may also be differences within strains – the incidence of MNCL seems much lower in Japanese F-344 rats than in those used by the NTP (Whysner et al., 1995). Of interest, the IARC categorized MNCL as "an unclassified leukemia with no known human counterpart" and substances which increase MNCL frequency as "not classifiable as to carcinogenicity in humans".

Chronic progressive nephropathy, a normal aging lesion in rodents, was seen in most of the rats, and not related to treatment of severity grade. Renal neoplasms were seen in 3 mid-dose and 2 high-dose male rats. The renal tumors were not statistically elevated when compared to controls and there was no evidence of any treatment related pre-neoplastic renal lesions. The underlying mechanism of the kidney tumors observed with the high-dose in male rats was determined to be an α2u-globulin mechanism of tumorigenesis which is not regarded as relevant to humans (EPA, IARC).

Kidney tumors have been observed in male rats exposed to high doses of DINP for two years, but not in female rats and not in mice of either gender. Male rats are known to be susceptible to formation of kidney tumors through a mechanism involving alpha 2u-globulin accumulation. The kidney tumors observed in the DINP study were malignant tubule cell carcinomas, found in male rats given high dietary doses but not in female rats or in mice of either sex. The tumors found were of a type associated with an alpha 2u-globulin process and also demonstrated the sex- and species-specific responses expected for an alpha 2u-globulin process. Subsequent studies have demonstrated that all the criteria established by the EPA and by IARC to verify that a carcinogenic response is the consequence of the alpha 2u-globulin mechanism are met for DINP (Caldwell et al., 1999; Schoonhoven et al., 2001). Because humans do not produce alpha 2u-globulin, such male rat kidney tumors are not relevant for human health assessment (EPA, 1991; Swenberg and Lehman-McKeeman, 1998).

Reproductive and Developmental Toxicity – Section 7.8

Effects on fertility
The reproductive and developmental toxicity of DINP was evaluated by Waterman et al. (1999; 2000). The reports describe both a one generation and a two generation reproductive toxicity study.

In the one generation study which was a range-finding test for the subsequent two-generation study, groups of 30 male or female Crl: CDBR, VAF Plus rats were administered DINP in the feed at doses of 0, 0.5, 1.0, or 1.5% w/w for 10 weeks prior to mating. The females were exposed throughout mating, gestation, and lactation until post natal day (PND) 21. The males were killed immediately after the mating period.

Parental effects included a statistically significant lower mean body weight, as well as suppression in body weight gain, primarily observed in the mid and high-dose groups. The greatest decrease from controls was observed during the postpartum period. Similarly statistically significant lower mean food consumption was observed primarily in the mid and high-dose groups. Statistically significant increases in the mean and absolute and/or mean relative liver and kidney weights of both male and female animals as all dose levels tested were observed. Males in the high dose group exhibited a

statistically significant increase in the mean absolute and relative right testis weight, left testis and right epididymis weights and the mean relative left epididymis and seminal vesical weights. High dose females showed a significant decrease in the mean absolute and relative right ovarian and mean absolute left ovarian weights.

No significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were noted. Mean days of gestation were unaffected by treatment as well as the mean sex ratio of the treated offspring when compared with controls.

Offspring effects were noted for a number of parameters. The mean live birth index, day 4 survival-index, day 14 survival index and lactation index of the high-dose offspring were statistically significantly decreased. Dose related decreases in mean offspring body weight were observed during the postnatal period (PND 0-21). There were statistically significant lower mean body weights in the high-dose males and females, mid dose females at all weighing intervals and in mean offspring body weight of the mid dose males on PND 0, 1, 7, 14 and 21. Statistically significant lower mean body weights in the low-dose males on PND 0, 1, 14, and 21 and low-dose females at all weighing intervals were also observed.

No effect was observed on fertility parameters indicating a reproductive NOAEL of 1000 mg/kg/day; however, a decrease of live birth and survival indices occurred at 1.5% which led to a developmental NOAEL of 1% (622 mg/kg/day for parental males during pre-mating).

A two generation study was designed based on the results of the one generation range finding study. Crl: CDBR VAF Plus rats (30/group) were fed DINP in the diet at 0.2, 0.4, or 0.8% (w/w) for 10 weeks prior to mating, and through gestation and lactation.

There were no treatment-related deaths and no clinical signs which were judged to be directly related to treatment with DINP in P1 and P2 animals.

During gestation, significantly lower mean food consumption in the P2 high-dose females compared with controls was noted without an associated decrease of the body weight change during gestation days 0-21. During the postpartum period, parental toxicity was limited to a lower mean body weight in the high dose P1 females on post partum days 14 and 21 which corresponded to significant body weight gain suppression during the overall postpartum interval and was associated with decreased mean food consumption. Lower mean body weights were observed in the P2 high-dose females with an associated decrease of mean food consumption but without an associated decrease of the body weight gain.

Statistically significant increases in the mean absolute and mean relative liver weights in P1 and P2 in both sexes at 0.4% and 0.8% were observed. Microscopic hepatic changes were noted from 0.2% in P1 and P2 animals. High-dose males exhibited a statistically significant increase of relative right and left epididymal weights in P2 animals with a concurrent increase (not statistically significant) of absolute epididymis weight.

There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity or female gestational indices in P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility, and female fecundity indices was observed in P2 generation. Mean days of gestation of the P1/P2 treated and control animals were equivalent.

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 or gross post-mortem findings. There were statistically significant, dose-related, lower mean offspring bodyweights in all treatment groups compared with controls during the F1 or F2 generations. However, when the litter size was taken into account (Waterman et al., 2000), effects were only significant in high-dose males on PND 0, in males and females of the mid and high-dose levels on PND 7 and 14 and in all treated animals on PND 21. In addition, the weights of all F1 and F2 treated offspring were within the historical control range of the laboratory with the exception of the F2 high-dose males and females on PND 0 and the F2 high-dose males on PND 1 (considering litter size). These findings were considered by the laboratory to be a result of maternal stress and/or direct effects of DINP via exposure through lactation. Studies with other phthalates concluded that these decreases were apparently due to decreased food consumption by the dams and changes in the quality or quantity of milk (Dostal et al., 1987). Thus the laboratory concluded that the lower body weights in the pups might have resulted from decreased milk consumption.

Developmental toxicity
Using Crl: CDBR mated female rats, DINP was administered by gavage at doses of 0, 40, 200, 500 or 1000 mg/kg/day on gestation day 6 through day 15 (Waterman et al., 1999). Overt signs of maternal toxicity were not apparent at any dose level. Similarly, there were no significantly elevated fetal observations or body weight changes at any dose level. Therefore, the maternal and fetal NOAELs were determined to be 1000 mg/kg/day.

The comparative developmental toxicity of a number of phthalates including three DINP compounds was evaluated by Hellwig et al., 1997. DINP was administered by gavage at 0, 40, 200, and 1000 mg/kg/day to 8-10 sperm-positive Wistar females/group on gestation day 6 through day 15. The dams were sacrificed on day 20 and implantation sites were examined. Fetuses were weighed and examined for external malformations; half of the fetuses were examined for skeletal malformations and the other half for visceral malformations.

Maternal toxicity at the high dose consisted of reduced food consumption and increased relative liver and kidney weights. There were no treatment-related effects on the number of live fetuses/dam or fetal weight. There was an increase in percent fetuses per litter with variations at the highest dose. These variations consisted of rudimentary cervical and/or accessory 14th ribs. A modest increase in dilated renal pelves in the high-dose group was also noted. There were no maternal or developmental effects at 40 or 200 mg/kg/day. A maternal and developmental NOAEL of 200 and LOAEL of 1000 mg/kg/day were determined.

The two-generation reproductive study by Waterman et al. (2000) suggests an adverse effect on weight gain in pups during the perinatal and pre-weaning period of life. F1 mean pup body weight was significantly reduced on PND 0 in males at 0.8% DINP (555 and 1,026 mg/kg bw/day during gestation and lactation, respectively, as calculated by study sponsors). On PND 7 and 14, mean male and female pup body weights were significantly reduced at 0.4% (287 and 539 mg/kg bw/day during gestation and lactation, respectively) and 0.8%, and by PND 21, mean male and female body weights were reduced at all dose levels. In the F2 generation, mean female pup body weights were significantly reduced at 0.4 and 0.8% on PND 4, 7, 14, and 21 and at 0.2% (143 and 285 mg/kg bw/day during gestation and lactation, respectively) at PND 7. Mean male pup body weights were significantly reduced at 0.4 and 0.8% at PND 7, 14, and 21. The LOAEL for developmental effects was therefore identified as 0.2% (143–285 mg/kg bw/day during gestation through lactation for changes in body weight).

Additional Information – Sections 7.9 and 7.12

Peroxisome Proliferation
DINP is in a class of chemicals known as "peroxisome proliferators" – chemicals that induce an increase in the size and number of a subcellular organelle known as a "peroxisome" in the liver cells of rodents. Many peroxisome proliferators are known to cause liver tumors in rodents. As observed with peroxisome proliferators, including DINP, rats and mice are susceptible to the morphological, biochemical, and carcinogenic effects of peroxisome proliferators, while non-human primates and humans are completely non-responsive or refractory.

Criteria have been established by IARC to make the determination that tumors resultant from peroxisomal proliferation are not relevant to humans (IARC, 1995 at 12-13):

(a) Information is available to exclude mechanisms of carcinogenesis other than those related to peroxisome proliferation.

(b) Peroxisome proliferation (increases in peroxisome volume density or fatty acid b-oxidation activity) and hepatocellular proliferation have been demonstrated under the conditions of the bioassay.

(c) Such effects have not been found in adequately designed and conducted investigations of human groups and systems.

The data for DINP meet all of these criteria. With respect to the first criterion, alternative mechanisms of carcinogenicity, IARC relies substantially on the same types of information considered by ILSI, i. e., is there evidence that peroxisomal proliferation does occur in the species which develop cancer, and, can a role for a genotoxic process be ruled out (Klaunig et al, 2003). As described above, DINP does produce tumors in livers of rats and mice (Moore, 1998a; b), and there is clear evidence of peroxisomal proliferation in the livers of both species (Moore, 1998a; b; Smith et al., 2000; Valles et al., 2003; Kaufmann et al., 2002). DINP is not genotoxic. In addition, there is no evidence of pathologic changes in the livers of these species unrelated to peroxisome proliferation which could provide an alternative explanation for tumor formation (Lington et al., 1997; Moore 1998a; b). Further, the electron microscopic evaluation in mice revealed exclusively findings related to peroxisome proliferation; no other degenerative findings on the subcellular level were observed in either sex (Kaufmann et al., 2002).

The second criterion requires that peroxisome proliferation and hepatocellular proliferation be demonstrated under the conditions of the bioassay. As indicated above, increases in peroxisomal volume density, fatty acid b-oxidation, and hepatocellular proliferation in livers of rats and mice treated with DINP have been documented (Barber et al., 1987; Moore, 1998a; b; Smith et al., 2000; BASF AG, 2001; Valles et al., 2003; Kaufmann et al., 2002). In the rat study (1998a), the tumors appeared only at the highest dose (1.2% in the diet or approximately 733 mg/kg/day in male rats and 885 mg/kg/day in females). As also documented in the laboratory report describing that study (1998a), DINP also caused significant increases in liver weight, peroxisomal enzyme induction, and enhanced cell replication at that level. An independent study (Smith et al., 2000) confirmed these observations at the same levels in the same strain of rats. Thus the requirement that peroxisomal proliferation be demonstrated under the conditions of the bioassay has clearly been met in rats.

In the mouse study, liver tumors were significantly increased in male mice given 4000 or 8000 ppm (approximately 740 and 1560 mg/kg/day) and in female mice given 1500, 4000 or 8000 ppm

(approximately 336, 910 and 1888 mg/kg/day) in the diet for two years (1998b). As defined by the study protocol, liver weights, peroxisomal enzyme induction and cell replication were examined in only the high dose group (8000 ppm) and the control, and all of these parameters were significantly elevated in the high dose group from that study (Moore, 1998b). An independent study also measured liver weight increase, peroxisomal enzyme induction, and enhanced cell replication in the same strain of mice treated at 6000 ppm (Smith et al., 2000), and again all of these parameters were significantly elevated with respect to control. To evaluate peroxisome proliferation at the 1500 ppm and 4000 ppm levels, another study was conducted to determine the dose-response relationships for peroxisomal volume density and peroxisomal enzyme induction in mice treated with DINP. The data indicated that both peroxisome volume density and peroxisomal induction were significantly elevated at the tumorigenic doses (Kaufmann et al., 2002). These new data provide direct evidence of peroxisomal proliferation under the conditions of the bioassay in the mouse as well as the rat. Taken together, these data demonstrate that, at every tumorigenic dose level in both rats and mice, there is a significant increase in peroxisome proliferation. Thus peroxisomal proliferation has been demonstrated under the conditions of the bioassay for DINP, meeting the second IARC criterion.

The third criterion requires evidence that peroxisome proliferation effects do not occur in "adequately designed and conducted investigations of human groups or systems. " For this, IARC normally relies on data from studies in primates and/or human hepatocytes in culture. There have been two studies in non-human primates; in one of these DINP had no effects on the liver and showed no other evidence of peroxisome proliferation in marmosets following 90 days of treatment at levels up to 2500 mg/kg/day (Hall et al., 1999). In the other, DINP had no effects on the liver and showed no other evidence of peroxisome proliferation in cynomolgus monkeys following 14 days of treatment at levels up to 500 mg/kg/day (Pugh et al., 2000). Similarly, there was no evidence of peroxisome proliferation in either human hepatocytes (Hasmall et al., 1999; Kamendulis et al., 2002; Shaw et al., 2002) or other primate hepatocytes tested under in vitro conditions (Benford et al., 1986; Hall et al., 1999; Kamendulis et al., 2002). Thus studies from several laboratories using hepatocytes from different individuals or different species of primates have demonstrated that a peroxisome proliferator response is not elicited by DINP in humans and other primates.

Additionally, work has shown that the DINP metabolite, MINP, has the ability to bind PPARa (Bility el al, 2004) while PPARa null mice do not exhibit the same effects as wild type controls to DINP (Valles et al, 2003).

In summary, DINP meets all three IARC criteria for identifying a peroxisome proliferator for which observed liver tumors in rodents are not relevant to humans.
An alternative mechanism for phthalate induced liver tumors has been recently proposed that is independent of PPARα activation (Ito et al., 2007). The hypothesis, based on studies using mice without functional PPARα, suggests increased production of reactive oxygen species as a result of increased oxidative stress in mouse hepatocytes due to DEHP exposure. The applicability of the Ito et al., 2007 results is limited in that a number of reports have indicated that PPARα null mice are more vulnerable to tumorigenesis in the absence of any chemical exposure due to fundamental mechanistic differences. As spontaneous tumors are known to occur in the PPARα null mice at 24 months, the utility of this mouse model to understand alternative mechanisms of tumorigenesis that are independent of PPARα is problematic and can not currently be used to assess relevance to humans. Therefore, the Ito et al., 2007 data are not sufficient to indicate there is a valid alternative mechanism of carcinogenesis other than that related to peroxisomal proliferation indicating that the first IARC criterion is met.

Endocrine Modulation

In Vitro Studies

A series of phthalate esters, including DINP, were screened for estrogenic activity using a recombinant yeast screen (Harris et al., 1997). In the recombinant yeast screen, a gene for a human estrogen receptor was integrated into the main yeast genome and was expressed in a form capable of binding to estrogen response elements, controlling the expression of the reporter gene lac-Z (when receptor is activated, the lac-Z is expressed). DINP was tested at concentrations ranging from 10-3 M to 5.10-7 M. DINP produced inconsistent results in the yeast screen. DINP was also tested for the ability to stimulate proliferation of human breast cancer cells (MCF-7 and ZR-75 cells). DINP produced no effects in the MCF-7 assay. In the ZR-75 cells, DINP at concentration of 10-5, 10-6 and 10-7 M induced proliferation to a significantly greater extent than the control, which is in contrast to the findings for this chemical using the yeast screen. It should be noted that these in vitro assays have investigated one mechanism of action only, the ability of phthalates to act as estrogen agonists. More importantly, it should also be noted that these were tests of phthalate diesters. Under in vivo conditions the diesters are metabolized to monoesters which are not estrogen receptor agonists. The in vitro data need to be evaluated very carefully as the tests may have involved either substances which for all practical purposes do not exist under in vivo conditions or may have employed non-physiological conditions.

The estrogenic activities of DINP were investigated by Zacharewski et al (1998) in vitro using estrogen receptor (ER) competitive ligand-binding and mammalian- and yeast-based gene expression assays. No significant responses were observed with DINP in any of the in vitro assays.

Additionally, there is no indication that MINP, a metabolite of DINP, binds to androgen receptors (McKee et al, 2004).

Taken as a whole, the available data indicate that DINP or MINP do not have significant interactions with the estrogenic or androgenic receptors.

In Vivo Studies

Uterotrophic assay/vaginal cell cornification assay

In an in vivo study, 20, 200, 2,000 mg/kg/d of DINP was administered by oral gavage once daily for a period of 4 days to ovariectomised Sprague-Dawley rats (10 females per dose, two experiments) (Zacharewski et al., 1998). Ethynyl Estradiol (EE) was used as a positive control. Body weight, uterine wet weight and percentage of vaginal epithelial cell cornification on each day were assessed. DINP did not produce any statistically significant increases in body weight. Additionally, DINP did not produce any reproducible, dose-dependant effect on uterine wet weight relative to vehicle control at any of the dose tested. DINP did not induce a vaginal cornification response at any of the doses tested. Accordingly, it can be concluded that DINP is not estrogenic under in vivo conditions.

Steroidogenesis assay

In a study designed to test effects on testosterone synthesis, 32 pregnant female rats were exposed to either 300 mg/kg-bw DEHP or 750 mg/kg-bw DINP, alone or in combination, from gestation day 7 to gestation day 21 (Borch et al., 2004). The dams were sacrificed on gestation day 21 and the pups were harvested for analysis of testicular testosterone production, testicular testosterone content, plasma testosterone levels, and plasma luteinizing hormone (LH) levels. The results indicate that testicular testosterone production and testicular testosterone content were significantly decreased in the DINP exposed pups while plasma testosterone and plasma LH levels were unaltered. However, the utility of this study for hazard identification and risk assessment is limited by several factors. First, the study

utilized only one very high dose of DINP. Second, there were no adverse phenotypic effects reported in the study. Therefore it is unclear if the observed decrease in testosterone content is in-fact a toxicologically significant response. Third, while DEHP and DINP alone appeared to induce a decrease in testosterone content, there was no indication of a modulating effect of DINP on DEHP when co-administered. Finally, the authors sampled testosterone levels on gestation day 21, a time point after the developmental surge of testosterone that occurs during gestation day 16-18 in the rat. After gestation day 18, plasma testosterone levels are naturally declining in the fetal rat. Thus, conclusions regarding reductions in testosterone synthesis are problematic when assayed at this point.

Contrasting the work by Borch et al (2003), the effects of developmental exposure to DINP (250 and 750 mg/kg) was examined on gestation day 19.5 in fetal male Sprague Dawley from dams exposed to DINP between gestation days 13.5 – 17.5 (Adammson et al, 2009). No effect on testicular testosterone levels (gd 19.5) were observed with DINP. The expression patterns of genes associated with steroidogenesis were also examined. An increase in activity in the 750 mg/kg/day male pups was observed with P450scc, a gene coding for the enzymatic cleavage of the alkyl side chain on cholesterol, a precursor step for testosterone synthesis. No changes were observed in expression of genes associated with membrane transport (StAR), testosterone synthesis (3β-HSD), or overall control of male reproductive tract development (SF-1). With the exception of SF-1, these genes are typically strongly down regulated following exposure to chemicals that interfere with testosterone synthesis. GATA-4 and Insl-3 mRNA levels, genes associated with development of the male reproductive tract were seen to increase in male pups exposed prenatally to 750 mg/kg/day DINP. Again, down regulation of these genes is typically observed with anti-androgenic substances that affect male reproductive tract developmental. No marked effect was observed in concentrations of these gene products. Overall the genomic analysis (both the transcriptome and the proteome) is inconsistent with that observed for other low molecular weight phthalates that produce marked antiandrogenic effects. Further no morphological change in the testis was noted. Therefore, no effect on testosterone synthesis, or expression of the genes and proteins associated with testosterone synthesis were observed in this study.

Taken as a whole, DINP does not modulate estrogenic or androgenic endocrine systems.
DINP and its major metabolite MINP are devoid of estrogenic activity in vitro; it shows no ability of binding to rodent or human estrogen receptors or to induce estrogen receptors-mediated gene expression. In vivo assays demonstrated that DINP does not increase uterine wet weight or does not give rise to vaginal epithelial cell cornification. DINP and MINP do not interact with the androgen receptor.

Anti-Androgenicity
A study during the late gestational period (Gray et al., 2000) was conducted with several phthalate esters, including DINP. Timed-pregnant rats were gavaged daily with DINP at single dose of 750 mg/kg/d in corn oil as vehicle from gestational day 14 through postnatal day 3. In contrast to the effects observed with low molecular weight phthalate estes (BBP and DEHP), DINP produced slight equivocal changes in phenotypic expression of antiandrogenic effects. No effect was observed on anogenital distance or testis weight following DINP treatment. The authors reported a small statistically significant increase in malformations of the genital tract in male rat exposed in utero to DINP (7.7%). This statistical result is questionable because statistical significance was achieved only by pooling several different effects and treating them as a single effect. Further, the statistical unit was the pup as opposed to the litter, the commonly accepted statistical unit for developmental toxicity studies. An increase in percentage of males with retained areolas was observed in the DINP dose group at day 13 of age (22% vs. 0% in controls). However, subsequent publications from this lab

indicated that retained areolas in control animals ranged as high as 14% (Ostby et al., 2001). The usefulness of these data for hazard and risk assessment is limited as a single high dose was utilized, effects were pooled to achieve statistical significance, and the only clearly statistically significant finding is suspect based on high incidence of this finding in other control groups. The authors, too, questioned the significance of the statistical power of their analysis.

Anti-androgenic parameters were also evaluated in a poorly reported study by Hass et al (2003). This study is only available as an abstract. Groups of 12 mated female Wistar rats were gavaged from gestation day 7 to PND 17 with 0, 300, 600, 750, or 900 mg/kg/day DINP. Anogenital distance in male pups was significantly decreased at 600, 750 and 900 mg/kg/day. However, birth weights were decreased at the same dose levels and when birth was included as a covariate in the statistical analysis, the anogenital distances were only significantly decreased at 900 mg/kg/day DINP. At doses of 600 mg/kg/day and above, dose-related increases in nipple retention were observed in the male offspring, however, incidence was not reported. In contrast to the study of Gray et al., 2000 no malformations of the male reproductive tract were reported. The poor reporting of the study makes it difficult to conclude on the significance of the findings observed at exceedingly high doses.

In contrast to the findings reported by Gray et al., 2000 and Hass et al., 2003, no anti-androgenic effects were observed in male offspring of pregnant rats exposed to higher levels of DINP in the diet (Masutomi, et al., 2003). DINP was administered to Sprague-Dawley rats at concentrations of 400, 4000, and 20,000 ppm from gestational day 15 to PND 10. Maternal intake as estimated for both the gestational and lactational phases. The intakes were 30.7 mg/kg. day, gestation, 66.2 mg/kg/day lactation, 400 ppm; 306.7 mg/kg. day, gestation, 656.7 mg/kg/day lactation, 4000 ppm; and 1164.5 mg/kg/day, gestation, 2656.7 mg/kg/day lactation, 20000 ppm. Offspring evaluations included anogenital distances, prepubertal organ weights, onset of puberty, estrous cyclicity, and organ weights and histopathology of endocrine organs at adult stage (week 11) as well as the volume of sexually dimorphic nucleus of the preoptic area (SDN-POA). DINP, at 20,000 ppm (~1165 – 2657 mg/kg/day) did not cause any developmental alterations, other than slight degeneration of Sertoli cells and meiotic spermatocytes noted in the male pups at the adult stage. DINP did not alter any parameters in the females except for slight ovarian changes in the adult stage (i. e. marginal decrease in the number of corpora lutea). In addition, no change in the volume of the SDN-POA was observed. In summary, no antiandrogenic effects were observed on the developing male reproductive tract in this study. Levels of exposure were similar to and exceeded those utilized in the studies of Gray et al., 2000 and Hass et al., 2003.

A study designed similarly to the Hershberger bioassay screen for anti-androgenic chemicals which is currently undergoing validation by OECD (Lee et al, 2007) also tested the antiandrogenic properties of DINP. This assay investigates whether the co-administered chemical treatment interferes with the bioactivity of the endogenously provided testosterone and affects the expected rapid and vigorous re-growth of 5 androgen dependent sex accessory tissues in the young castrated male rat. In accordance with OECD, seven days after surgical castration (removal of testes and epididymides, followed by recovery and growth regression), young male rats were administered 0.4mg/kg/d testosterone propionate (sc) plus an oral gavage dose of a phthalate (DINP) at one of 3 dose levels (20, 100 or 500mg/kd/day). This treatment was repeated for 10 days, after which the animals were sacrificed and target organs weights collected.

DINP did not induce consistent changes in the absolute weight of all 5 androgen sensitive tissues (seminal vesicles, ventral prostate, levator anti-bulbocavernous muscle, Cowper's glands and glans penis). DINP showed significant reductions in seminal vesicle weight at all dose levels, but not in a

dose-related manner. DINP did not induce a significant change in Cowper's gland, glans penis weights, on serum testosterone levels, LH levels or produce clinical signs of toxicity or mortality. Overall, these data indicate that DINP does not meet the OECD criteria for androgen antagonists as the weights of the sex accessory tissues from the administered groups showed no consistent statistically significant differences from the testosterone-only animals.

Collectively, the data for antiandrogenicity of DINP are based on limited study designs with no or only minor effects being observed at very high doses with no dose response observed. Based on the comprehensive 2-generation reproductive, sub-chronic, and chronic studies it can be concluded that DINP is not an endocrine disrupter as defined by the Weybridge, IPCS and REACH guidance definitions.

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Endpoint Summaries of Environmental Toxicity Data
Submitted in the REACH Registration for DINP

Stability – Section 5.1

DINP has the potential to photodegrade in air at a relatively rapid rate (5.5 hour half-life based on a 12-hour day). However, it is not expected to partition to the air to a great extent where this process could significantly influence its fate. Therefore, abiotic degradation processes including hydrolysis and phototransformation in water will not significantly contribute to the removal of DINP from the environment.

Biodegradation – Section 5.2

DINP is readily biodegradable and is not expected to persist in the environment, based on results from standard ready biodegradation tests, simulation tests, and a study that assessed DINP loss from soil. The biological half-life of DINP in the aquatic environment is 10.3 days, approximately 1 day under wastewater treatment conditions, and 51 days in the soil compartment. Studies are not available to assess the biodegradability of DINP in sediment. However, the monoester of DINP (mono-isononyl phthalate) demonstrated an average half-life of 23 hours in aerobic marine sediments. Because the formation of the monoester occurs as the first step in the biotic degradation of DINP and because this step does not appear to be rate limiting, as evidenced by the high extent of biodegradation demonstrated by DINP in a ready test, the degradation of the diester in aerobic sediment is expected to occur at a similar high rate as demonstrated in soil.

Bioaccumulation – Section 5.3

DINP has a low potential to bioaccumulate in the environment based on results from a biomagnification food-web field study, a bioaccumulation dietary lab study with a fish, and a measured biota-soil accumulation factor (BSAF) for a soil-dwelling invertebrate. An aquatic food-web study that included 18 marine species showed that DINP did not biomagnify, but rather decreased in tissue concentration in organisms of increasing trophic position. Decreasing concentrations also referred to as biodilution, can be quantified by food-web magnification factors (FWMFs). The FWMF for DINP was 0.46. A FWMF that is greater than 1.0 is an indication of chemical biomagnification within a food-web, whereas a value of less than 1.0 indicates biodilution or dilution from lower to higher trophic levels. The FWMF is consistent with a laboratory fish bioaccumulation study in which rainbow trout were fed a DINP spiked diet for 14 days. Results demonstrated limited bioaccumulation with a lipid normalized biomagnification factor (BMF, concentration ratio in tissue to that in diet) of 0.1 and rapid subsequent depuration with a tissue elimination half-life of 1 day. The half-life of 1 day was used to calculate a BCF in fish of <3 L/kg for DINP. Data to assess the potential for terrestrial bioaccumulation of DINP were reported in a 14-day earthworm toxicity study. The BSAF as measured in a natural soil was 0.018. A BSAF value of <1 indicates a lack of bioaccumulation.

Aquatic Toxicity – Section 6.1

DINP has been tested with multiple aquatic organisms in acute and chronic studies. The results demonstrate that DINP does not cause any adverse effects within the limits of water solubility. In addition the test conducted with amphibia (Moor frog) also did demonstrate the absence of adverse effects on the development of the larvae exposed up to 858 mg/kg sediment.

DINP does not produce acute or chronic aquatic toxicity in fish, invertebrates, and algae. It has also been shown not to adversely impact the population parameters evaluated in a multi-generation study with a fish species, demonstrating that DINP does not have the potential to cause endocrine disruption in the aquatic environment. The absence of toxicity demonstrated by DINP is, at least in part, due to its low water solubility, resulting in low exposure potential, as well as the ability of organisms to metabolize DINP at a rate that prevents a critical body burden from being reached. The data for DINP show that it is not toxic at its maximally attainable water solubility level, which varies dependent on the conditions of study. Since DINP does not cause acute or chronic aquatic toxic effects at the limits of water solubility, it is not possible to derive NOEC or PNEC values needed for quantitative risk assessment. However, it is possible to qualitatively conclude based on low solubility and the results of acute and chronic aquatic toxicity tests that DINP does not pose an unacceptable risk to the aquatic compartment.

Short-term Toxicity to Fish – Section 6.1.1

All acute freshwater and marine fish studies are considered to be valid. The overall conclusion is that DINP does not cause any acute toxicity to fish within the water solubility of the test material.

Long-term Toxicity to Fish – Section 6.1.2

The result of long-term toxicity study is reported as a NOEC value for chronic fish toxicity. The NOEC value from this feeding study represents the highest concentration tested and measured under the conditions of the study. The results show that DINP does not cause toxicity. Additionally, the results can be used to assess the potential for endocrine disruption in fish. The multi-generation feeding study with Oryzias latipes (Medaka, 284-days) was conducted by adding DINP to dry flake food at 20 mg/kg. Evaluation of F1 and F2 embryos showed normal development except for a transient decrease in red blood cell pigmentation, which was observed in both the acetone control and DINP treatment groups. The only histopathological change observed in the F0 adults was a minor alteration in hepatocellular staining around the central vein. The male to female ratios (3:1) in all groups were similar. Phenotypic gender classifications of male and female fish were histopathologically confirmed to be 100% correct. The gonadal and liver somatic indices were not significantly different in the DINP treated group. There were no statistically significant changes in mortality, fecundity, or egg production between the treatment groups.

Short-term Toxicity to Invertebrates – Section 6.1.3

All acute freshwater and marine invertebrate studies are considered to be valid. The overall conclusion is that DINP does not cause any acute toxicity to invertebrates within the water solubility of the test material.

Long-term Toxicity to Invertebrates – Section 6.1.4

Results from long-term toxicity studies with an invertebrate show that DINP does not cause chronic toxicity at the maximum achievable aqueous concentrations investigated in these tests (i.e. in excess of water solubility). The absence of toxicity has been demonstrated both in testing procedures with and without solubilizer.

Toxicity to Aquatic Algae and Cyanobacteria – Section 6.1.5

All algae studies are considered to be valid. The overall conclusion is that DINP does not cause any toxicity to algae within the water solubility of the test material

Toxicity to Microorganisms – Section 6.1.7

The results of toxicity studies with microorganisms single and as communities of sewage treatment plants show that DINP does not cause any toxicity within its water solubility limits.

Toxicity to Other Aquatic Organisms – Section 6.1.8

The effects of DINP in a long-term toxicity study on the moor frog (Rana arvalis), a vertebrate, was evaluated by Solyomet al. (2001) in a natural sediment. The authors assessed egg hatchability followed by tadpole survival and growth over a 35-day exposure period. DINP at a measured concentration of 858 mg/kg sediment (dry weight) did not demonstrate a significant effect on frog egg hatching success following a 21-day exposure and tadpole survival and growth following a 35-day exposure. The 21- and 35-day NOEC values were each 858 mg/kg sediment, which was the highest concentration tested.

Sediment Toxicity – Section 6.2

DINP has been tested in sediment toxicity test in concentrations up to 3000 mg/kg. No adverse effects had been observed with two sediment dwelling organisms.

Terrestrial Toxicity – Section 6.3

DINP does not produce acute or chronic toxicity to earthworms or plants at high concentrations of DINP in soil. Since DINP does not pose an acute or chronic hazard to the terrestrial compartment, it is not possible to derive NOECs or PNECs needed for quantitative risk assessment. However, it is possible to qualitatively conclude based on available effects test data that DINP is not harmful to terrestrial organisms.

Toxicity to Soil Macroorganisms Except Arthropods – Section 6.3.1

The short-term toxicity of DINP was measured by mortality to the earthworm (Eisenia fetida) in a 14-day study using natural and artificial soils. The long-term toxicity of DINP was measured by reproduction to the earthworm (Eisenia fetida) in a 56 day study using an artificial soil. No significant mortality was observed in natural and artificial soils dosed with DINP after 14 days at a nominal loading rate of 10,000 mg/kg soil, which measured 7270 mg/kg natural soil and 7372 mg/kg artificial soil (concentrations are from analyses of soils at test initiation). DINP did not effect earthworm (Eisenia fetida) reproduction, based on a 56-day limit study in artificial soil at a high measured concentration of 982.4 mg/kg soil.

Toxicity to Terrestrial Arthropods– Section 6.3.2

In accordance with REACH Chapter R.7C Endpoint Specific Guidance, specifically R.7.11.6.3 Testing Strategy (Table R.7.11-2), data to characterize toxicity to terrestrial arthropods is waived for the following reasons. Di-isononyl phthalate ester (DINP) is ready biodegradable, consequently it is considered to degrade rapidly in the environment and not persist. DINP does not cause acute or chronic

aquatic toxicity at its maximum water solubility, consequently it does not pose an acute or chronic aquatic hazard, and it is not possible to derive NOEC or PNEC values needed for quantitative risk assessment. However, it is possible to qualitatively conclude based on low solubility and available effects test data that DINP is not harmful to aquatic organisms. Acute and chronic toxicity data for soil macro-organisms, earthworms, also show that DINP does not cause effects at high soil loading rates. Therefore, based on these considerations, additional short and long-term toxicity testing for soil organisms is not needed.

Toxicity to Terrestrial Plants – Section 6.3.3

The data used to characterize the terrestrial plant toxicity of DINP show that DINP does not cause toxicity to terrestrial plants at a high soil loading level, 8630 mg/kg for ryegrass (Lolium species) and 1080 mg/kg for lettuce (Lactuca sativa), based on germination. The chronic toxicity of DINP to plants has been measured in a 28 day test based on germination and growth of lettuce (Lactuca sativa) seeds in natural soil and shown not to be toxic at the highest level tested, 1387 mg/kg dry weight.

Results from 5-day plant toxicity studies show that DINP does not cause toxicity at high soil concentrations, based on germination, as determined in natural and artificial soils. Effects were observed with Lactuca sativa in only one germination test: 16 and 33% inhibition at 3,000 and 10,000 mg/kg, respectively. The chronic toxicity of DINP to plants has been measured in a long-term 28 day test based on germination and growth of lettuce (Lactuca sativa) seeds in natural soil and shown not to be toxic at the highest level tested, a concentration of 1,500 mg/kg.

Toxicity to Soil Microorganisms – Section 6.3.4

Results from a 33 day study with a population of microorganisms from natural soil show that DINP does not inhibit respiration at the highest tested concentration (9616 mg/kg). This result is supported by short-term toxicity studies on sewage treatment organisms and microorganisms, which also did not show adverse effects at their highest tested concentrations.

Toxicity to Birds – Section 6.3.5

Studies directly assessing the toxicity of DINP to birds have not been conducted. However, based on the lack of toxicity reported in mammalian toxicity testing, DINP is not expected to produce toxic effects in birds.