Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles

(CAS No. 68515-48-0 / EINECS No. 271-090-9, 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich; CAS No. 28553-12-0 / EINECS No. 249-079-5 di-isononyl phthalate)

European Council for Plasticisers and Intermediates
Technical Report 2009-0601-DINP
June 2009

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Executive Summary

This report provides a review of recent scientific data on di-isononyl phthalate (DINP) and a risk characterization for its use in toys and childcare articles. New data includes information relating to the relevance of spongiosis hepatis in rats as an endpoint for human risk assessment, as well as data on the exposure estimation of children to DINP from toys and childcare articles. In addition recent results on urinary biomonitoring are included. For completeness a review of relevant toxicology data is included in this report.
Recent hazard data has confirmed the appropriateness of the rat no observed adverse effect level (NOAEL) used in the EU Risk Assessment of 88 mg/kg/day. This NOAEL is conservative in view of the fact that two repeat dose primate feeding studies have shown NOAELs of 500 mg/kg/day with only minor effects being seen at 2500 mg/kg/day. Recent exposure data including biomonitoring of urinary metabolites has shown that previously estimated exposures to DINP have been overestimated. This is consistent with the fact that phthalates are physically bound within the polymer matrix and with the fact that high molecular weight phthalates such as DINP migrate to a much lower degree than low molecular weight phthalates.
Based on these most recent hazard, exposure and biomonitoring data a risk characterization has been carried out and margins of safety have been calculated for the use of DINP in toys and childcare articles. These show margins of safety of at least 1000 based on the most conservative exposure data. These data remove previous conflicts and uncertainties with regard to DINP and lead to the conclusion that the precautionary principle no longer needs to be applied in the case of DINP. All of these data confirm the positive conclusion for the use of DINP in toys and childcare articles which was reached in both the EU Risk Assessment Report (2003) and the Consumer Product Safety Commission (2002).
The following studies in particular should be noted:

- Karbe and Kerlin (2002), and MacSween et al (2002) – provides evidence that the critical endpoint highlighted by the CSTEE, namely spongiosis hepatis, is a spontaneous degenerative change seen in aging rats without a counterpart in human hepatic pathology and therefore is not relevant to the assessment of risk in children. This information confirms that the appropriate NOAEL for risk characterization in children is the NOAEL of 88 mg/kg/day selected in the EU Risk Assessment report. This information addresses the conflicting opinion between the EU Risk Assessment and the CSTEE.
- CPSC (2002); risk assessment subsequently published as Babich et al. (2004) – report on children's exposure to DINP from toys. This report provides exposure estimates that are significantly lower than those assumed by the CSTEE Opinion and the EU Risk Assessment Report.
- Sugita et al (2003) – report on exposures to DINP from soft plastic toys and pacifiers. This study also estimates exposures lower than those assumed by the CSTEE and the EU Risk Assessment Report.
- Center for Disease Control 2005, Wittasek et al 2007, Wittasek and Angerer 2008 provide supporting evidence based on urinary measurements of DINP metabolites that exposures to DINP are significantly lower than those assumed in the CSTEE Opinion and the EU Risk Assessment Report. This addresses uncertainties with regard to exposure from sources other than toys.

- The new exposure information shows that actual exposures to DINP are much lower than previously estimated. This is consistent with the fact that DINP is physically bound within the polymer matrix and migrates to a much lesser extent than low molecular weight phthalates.
- The SCHER Opinion on Phthalates in School Supplies concluded: "the phthalates in the articles tested do not significantly contribute to the body burden of phthalates in children. Analysis of exposure data on phthalates based on biomonitoring showed that exposures to DEHP and other phthalates in the general population, except di-n-butyl phthalate, are below the TDIs based on the comprehensive database on the toxicology of these compounds".

The current report also includes a review of the toxicology of DINP for the purposes of completeness. The review also highlights clear differences in sensitivity between rodents and primates in their response to DINP. Rodents are clearly much more sensitive to the liver and kidney effects with NOAELs in rats of 88 mg/kg/day and NOAELs in two separate primate studies of 500 mg/kg/day. The only effect seen in the primate studies is a small effect on bodyweight of exposed animals. This underlines that using the rat NOAEL of 88 mg/kg/day is already a conservative approach.
It should also be noted that the EU Risk Assessment (2003) and associated classification reviews led to the conclusion that DINP is not classified and labelled under the EU Dangerous Substances Directive (67/548/EEC). Hazard studies conducted since the completion of the EU Risk Assessment do not justify any change in this conclusion. This data will also be included in the REACH registration dossier for DINP.
Based on the most appropriate data, margins of safety (MOS) are calculated which confirm the positive conclusion for the use of DINP in toys and childcare articles which was reached in both the EU Risk Assessment Report (2003) and the Consumer Product Safety Commission assessment (2002).

I. Introduction

This report provides a review of recent scientific data on di-isononyl phthalate (DINP) and a risk Characterisation for its use in toys and childcare articles. New data includes information relating to the relevance of spongiosis hepatis in rats as an endpoint for human risk assessment, as well as data on the exposure estimation of children to DINP from toys and childcare articles. In addition recent results on urinary biomonitoring are included. For completeness a review of relevant toxicology data is included in this report.

Under the REACH regulation (Regulation No 1907/2006) exposure assessments and risk Characterisation are required for substances classified as dangerous under the EU Dangerous Substances Directive (67/548/EEC) or which are assessed to be PBT or vPvB. DINP is not dangerous under the EU Dangerous Substances Directive nor is it a PBT or vPvB. Therefore exposure assessments and risk Characterisations are not required for DINP under REACH. Nevertheless, because of the existing restrictions on DINP in toys and childcare articles which can be placed in the mouth based on the precautionary principle (2005/84/EEC) and because of the re-evaluation of those restrictions risk Characterisation has been carried out for toys and childcare articles and are included in this report.

II. Background

The European Union (EU), under the European Commission (EC) guidance, produced a rigorous risk assessment report on DINP in 2003 (ECB, 2003) concluding that DINP poses no risk to either human health or the environment. The risk assessment report clearly states there is no need for any further measures to regulate the use of DINP. The risk assessment was carried out in accordance with Council Regulation (EEC 793/93) on the evaluation and control of the risks of "existing substances." The European Food Safety Authority (EFSA) also re-evaluated DINP (EFSA, 2005), and concluded that the estimated dietary intake (0.17 ug/kg/day) based on food and diet in the UK and a maximum intake based on conservative computer modelling (10 ug/kg/day) were well below the tolerable daily intake (EFSA, 2005). DINP is permitted for use in food contact applications under Commission Directive 2007/19/EC.

An assessment focusing specifically on the potential risks to young children from exposure to DINP from toys use was conducted at approximately the same time as the EU Risk Assessment by the U.S. Consumer Product Safety Commission (CPSC, 2002). The CPSC concluded that "oral exposure to DINP from mouthing soft plastic toys, teethers and rattles is not likely to present a health hazard to children."

The EU risk assessment was then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE), which gave its opinion to the EC on the quality of the risk assessment (CSTEE, 2001). In this opinion the CSTEE commented that the "health part of the document is of excellent quality". CSTEE did however disagree with the use of a NOAEL for repeated dose toxicity in the EU Risk Assessment and preferred to a select a different NOAEL based on a liver effect observed in aging rats (spongiosis hepatis). Because of this difference of opinion between the EU Risk Assessment Report and the CSTEE Opinion and because of uncertainties with regard to exposure to DINP, the European Commission decided to apply the precautionary principle and restrict the use of DINP in toys that can be placed in the mouth. This led to the temporary restrictions on DINP being formalized as an amendment to the

Marketing and Use Directive (Directive 2005/84/EC amending Directive 76/769/EEC) stating that DINP (and DIDP, DNOP) shall not be used as a constituent of greater than 0.1% by mass of the plasticized material in toys and childcare articles which can be placed in the mouth of children. It was specifically noted that the restrictions for DINP, DIDP, DNOP should "be less severe than the ones proposed for DEHP, DBP, BBP for reasons of proportionality". It should also be clearly noted that the restrictions on DINP, DIDP, DNOP were based on conflicting and uncertain information and not on an established scientific identification of a risk for the health of children. Directive 2005/84/EC also states that "In line with the Commission Communication on the Precautionary Principle, the measures based on this principle should be subject to review in the light of new scientific information". With regard to timing the directive states the following: "The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this point in the light of new scientific information on such substances and their substitutes, and if justified, these measures shall be modified accordingly." As of June 1, 2009 these restrictions are now regulated as part of Annex XVII of the REACH regulation (Regulation (EC) 1907/2006).

III. Hazard Characterisation

Toxicokinetics

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, 1983; 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 appears to be process which can be saturated. 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).

Metabolism

Once absorbed, DINP is de-esterified to the monoester and then further metabolised by side-chain oxidation 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 metabolised 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.

In humans, DINP is also rapidly metabolised to the simple monoester, mono-iso-nonylphthalate (MINP), and oxidized isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups (Koch and Angerer., 2007).

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.

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 was recovered in urine 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.

Basic Toxicokinetics Summary

The acute exposure toxicokinetic studies conducted in F344 rats by oral administration showed that, at a low dose (50 mg/kg), approximately half of the DINP was excreted in the urine within

about 24 hours. The remainder of the dose was excreted in the feces within 96 hours. At the high dose (500 mg/kg) the fraction excreted in the urine was about 40% of the administered dose. In the repeated dose studies (5 daily doses of 50, 150, and 500 mg/kg) approximately 60% of the administered dose was excreted at all doses, suggesting an elevation of esterase activity and more rapid conversion to monoester following repeated treatment. Based on these urinary excretion data, the half-time for elimination of absorbed phthalate was about 7 hours. The dermal absorption study (approximately 0.2 ml/rat), by contrast, indicated that absorption was very slow, with 2-4% of the applied dose being absorbed within 7 days. However, the data indicated that DINP was rapidly metabolised and excreted once it was absorbed (McKee et al., 2002).

As shown in McKee et al (2002), most of the orally administered DINP was recovered in urine (52-59%) and feces within 48 hours of administration. Urinary metabolites were primarily oxidation products of MINP (monoisononyl phthalate) and phthalic anhydride. There was little, if any, un-metabolised DINP or MINP in the urine. The majority of the material recovered from the feces was unmetabolised DINP. Measurements of phthalate (as total radioactivity) in tissue indicated that the majority of the absorbed material went into the blood, liver and kidney compartments with little radioactivity elsewhere. In the liver, the major metabolites were MINP and oxidized MINP. In general, the highest levels of radioactivity in these compartments were found 2 to 4 hours after oral dosing, and declined thereafter. Estimated elimination half-times from the blood and tissue compartments were 3.5 to 4.5 hours. Repeated dosing caused no accumulation of DINP and/or its metabolites in blood and tissue, but resulted in increased formation and elimination of the monoester-oxidation products. Similar results have been observed in other studies (Silva et al., 2006a).

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 was recovered in urine 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 identified MINP and oxidative metabolites (Silva et al., 2004, 2006b), in agreement with the work of Koch et al (2007). Thus, in humans, as in animals, approximately half the ingested DINP is absorbed and then rapidly metabolised and excreted in urine and feces.

There are data available on di-2-ethylhexyl phthalate that suggest younger rats (25 days old) absorbed approximately twice as much of an administered dose (1 g/kg) of di-2-ethylhexyl phthalate compared to 60 day old rats (Sjoberg et al., 1985). Absorption, estimated by cumulative excretion of the administered dose, was approximately 44% in the younger animals and 26% in the older animals. The impact of these data on an assessment of DINP kinetics is uncertain and application of a scaling factor of two across age groups is not appropriate due to

the differences in percent absorbed at lower dose levels. Additionally, for the purposes of this assessment and development of No Observed Adverse Effect Levels (NOAELs) 100% absorption is assumed.

In summary, studies in both laboratory animals and humans demonstrate that DINP is rapidly absorbed from an oral route of exposure and quickly metabolised into the mono-ester (MINP) which can then be further transformed into oxidative metabolites. The dermal absorption 14C-DINP was determined to be slow in rats, but it occurred at a steady rate as evidenced by the increased amounts of radioactivity recovered in urine, feces, and tissues. The total amounts absorbed during a 7-day period ranged from 2 to 4% of the applied doses. Therefore, there is a low potential for dermal absorption of DINP since most of the dose remains unabsorbed at the application site.

Acute Toxicity

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 maximum amounts that can be administered to test animals (ExxonMobil., 1968a,c; BASF, 1981). 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, 2003).

Irritation and Sensitisation

Acute skin irritation studies with DINP have been conducted in both humans and rabbits (ExxonMobil, 1995a; 1996). In laboratory animals, DINP is slightly irritating to the skin and eyes (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.

Two sensitisation 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. The strong irritant effect during induction phase, only observed in this assay, is also surprising, considering the minimal evidence for irritancy in other studies.

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 sensitiser in humans and is not classified.

Repeat Dose Effects

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

The key study for which the NOAEL is derived for effects observed from repeated dose administration, is a well documented study reported by Moore (1998). 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. 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 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.

Spongiosis hepatis is a spontaneous lesion in the livers of ageing rats, appearing most often in the second year of life, with a strong predilection for male animals. The incidence of spongiosis hepatic can reach 34% in male Fischer rats as a spontaneous lesion and it can be increased and the age of onset reduced by exposure to a diverse number of chemicals. Some of these chemicals are genotoxic, but also a number of non-genotoxic agents as well, including di(2-ethylhexyl) phthalate (DEHP), a known peroxisome proliferator, have been shown to produce spongiosis hepatis (Karbe and Kerlin, 2002; David *et al.,* 2000; Moore *et al*., 1998; Lington *et al*., 1998).

The lesion is composed of altered sinusoidal lining cells interpreted as hepatic stellate cells (Ito cells or fat-storing cells). The characteristic histologic appearance, as first described by Bannasch and co-authors in rats treated with carcinogens, is that of a uni- or multilocular cyst-like formation filled with finely granular or flocculent acidophilic material (Bannasch, 1981). The lesion tends to replace lost hepatic parenchyma or interdigitate between hepatocytes, rather than compress adjacent parenchyma. The cystic spaces are lined not by endothelium or epithelium, but by extracellular matrix components typical of those produced by hepatic stellate cells. These elements include collagen III and IV, notably not collagen I as would be typical for activated hepatic stellate cells. This extracellular matrix abuts flattened fibroblastic-like cells that contain small lipid vacuoles typical of hepatic stellate cells. Cell markers typical of hepatic stellate cells include desmin and occasionally smooth muscle actin. The contents of the cyst-like structures are suggestive of proteoglycans, consistent with products of stellate cell metabolism.

Ultrastructually, cells forming the walls of the lesions resemble hepatic stellate cells both in conformation (interdigitations between hepatocytes and a subendothelial location in early lesions) and on the basis of cytoplasmic lipid droplets. Spontaneous spongiosis hepatis is less

likely to be multifocular and is less often associated with stellate cell aggregates. Spongiosis hepatic lesions can, in carcinogen-treated rats, be associated with or found within aggregates of hepatic stellate cells. Spongiosis hepatis is often found within other hepatic lesions such as altered foci and hepatocellular neoplasms. One group of authors associated with the Bannasch laboratory regards spongiosis hepatis to be a preneoplastic or neoplastic lesion (Bannasch and Zerban, 1986;1997) based primarily on its association with exposure to hepatic carcinogens, the increased rate of cell proliferation and the persistence of cell proliferation following withdrawal of the initial chemical treatment. In contrast, Karbe and Kerlin, (2002) have argued that there is insufficient evidence to warrant the designation of spongiosis hepatis as a pre-neoplastic lesion, since these characteristics are not necessarily indicative of a neoplastic process and that spongiosis hepatis is more likely a degenerative response.

The broad consensus of pathologists appears to support the view that spongiosis hepatis is a degenerative change. In the definitive text written largely by pathologists from the National Toxicology Program, Pathology of the Fischer Rat and published in 1990, spongiosis hepatis is described as a degenerative lesion (Boorman *et al*., 1990). It is clear that the response is multifocal and represents altered metabolism and proliferation of a population or populations of subendothelial lining cells of the sinusoids.

The cell of origin of spongiosis hepatis, regarded as the hepatic stellate cell, is probably less clear than assumed. In recent years, several fibroblastic cell types in addition to hepatic stellate cells have been described in the liver sinusoids and connective tissue of the portal tract and perivenular region of the central veins. These include, in addition to the hepatic stellate cell, transitional cells and myofibroblasts. All are reported to be present in the perisinusoidal spaces (Space of Disse). In addition, other lineages with distinct immunohistochemical signatures, septal myofibroblasts and interface myofibroblasts have been recently described in rats (Cassiman and Roskams, 2002). Thus, there are a number of cells, some of which may be species-specific (i.e. rat only) or have species-specific responses, within the group that had previously been aggregated into a single category of hepatic stellate cell.

Importantly, spongiosis hepatis is a lesion that appears to be confined to rats, particularly male rats, and teleost fish. Careful review of rodents over the last twenty or more years by the National Toxicology Program has led to only a rare incidence of neoplasms arising from stellate cells in mice (13 cases from more than 90,000 mice), but these lesions differ morphologically from spongiosis hepatis. There was no evidence of a lesion resembling spongiosis hepatis in a review of 163 human livers conducted by members of the Bannasch laboratory (Su *et al*., 1997). Indeed, in the chapter on liver neoplasia from a definitive text on human liver disease, Pathology of the Liver edited by R.N.M. MacSween *et al (2002).,* the authors, including Dr. Bannasch, state: “To the best of our knowledge no human counterpart of the spongiotic pericytoma [spongiosis hepatis] has ever been described.” Reports of lesions with similar characteristics in humans or non-human primates are also not found in the literature. This lesion or lesions with similar appearances are not described in any of a number of standard texts on neoplasia or systemic pathology in domestic animals, and there are no reports of this lesion in dogs. The only other species in which this lesion has been reported is the teleost fish (Couch, 1991). Given the large number of laboratory dogs and primates that have been exposed to a broad variety of

chemicals over a considerable number of years, the absence of descriptions of this lesion would support the view that spongiosis hepatis is primarily confined to male rats and teleost fish.

In light of the above review, the conclusion that DINP poses or can be reasonably anticipated to cause serious or irreversible hepatic toxicity in humans is not supported by the DINP findings or the literature (or lack of literature) on the biology of spongiosis hepatis. The relationship to human health is, at most, unclear, and based on the scientific evidence discussed, it is highly doubtful that there is any relationship. In view of these conclusions on the lack of relevance of spongiosis hepatis in rats to human health assessment, the use of spongiosis hepatis as an endpoint and as the basis for selecting a NOAEL from rodent studies is not appropriate. This calls into question the use of spongiosis hepatis and the benchmark dose of 12 mg/kg/day by both the CPSC CHAP and the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001). For further information on spongiosis hepatis see the Appendix to this report.

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.

Chronic progressive nephropathy, a normal aging lesion in rodents, was seen in most of the rats, and not related to treatment or 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 generally 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 extrapolation, 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 study by 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 (, 1998). 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 (ExxonMobil, 1986) 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). 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 localised response of slight to moderate erythema. No systemic toxicity was observed (ExxonMobil, 1969).

Repeat Dose - Carcinogenic Effects
The neoplastic lesions observed in these chronic studies are not relevant to humans.

A clear increased incidence of mononuclear cell leukemia (MNCL) is also 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 categorised MNCL as "an unclassified leukemia with no known human counterpart" and substances which increase MNCL frequency as "not classifiable as to carcinogenicity in humans".

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).

Repeat Dose – Peroxisome Proliferation

Rats
A 14 day toxicity study was conducted in Fischer 344 rats where doses of 25, 75, 150 and 1500 mg/kg/day DINP were administered by gavage (Huls, 1992). Although three DINP compounds with three separate CAS#s were tested, only results from CAS# 68515-48-0 are reported here.

A slight increase of the relative and absolute liver weight was observed with 1500 mg/kg/day. Decreases of the albumin and total protein concentrations were observed at all doses. A slight

decrease in triglyceride values was noted for the 1500 mg/kg/day group which is consistent with the hypolipidemic activity of phthalates. Slight changes in hematology were observed at doses up to 150 mg/kg/day.

Analysis of the microsomal fraction of the liver showed no effect on ethoxyresorufin O-desalkylase (biochemical marker for the isozyme of the P450IA1 and IA2) and p-nitrophenol hydroxylase (biochemical marker for the isozyme P450IIE1). Pentoxyresorufin O-desalkylase (biochemical marker for the P450IIB subfamily) was enhanced fourfold in the highest group, the lauryl-CoA activity was increased by a factor of ~6 in the highest dosage group and the dodecanoic acid 12-hydroxylase activity (DOS) was increased by a dose-effect manner from the lowest dose to the highest dose tested. The statistical assessment performed on the DOS activities at the lowest doses showed that no NOEL was calculable for DOS activity, only a LOEL of 25 mg/kg/day can be derived for DOS activity. In comparison with two other DINP compounds tested, no significant effect was observed for DOS activity at the dose of 25 mg/kg/day; therefore, overall it can be considered that the NOEL is close to 25 mg/kg/day. These changes are all associated with peroxisomal proliferation, which is considered a rodent specific effect and thus not relevant for human hazard risk assessment (For more information please see Appendix I).

Dose-response relationships for peroxisomal proliferation and related effects of DINP were investigated in a 21 day feeding study in Fischer 344 rats (BIBRA, 1985). Rats were fed diets containing 0, 0.6, 1.2, and 2.5% DINP corresponding to 0, 639, 1192, and 2195 mg/kg/day in males and 0, 607, 1193, and 2289 mg/kg/day in females.

Both sexes of rats exhibited weight loss with the mid and high concentrations. There was a statistically significant reduction of food intakes in both sexes with the high dose, although females returned to normal levels as the study progressed. A statistically significant increase of the absolute and relative liver weight was observed from the lowest dose 0.6%. Histologically, a reduction in hepatocytes cytoplasmic basophilia was detected from 1.2% associated at 2.5% with an increase in eosinophilia. Serum triglyceride levels were reduced in all treated males with a clear dose response. In the female treated groups serum triglycerides were elevated. Cholesterol concentrations were significantly reduced in all treated groups but without an apparent dose relationship. There was a dose-dependent increase in cyanide-insensitive palmitoyl CoA oxidation in both sexes and the difference became significant with 1.2% DINP. Males exhibited increases in lauric acid 11- and 12-hydroxylase activities at lower treatment levels than females; significant differences were noted at all dose levels in males, but in females, only in those receiving 2.5% DINP in the diet were lauric acid hydroxylase levels increased. Total hepatic protein levels were increased in both sexes from the lowest tested dose. Microsomal protein levels were significantly increased in females given DINP at 0.6 and 1.2%, but no dose relation was observed. Electron microscopic examination showed that 2.5% DINP produced a very marked increase in peroxisomes in males and a marked increase in females.

A NOEL was not identified in this study; however, the LOEL was determined to be 0.6% (607-639 mg/kg/day) based on the increase of liver weights associated with the increases in lauric acid 11- and 12-hydroxylase activities and reduction in the cholesterol and triglycerides observed at this dose.

A similar study was performed in F344 rats for 21 days in which 5 male and 5 female rats per group were fed 0, 0.3, 1.2, or 2.5% DINP (Lin, 1986; 1987). Electron microscopic examination of liver centrilobular and periportal peroxisome proliferation was performed. A peroxisome proliferator index was constructed from a linear combination of all usable parameters that yielded the best geometric separation among the 3 peroxisome conditions (low, moderate, and high). It was found by linear discriminant analysis that the best predictive end point is PCoA, with the relative liver weight performing nearly as well. This analysis also confirmed that females have less peroxisome proliferation than males after adjusting for body weight. The statistically predicted dose of DINP that would not induce peroxisome proliferation in 99% of rats was found to be 114.5 mg/kg/day for a 200 g rat.

Marmosets

In a study using marmosets, four groups of marmosets (4 marmosets/sex/group) were administered DINP by gavage at dose levels of 0, 100, 500 or 2500 mg/kg/day for 90 days (Hall, 1999). An additional group was treated with 500 mg/kg/day clofibrate to act as a reference control.

Body weight losses or low body weight gains were observed for males and females at 2500 mg/kg/d. There were no treatment-related changes in biochemical parameters or in hormonal concentrations assessed. No histological findings were considered to be treatment-related. No changes in weights or macroscopic/microscopic findings were observed for testes.

For peroxisomal parameters assessed, clofibrate treatment led to a statistically significant increase in microsomal protein and cytochrome P450 concentrations in females only and an increase in palmitoyl CoA oxidase and lauric acid hydroxylase activities was seen in both sexes. With 2500 mg/kg/day DINP, a slight increase in palmitoyl CoA oxidase activity, lauric acid 11-hydroxylase and lauric acid 12-hydroxylase was observed in both males and females. These increases were not statistically significant.

A conservative NOAEL of 500 mg/kg/day was determined based on minor decreases of body weight and body weight gain with 2500 mg/kg/day DINP.

Monkeys

The effects of DINP were evaluated in young adult male cynomolgus monkeys after 14 days of treatment, with emphasis on detecting hepatic and other effects seen in rodent studies after treatment with high doses of phthalates (Pugh *et al*., 2000). Groups of 4 monkeys received DINP (500 mg/kg/day) or the vehicle (0.5% methyl cellulose, 10 ml/kg) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg/day), a hypolipidemic drug used for cholesterol reduction in human patients was used as a reference substance. DINP had no effect on behavior, body weight or liver weights and histopathological examination of tissues from DINP treated animals revealed no distinctive treatment-related effects in the liver, kidneys, or testes. There were also no changes in any of the hepatic markers for peroxisomal proliferation, including peroxisomal beta-oxidation (PBOX) or replicative DNA synthesis. Additionally, DINP had no effect on gap junctional intercellular communication (GJIC) and it did not produce any

toxicologically important changes in urinalysis, hematology, or clinical chemistry. A NOEL of 500 mg/kg/day was determined for this 14 day study in cynomolgus monkeys.

Genetic Toxicity

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; 1995c; 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 show that DINP is not genotoxic in vivo or in vitro.

Reproductive and Developmental Toxicity

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, 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 at 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 was also observed.

Based on increases in liver and kidney weights from 0.5%, no NOAEL could be determined for parental systemic toxicity. 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 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.

No statistically significant differences were observed in reproduction indices indicating a reproductive NOAEL of 0.8% (1000 mg/kg/day). Based on the microscopic liver changes observed from 0.2%, the NOAEL for parental systemic toxicity is considered to be lower than 0.2% (114 to 395 mg/kg bw/day seeing that received doses are widely dependent on the period considered). No NOAEL can be derived from this study, but a LOAEL for offspring might be considered as 0.2%, emphasizing a trend observed similarly in males and females, based on the dose dependent reduced mean body weights of the treated offspring. The LOAEL remained approximate since pups switched diet from milk to solid food between PND 14 and 21 but may be estimated to be 159 mg/kg/d, the lowest dose of the maternal estimated range (159 - 395 mg/kg/d) during post-partum.

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. Transient signs of maternal toxicity at 1000 mg/kg/d, as indicated by slight reductions in body weight gain and food consumption were observed; however, normal weight and food consumption patterns were observed during the late gestation period, after exposure ceased, possibly indicating a recovery effect. A significant increase in fetuses with skeletal lumbar rudimentary ribs and with visceral (dilated renal pelves) variations at 1,000 mg/kg/d on a per litter basis was observed. Therefore, the maternal and fetal NOAELs were determined to be 500 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. The only foetal effects were evident at the highest dose by a statistically significant increase in percent fetuses per litter with variations. These variations consisted of rudimentary cervical and/or accessory 14^{th} ribs. A modest increase in dilated renal pelves in the high-dose group was also noted. There were no maternal or foetal effects at 40 or 200 mg/kg/day. A maternal and feotal NOAEL of 200 mg/kg/day was determined.

Endocrine Modulation

In vitro

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 $5x10^{-7}$ M. DINP behaved un-reproducibly 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 concentrations 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 metabolised 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 vitro 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).

In vivo

DINP was administered by oral gavage once daily for a period of 4 days to ovariectomized SD rats at doses of 20, 200, or 2000 mg/kg/d (Zacharewski *et al*., 1998). Ethinyl estradiol was used as a positive control. Body weight, uterine wet weight and percentage of vaginal epithelial cell cornification on each day were assessed. Increases in body weight were observed with DINP in only one of two identical experiments. DINP did not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovarectomized rats.

A study designed similarly to the Hershberger bioassay screen for anti-androgenic chemicals which is currently undergoing validation by OECD provided an assessment of the anti-androgenic properties of DINP (Lee and Koo, 2007). 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 20, 100 or 500 mg/kg/day DINP. This treatment was repeated for 10 days, after which the animals were killed and target organs weights collected. DINP showed significant reductions in seminal vesicle weight at all dose levels, but not in a dose-related manner. The biological plausibility of these data cannot be further investigated without the individual animal data or the historical data

for these endpoints in these laboratories Overall, these data indicate that DINP does not meet the OECD criteria for an androgen antagonist.

Evaluation of potential anti-androgenic effects for DINP have been reported in the peer-reviewed literature. A study on the effects of fetal exposure during the late gestational period (Gray *et al*., 2000) was conducted with several phthalates. Timed-pregnant rats were gavaged daily with DEHP, BBP, DINP, DEP, DMP and DOTP at single dose of 750 mg/kg/d in corn oil as vehicle from gestational day 14 through postnatal day 3. Data for DINP indicated that at 13 days of age, infant males with areolas were observed at an incidence of 22% compared with controls (0%).. Adult males exposed perinatally to DINP (4/52 pups) had malformations of testis, epididymis, accessory reproductive organs and external genitalia. The low incidence of reported effects was without any dose response and with effects of unclear significance using a small number of rats. The incidence of effects in DINP treated animals was 7.7%. (compared to 82% with DEHP treated animals). The range of historical control values is important for understanding the low incidence effects. In this study the control incidence for areola retention was reported to be zero, but in a subsequent study, control values are reported as 14% (Ostby *et al*., 2001).

As infants, males in the DEHP, BBP and DINP groups were reported as displaying areolas (87, 70, 22% respectively and reported as statistically significant). No other single endpoint (nipple retention, epididymal agenesis, fluid filled testes, testes weight) on its own was significantly different from control values. In addition different effects were pooled to produce the 7.7% incidence. Only by pooling of these different effects was statistical significance demonstrated. This type of data manipulation is not routinely performed in toxicological safety evaluations, nor is it considered good statistical practice. It should also be noted that Gray *et al*., 2000 did not see any effects on anogenital distance or on reduction of testosterone levels in the blood with DINP treated animals. Based on the above points the significance of the reported findings is questionable.

Anti-androgenic parameters were also evaluated in a study by Hass *et al*., 2003, abstract only available. 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,

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. Offspring were examined in terms of anogenital distance, 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 the very high dose of 20,000 ppm (~1165 – 2657 mg/kg/day) exerted severe toxic effects on both dams and offspring, but did not cause any developmental alteration, other than slight degeneration of

Sertoli cells and meiotic spermatocytes noted in 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), providing additional evidence that DINP does not cause estrogenic effects. In addition, no change in the volume of the SDN-POA was observed.

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 indicated 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, no mechanism of toxicity can be determined from this paper since it is limited by several confounding factors. First, there were no adverse phenotypic effects reported in the study. Second, the authors sample 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. Therefore it is unclear if the decrease in testosterone content is in-fact a toxicologically significant response.

In contrast, in a recent study designed to test the effects of DINP on steroidogenesis in the fetal rat testis and adrenal gland, DINP was administered by gavage at doses of 250 and 750 mg/kg/day to Sprague-Dawley rats on gestational day 13.5 to 17.5 (Adamsson *et al*., 2009). No effects on testicular testosterone levels (gd 19.5) were observed with DINP. Gene expression of P450scc was increased at 750 mg/kg/day; however no changes in relative StAR, 3β-HSD or SF-1 mRNA levels were seen in either dose of DINP. GATA-4 and Insl-3 mRNA levels were seen to increase with 750 mg/kg/day. Changes in protein levels were largely inconsistent with the gene expression data, and no pathologic change in the testis was noted. Therefore, this study provided no evidence that *in utero* exposure to DINP down-regulates testicular or adrenal steroidogenesis .

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 definitions.

Human Data

Several studies in humans in which development of the male reproductive system has been evaluated with respect to phthalate exposure during pregnancy or early childhood have been published. Of those studies, only one, Main *et al*., 2006, has evaluated exposure to DINP in an attempt to associate phthalate monoester levels with reproductive hormone levels and cryptorchidism in male infants. Pooled milk samples were obtained from each of 130 women when their children were 1-3 months old. Milk was analysed using HPLC-MS for the monoesters of di-(2-ethylhexyl) phthalate, di-methyl phthalate, di-n-butyl phthalate, butylbenzyl

phthalate and DINP. There were no significant differences in milk phthalate concentrations between the 62 mothers of sons with cryptorchidism and the 68 controls. The children had venous blood sampled at 3 months of age for determination of sex hormone-binding globulin, total and free testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and inhibin B. Individual hormone levels were used to calculate LH/testosterone, LH/free testosterone, and FSH/inhibin B ratios. MINP was found in all milk samples. Of the parameters tested, MINP was significantly associated with increased serum LH levels. The authors implied that testosterone levels were likely decreased relieving the negative feedback to the pituitary and thereby increasing LH levels. However, no alteration in free or total testosterone was observed (in fact an increase in free testosterone was observed). Further, this association could simply have been a statistical consequence of multiple comparisons to a common control. Overall, the authors concluded that there were "subtle, but significant, dose-dependent associations between neonatal exposure to phthalate monoesters in breast milk and levels of reproductive hormones in boys at three months of age." In 2006, the NTP-CERHR evaluated this study and indicated a number of weaknesses including confounding and possible contamination of breast milk samples. According to Calafat *et al*., 2004, a special treatment of the milk is required upon sample collection to denature milk enzymes and avoid overestimating the concentrations of phthalate metabolites in milk caused by contamination from the ubiquitous phthalate contaminants that may have been incorporated in the milk during the collection, storage, and measurement process. These considerations limited the usefulness of this study in the NTP-CERHR evaluation process.

The report of Swan *et al.*, 2005 investigated the link between metabolites of certain phthalates (DMP, DEP, DBP, BBP, DiBP, DnOP, DEHP) in the urine of pregnant women and changes in the reproductive systems of the male infants they later gave birth to. DINP was not assessed in this study. The author was careful to state that no adverse health effect was detected just changes in the infants' ano-genital distance (AGD) index, a correction of ano-genital distance (AGD) of age and size of the infant at time of measurement. Contrary to media reports, she reported no correlation between changes in penis size and maternal phthalate exposure, but only to the changes in the AGD measure.

The report (Swan *et al*., 2005) has been heavily criticised by scientists and statisticians. From a toxicological perspective, there is a difficulty in that the strongest association was with di-ethyl phthalate, a substance which when tested in rats had no effects on the development of the male reproductive system. From a clinical perspective, the attempt to convert AGD into a kind of index for adverse human health effects is not recognised in human biology. Further there are questions regarding the legitimacy of the AGD measurements in this study, particularly whether there was adequate compensation for the wide variations in age and weight of the measured infants (assuming that the infants could be accurately measured in the first place). An EPA expert panel reviewing the safety profile of di-n-butyl phthalate, one of the cited phthalates, considered the Swan study, but rejected its conclusions due to its numerous flaws. Recently, in an updated study Swan, 2008 presented a revised mathematical analysis for measuring changes in AGD and applied this new methodology to a further population of infant boys. Interestingly, the new data set resulted in contradictory results to some of the previous findings adding further doubt to the validity of the studies.

IV. Biomonitoring of DINP Metabolites

Specifically for young children, oral ingestion and dermal contact are the two most relevant routes of exposure to phthalate plasticisers where oral ingestion via mouthing children's objects has been the primary contributor (ECB, 2003; Gill *et al*., 2001; Clark, 2008). The most recent evaluation by Clark, 2008, supports oral ingestion as the primary source for estimated intake for children, toddlers and infants. Furthermore, studies have estimated that >40% of estimated intakes of DINP to the toddler and infant were due to mouthing children's products (Gill *et al*., 2001; Wormuth *et al*., 2006). In countries and regions where restrictions have been introduced on the use of DINP in mouthing toys this has changed. In comparison to oral ingestion, dermal exposure is a minimal contributor to the overall exposure of children (Clark, 2008). The human dermal absorption rate for HWM phthalates has not been determined; however studies (Deisinger *et al*., 1998; Elsisi *et al*., 1989) performed in rats indicate that the estimated dermal absorption rate is low and calculated maximal daily intakes attributable to dermal contact are significantly less than those attributable to oral ingestion (ECB, 2003). In addition, the physical size of the HMW phthalates impedes the passage of the molecule through the skin. Exposure to children via indoor and outdoor air is considered negligible due to the very low vapor pressure of HMW phthalates (Clark, 2008; Wechsler, 1984; Tienpont, 2000).

In general, phthalates are metabolised and excreted quickly and do not accumulate in the body (Anderson *et al*., 2001). Phthalate diesters are initially hydrolysed in the intestine to their corresponding monoesters, which are then absorbed (Albro *et al*., 1982; Albro and Lavenhar, 1989). The absorbed monoester metabolites may be further oxidized in the body. In the case of DINP, the hydrolytic monoester, monoisononyl phthalate (MINP) has been used for human exposure assessment. Based on Blount *et al*., 2000, external exposures to DINP from creatinine-corrected urinary metabolite measurements of MINP were calculated and reported by David, 2000 and Kohn *et al*., 2000. Estimates, as identified by Wittassek *et al*. 2007, were a median intake of 0.37 µg/kg/day and a 95th percentile of 1.5 µg/kg/d. Other reports showed a geometric mean of 0.21 ug/kg/day and a 95th percentile of 1.1 ug/kg/day (David, 2000) and a 95th percentile of 1.7 µg/kg/d (Kohn *et al*., 2000).

Data from the CDC (2005) in conjunction with the National Health and Nutrition Survey (NHANES) indicated the frequency of detection of MINP was very low compared with other phthalate metabolites. In the most recent NHANES 2001-2002 sub-sample, urinary concentrations of MINP were below the level of detection for all groups except Mexican Americans and African Americans corresponding to calculated exposures for the 95th percentile of 0.51 µg/kg/d and 0.36 µg/kg/d, respectively. Notably, exposure to DINP was seen to be negligible in children as mean measurements were again below the limit of detection (i.e. 0.8 µg/L for MINP). Exposure for children aged 6-11 calculated from the 95th percentile data of the NHANES 1999-2000 sub-sample was 1.32 µg/kg/day.

This low frequency of MINP detection has been hypothesised to be due to the fact that MINP further metabolises to form oxidative metabolites before being excreted in the urine (Silva *et al*., 2006a) and therefore is unlikely to be a prominent urinary metabolite of DINP. MINP is estimated as representing ~ 2% of the overall urinary metabolite levels in Koch and Angerer, 2007. It has been proposed that calculations be based on the oxy- and hydroxy- metabolites which, together, represent approximately 29% of the metabolites (Silva *et al*., 2006a). Based on

these more recent studies of urinary metabolites, the earlier exposure assessments based on MINP levels have been criticised. However, it should be noted that MINP has been shown to be a urinary metabolite of DINP, albeit not the most prominent and that exposures calculated from the MINP data are in line with more recent calculations based on other metabolites. For example, Wittassek *et al.,* 2007 measured oxidative metabolites in the urine of 240 students sampled during 1988-1993 and an additional 119 sampled between 2001 and 2003 and calculated exposure levels based on the measured levels of these oxidative metabolites. The 50th percentile levels are 0.22 and 0.37 µg/kg/day respectively with 95th percentile values of 1.6 and 1.5 µg/kg/day. These are similar to the previous calculations based on MINP and reported by David, 2000 and Kohn *et al*., 2000.

An additional study based on 102 Germans aged 6-80 years measured metabolite concentrations of a number of phthalates including DINP (Wittasek and Angerer, 2008). By applying urinary excretion factors determined from previous human metabolism studies, the median estimated amounts of DINP exposure were determined to be 0.6 µg/kg/day. The CDC has also reported one study in which levels of the oxy-, hydroxy- and carboxy- metabolites were measured along with MINP in the urine of 129 adults (Silva *et al*., 2006a). The levels of the oxy metabolite were similar to those of Wittassek *et al.* 2007. The levels of the hydroxy metabolite were significantly higher. Wittassek *et al.* 2007 did not report levels of the carboxy metabolite or of MINP so no direct comparison could be made. Wittassek *et al*. 2007 noted that levels of hydroxy metabolites were different and suggested that it might be due to differences in analytical methods. At this point it is not clear whether levels of exposure in the US are similar to or different from those in Germany, taking these data as representative (which also may not be the case). Recalculation of the data is also not straight forward. Wittassek *et al*., 2007 presented data as µg/l and used 24 hour urine volumes (not provided) to run their calculations. Silva *et al*. 2006a provided raw numbers but did not provide creatinine corrected values.

In light of this information, the current best estimates of DINP exposure to European adults, as given in Wittassek *et al*., 2007 where for the 50th percentile daily intakes are less than 1 ug/kg/day with 95th percentile values of less than 2 µg/kg/day. Levels of three phthalates in children aged 2 – 14 have been reported by Becker et al (2004) and Koch et al (2007). These showed median and maximal exposures which are higher than those in ages 6+ but still much lower than the exposures assumed in the EU Risk Assessment for children's exposure from toys and childcare articles. While these measurements did not include DINP it is expected that DINP exposures would be lower in view of the much lower migration of DINP from plasticised articles (Sears and Darby, 1984).

V. Exposure Assessment for Children's Contact with Toys

Exposure Assessment Studies

A number of fairly detailed studies have been performed to estimate potential child exposure from mouthing of toys that include both observational studies of child mouthing and measurements of DINP migration rates from toy products. These include RIVM (1998), CPSC (2002) (risk assessment subsequently published as Babich et al. (2004)), and Sugita et al., 2003. The mouthing data utilised in these studies (Groot *et al*., 1998, Kiss 2002, Greene 2002a, Sugita

et al. 2003) represent the most recent, comprehensive data sets for mouthing relevant to toy exposures. Other recent mouthing studies are available, but generally include data for frequency only (number of times hand-to-mouth or object-to-mouth events occurred per day); most provide minimal differentiation of mouthing objects and mouthing duration (USEPA, 2008). One more extensive mouthing study of UK children (Norris and Smith, 2002) is also available and discussed below. In addition to the detailed exposure studies indicated above, an upper bound estimate of exposure was developed during the CHAP assessment (utilising mouthing data from Juberg, 2001) as well as the conservative estimate utilised in the EU Risk Assessment for DINP (2003). These exposure assessment and mouthing studies are discussed in chronological order within this section. In general, as additional mouthing data have been collected over time, the ability to focus on toy-specific mouthing events has led to reduced estimates of exposure for child mouthing of plastic toys. In comparing exposure estimates across studies, it is thus important to consider which mouthing events (i.e., toys, fingers, clothing, etc.) were included in the mouthing time estimate.

RIVM 1998 including Groot et al., 1998 Mouthing Study
RIVM (1998) estimated potential exposures from child mouthing of plastic toys utilising adult in vivo migration data for DINP-containing toy sections (Meuling and Rijk, 1998) and mouthing duration data from an observational study of 42 Dutch children aged 3-36 months (Groot, 1998). Results from the study are summarized in Table 1. The study found that that in 99% of cases exposure would be below 100 ug/kg/day, and in 95% of the cases below 40 ug/kg/day. The study concluded that: for children > 12 months exposure levels from mouthing of toys are well below 150 ug/kg/day; for children age 3-12 months, in rare cases exposure may approximate or exceed 150 ug/kg/day if the samples for which migration rate data are available are representative of those on the market.

The age specific mean exposures in Table 1 range from 1.7 – 14.4 ug/kg/day; they are highest for the 3-6 month age group and decline with age. The exposure estimate is based upon total time mouthing excluding pacifiers. Note that for children in the age group with the greatest mean exposure (3-6 months), excluding pacifiers, more time is spent mouthing fingers than toys (Table 2). Thus, the approach of using all non-pacifier mouthing time is a conservative one for assessing exposures specific to toys. Additional review of the Groot data indicate that it has a skewed distribution with most children having short mouthing times, while a few have long mouthing times (CPSC 2002 citation of CPSC 1998). From Table 2, the age-weighted average time mouthing toys (toys intended for mouthing and other toys) is about 24 minutes for the 3-12 month age range. CPSC (2002) reanalysis of the data found a geometric mean mouthing time of 12 minutes for the 3-12 month olds and 2 minutes for older children.

Table1. RIVM 1998: Oral exposure from Soft PVC Baby Toys

Basis:	• Total mouthing time excluding pacifiers of the study of Groot et al., 1998 • Migration rate from a teether, adult in vivo study.	
Exposure estimates:		
Age in Months	Exposure in ug/kg/day - Mean (95^{th} percentile)	Mouthing Time in minutes/day – Mean and Max
3-6	14.4 (39.7)	36.9 min/day, max = 67.0 (N=5)

6-12	11.6 (38.9)	44.0 min/day, max=171.5 (N=14)
12-18	3.4 (16)	16.4 min/day, max=53.2 (N=12)
18-36	1.7 (6.4)	9.3 min/day, max = 30.9 (N=11)

Additional Information:

Exposure calculated using product leaching scenario of CONSEXPO. Distributions used for age-specific body weight (normal distribution assumed), mouthing duration and migration rates as empirical distribution from which random sampling was assumed.

Migration Rate: Adult in vivo migration rates were measured for 3 specimens of 10 cm^2. The specimen with the highest migration rate, the finger of a teether, was considered the most realistic. Exposures were calculated for all 3 specimens, but the results cited in the main text of the final report (and included in this table) are that of the specimen with the highest migration rate. Mean rate for this specimen = 2.44 ug/10 cm^2/min. range 0.9 – 8.9 ug/10 cm^2/min

Mean child body weights by age group were 6.25, 9.25, 11 and 13.5 kg, respectively.

Table 2. Mouthing Durations from Groot et al., 1998

	Mean (range) minutes/day by object category			
Age (Months)	3-6	6-12	12-18	18-36
N	5	14	12	11
Dummy/pacifier	94.9 (0 – 214)	27.3 (0 – 113)	17.3 (0 – 95)	20.8 (0 – 155)
Non-toys	2.8 (0 – 7)	9.4 (0.2- 26)	7.2 (0 – 50)	2.0 (0 -12)
Toys for mouthing	3.4 (0 -12)	5.8 (0 – 40)	0 (0 – 0.4)	0 (0)
Other toys	11.3 (0.6 – 27)	22.1 (0.4 – 102)	3.6 (0 – 10.4)	1.1 (0 – 3.8)
Fingers	20.5 (1.6 – 51)	7.5 (0 – 42)	5.8 (0 – 53)	6.3 (0 – 26)
Total time excluding pacifiers	36.9 (15 – 67)	44.0 (2 – 172)	16.4 (0 – 53)	9.3 (0 – 31)
Total time all categories	131.8	71.3	33.6	30.1

Additional Information:

- Observers: Parents
- Observation Periods: Ten times, 15 minutes each time, at two different days.
- Toys classified as toys meant for mouthing and toys not meant for mouthing based upon definition of toy producers.
- Total mouthing time is extrapolated to the time the child is awake and not eating.

CHAP 2001

In 2001, the Chronic Hazard Advisory Panel (CHAP), developed an estimate of a plausible upper bound on the extent of potential DINP exposure from children's toys. To develop this estimate, they utilised several conservative assumptions:

- a mouthed surface area of 11 cm^2 (10% greater than the typical 10 cm^2 surface area default)

- a mouthing time representing the upper bound mouthing for all non-pacifier toys, defined as the value exceeded by more than one child in the observational study of Juberg et al. (2001). Mouthing durations used were 3 hours/day for 0-18 month olds and 1 hr/day for 19-38 month olds.
- for migration rate, the 95% upper bound of the confidence interval on estimated DINP extraction using in vivo data, which was 60 ug/cm^2/hr. This is about a factor of four greater than the in vivo migration rate utilised in the RIVM (1998) analysis.

The resulting estimates of upper bound exposures were 280 ug/kg/day for the 0-18 month age group and 66 ug/kg/day for the 19-38 month age group. CHAP indicated a number of areas of uncertainty in the exposure estimate which could be addressed to develop a more definitive estimate. CHAP concluded that for children age 0-18 months who mouth DINP containing toys for 3 hours/day, the upper bound exposure estimate exceeded 120 ug/kg/day. Further, CHAP concluded: "This implies that there may be a risk of health effects from DINP exposure for any young children who routinely mouth DINP-plasticised toys for 75 minutes/day or more. For the majority of children, the exposure to DINP from DINP containing toys would be expected to pose a minimal to non-existent risk of injury. Further research addressing topics listed above [research areas to reduce uncertainty in the exposure estimate] could reduce the uncertainty as associated with this Characterisation of DINP risk from consumer products."

Since the CHAP 2001 assessment, a number of these areas of uncertainty have been addressed. Specifically, the subsequent CPSC 2002 analysis includes consideration of data developed on the portion of toys that contain DINP, additional migration data specific to toy products, and a new observational study that include better categorisation of mouthing activities by item mouthed.

CPSC 2002 including the Kiss 2002 and Greene 2002a Mouthing Study

In 2002, CPSC completed a detailed assessment of potential child exposure from mouthing of plastic toys. The document indicates: "The staff concluded that oral exposure to DINP from mouthing soft plastic toys, teethers and rattles is not likely to present a health hazard to children. Since children mouth other children's products less than they do toys, teethers and rattles and since dermal exposure is expected to be minimal, staff does not believe that other children's products are likely to present a health hazard to children." In support of this statement, CPSC cites exposure estimates determined by CPSC staff based upon the new observation study and the new migration data (Tables 3 and 4 below). CPSC indicates that these include a number of conservative hypothetical analyses.

As indicated above, to develop these refined exposure estimates CPSC undertook several significant endeavors to address the uncertainties cited by CHAP (2001), including a new behavioral observation study (Kiss 2002 and Greene 2002a), and a phthalate migration and toy screening study.

The observational study (Kiss, 2002; Greene, 2002a) represents one of the largest mouthing studies to date with the greatest level of categorisation. The survey included 169 children aged 3-36 months. Trained observers watched each child for 12- twenty minute periods over 2 days. Items mouthed were placed into one of 13 categories. Most importantly, this study included soft plastic toys as a specific category. This study found that the largest single non-pacifier category

was anatomy (fingers, hands, skin). Soft plastic toys represented only a small part of mouthing time. CPSC (2002) also presented a comparison of results from this study with other mouthing studies for the category of non-pacifier mouthing times (indicated to be the smallest category that was the same for all 3 studies examined) (Table 5). This comparison shows that on an equal category basis, the CPSC results are not lower than the other studies, rather they are similar or greater. The comparison also shows that for this category, for all 3 studies mouthing duration is lower in the older age group.

The newer studies enabled CPSC to address the CHAP comments related to exposures that may exceed 75/minutes/day: "Based upon the results of the CPSC observation study which was not complete when the CHAP met, CPSC staff believes it is very unlikely that children will mouth soft plastic toys for more than 75 minutes per day. " Further, the results of the new observational study indicate a mean mouthing time for soft plastic toys of 1.3 minutes/day for the 3-12 month age group and 1.9 minutes/day for the 12-24 month age group (the ages group with the highest mouthing time) (Table 4; Kiss 2002, Greene 2002a, 2002a). The new study of DINP migration rates for 41 children's products resulted in a range of 1.0 – 11.1 ug/10 cm2/min.

Table 3. CPSC 2002

Basis:	• Item specific mouthing time – Greene 2002a • Migration rates for plastic toys, in vitro data adjusted to expected in vivo rates		
Age in Months	Exposure in ug/kg/day - Mean (95^{th} percentile)		Mouthing Time in minutes/day – Mean (95^{th} percentile)
	Basic Case – Soft Plastic Toys, 42% with DINP	Hypothetical Case – Soft Plastic Toys, 100% with DINP	
3-<12	0.07 (0.44)	0.17 (0.94)	1.3 (7.1) N=54
12-<24	0.08 (0.53)	0.22 (1.11)	1.9 (8.8) N=66
24-<36	0.03 (0.12)	0.07 (0.27)	0.8 (3.3) N=49

Additional Information:

- Observers: Trained observers
- Observation Periods: 12- twenty minute periods over 2 days

Total mouthing time is extrapolated to the time the child is awake and not eating.

Migration Rate:

- Used distribution of migration rates for various DINP containing toys, mean =4.1 ug/10 cm^2/min, range 1-11; Values of zero added to distribution to approximate 42% market fraction of DINP
- Product specific in vitro migration rates were adjusted to in vivo conditions by sampling from the distribution of the factor M_{human}/M_{lab}. (M_{human} 1.17 ± 0.38 (N=19), M_{lab} 4.18 ± 0.45)

Table 4 . CPSC 2002 – Additional Hypothetical Cases

	Soft plastic toys, teethers, and rattlers, 100% with DINP*		All soft plastic items (excluding pacifiers), 100% with DINP		All toys, teethers and rattlers, 100% with DINP	Pacifiers, 100% with DINP*	
Age in Months	Exposure in ug/kg/day - Mean (95th percentile)	Mouthing Time in minutes/day – Mean (95th percentile)	Exposure in ug/kg/day - Mean (95th percentile)	Mouthing Time in minutes/day – Mean (95th percentile)	Exposure in ug/kg/day - Mean (95th percentile)	Exposure in ug/kg/day - Mean (95th percentile)	Mouthing Time in minutes/day – Mean (95th percentile)
3-<12	0.45 (2.15)	3.1 (17.4)	0.63 (2.90)	4.4 (17.5)	2.91 (10.71)	4.75 (24.55)	33 (190)
12-<24	0.22 (1.12)	2.0 (9.3)	0.41 (1.69)	3.8 (13.0)	0.84 (3.35)	2.82 (17.44)	27 (201)
24-<36	0.08 (0.33)	1 (2.3)	0.37 (1.70)	4.2 (18.5)	0.28 (1.25)	1.71 (5.41)	19 (51)

Mouthing times from Kiss, 2002; Greene, 2002a.
*For these categories, mouthing times were only provided in min/hr, they were converted to min/day as per the equation listed in Babich et al., 2004, using the average age in months for the age group of interest

Table 5 : From CPSC 2002, Tab H, Greene 2002b:
Daily average non-pacifier* mouthing times from various studies in minutes per day

Age group (months)	Groot et al 1998	Juberg et al 2000	CPSC
0 – 18	32.4	36.0	61.0
19-36	9.3	5.0	39.5

*Smallest category of objects that is the same for all 3 studies

Smith and Norris, 2002 – Observational Mouthing Study

Results from this study for the 1 – 24 month age groups are summarized in Table 6. These data have not been used in any of the available risk assessments. The study included children up to 5 years of age. The study found that total mouthing time had no clear pattern with age. Age groups that spent the most time mouthing vary, depending upon if ranking is based upon mean or maximum mouthing times. The highest mean mouthing time by category occurs in different ages, but all items are mouthed most by children < 1 year except for pacifiers. The age category 18-21 months had the highest mean mouthing time for pacifiers.

This study did not have the level of categorisation seen in the CPSC (2002) study. The toy category included all types of toys, the most commonly mouthed ones were animals, vehicles, toy food and building blocks. For the combined toys and objects category, the highest mouthing time was for the 6-9 month old group, the least for the 1-3 month group. After the age of 6-9 months mouthing on toys and other objects generally decreases. Plastics represent about 49%

of the composition of toys and other objects mouthed for age groups greater than 3 months. For 1-3 month olds, fabrics represent 80% of the composition of toys and other objects mouthed.

Table 6 : Smith and Norris (2002) Mouthing Study

	Time - rounded to minutes/day - average (max)							
Age (months)	1-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24
N	9	14	15	17	16	14	16	12
Items:								
Pacifier	47 (175)	28 (153)	15 (100)	42 (324)	60 (212)	25 (220)	69 (318)	25 (114)
Fingers	18 (51)	49 (96)	17 (77)	14 (99)	8 (36)	10 (39)	19 (81)	36 (113)
Toys	0.25 (1)	28 (155)	39 (227)	23 (64)	15 (44)	17 (58)	11 (33)	16 (102)
Other Objects	5 (28)	13 (37)	25 (70)	16 (91)	12 (63)	23 (98)	20 (66)	13 (40)
Not Recorded	1 (7)	0.5 (3)	-	0.02 (0.15)	0.03 (0.5)	0.13 (2)	0.18 (2)	14 (171)
Total mouthing all items	72 (212)	118 (216)	95 (317)	95 (413)	96 (257)	75 (314)	119 (412)	104 (395)
Mean total # of toys and other objects mouthed per child*	3	10	26	23	20	17	16	10
Total Active Time (not eating or sleeping) – hr:min	8:22	9:09	9:21	9:06	9:15	9:50	10:10	10:12

Observers: Parents, 20 fifteen minute periods over two weeks., split out equally over time zones within a day and weekday to weekend ratio of 4:1. No significant difference by time fo day. lots of info requested
All observations in home.

Data for 2-5 year olds also available. Highest mean total times fall within the months covered. Maximum times were in the 2, 3 and 5 year category.

*Estimated from Figure 11 of Smith and Norris, 2002

EU RA 2003

The EU RA utilised the highest migration rate of DINP from toys reported in RIVM (1998)- a value of 8.9 ug/10cm^2/min, an 8 kg body weight, and a mouthing duration of 3 hours to develop an estimate of 200 ug/kg/day. The more detailed analyses currently available can be considered for improved understanding of potential exposures from toys.

Sugita et al., 2003

For this assessment, a mouthing observation survey was performed for 25 Japanese children. Adult in vivo migration rate data were also obtained for 3 plastic specimens. The resulting exposure estimates are summarized in Table 7. The authors concluded that mean exposure values were approximately 10 -14% of a Tolerable Daily Intake of 150 ug/kg/day). The 95th percentile exposure values were approximately 24-44% of TDI (ranges represent exposure estimates based upon mouthing durations with and without pacifier use, respectively). They also indicated that : "the maximum value obtained using the total mouthing time (177 µg/kg/day) exceeded the TDI. Such a high value is likely to be reached only under extreme conditions, although the possibility of exposure to DINP at a level that is close to the TDI cannot be discounted." It should be noted that the exposure estimates these conclusions are based upon represent total mouthing time with and without pacifier. Table 8 summarizes mouthing durations for the categories included in this study: toys, plastics other than toys, fingers/body parts, others (cloth, paper, etc.), and pacifiers. For all age groups, consistently around 20 – 25 minutes of mouthing time is for fingers/ body parts. Based upon mean mouthing duration values, subtracting this time would reduce exposure estimates by 13 – 34 % for total mouthing including pacifiers and 21-35% for total mouthing excluding pacifiers. The mean mouthing duration for all toys ranged from about 18 – 37 minutes/day across age categories (as estimated from Figure 1 of Sugita et al., 2003). Using the toy value would result in mean exposure 57-89% lower than those calculated using total mouthing time included pacifiers, and 57-73% lower than those calculated using total mouthing time excluding pacifiers.

Table 7. Sugita et al. 2003

Mouthing Time Basis:	• New observational study of Japanese children • Adult in vivo migration rate data for 3 specimens. Exposure estimates below represent the ranges using migration rate data for the 3 specimens.			
Exposure estimates:				
Age in Months	Exposure using total mouthing time excluding pacifiers – Range of results for migration rates of the 3 specimens		Exposure using total mouthing time including pacifiers – Range of results for migration rates of the 3 specimens	
	Exposure in ug/kg/day - Mean range (95th percentile range)	Mouthing Time – Mean minutes/day	Exposure in ug/kg/day - Mean range (95th percentile range)	Mouthing Time – Mean minutes/day
Body weight data for 3-10 month olds, mouthing for 6-10 month olds	13.4 – 16.6 (36.0 – 43.1)	73.9 (range 11.4-136.5)	19.1 – 23.6 (57.8 – 68.7)	105.3 (range 11.4 -351.8)
Additional Information: Adult in vivo migration rates for 3 toy types – a mouthpiece for chewing, a pacifier toy, and a rattle. All tested with a 15 cm^2 surface area rectangular specimens, the chewing mouthpiece also tested with an 8.5 cm^2 surface area disk. Subjects asked not to compress strongly with teeth but				

rather to suck or lick the specimens. Release rates expressed as amounts per 10 cm^2. DINP measured by HPLC analysis.

Note, interlab and intralab differences reported in mean migration rates. Average DINP migration rates used were 92.4 ug/10 cm^2/hr for the chewing mouthpiece, 107.0 ug/10 cm^2/hr for the pacifier toy, and 86.8 ug/10 cm^2/hr for the rattle.

Mean infant body weight 3-10 months = 7.96 kg based upon Maternal and Child Health Division of the Ministry of Health and Welfare 1990 survey.

Exposure assessment:
Distributions used for release amounts and mouthing times assuming normal distributions based upon mean and standard deviation.

Table 8 . Sugita 2003 Mouthing Observations by Category

	Minutes/day by Age (months)					
	6	7	8	9	10	6-10
N	5	5	5	5	5	25
Toys	***35****	***37***	***18***	***25***	***20***	
Plastics other than toys	***5***	***30***	***15***	***15***	***15***	
Fingers/body parts	***28***	***20***	***22***	***23***	***25***	
Others (cloth, paper, etc.)	***14***	***6***	***13***	***15***	***12***	
Pacifiers	***0***	***5***	90	54	***10***	
Estimated total time including pacifiers	***82***	***98***	***158***	***132***	***82***	
From Sugita: Total Mouthing time including pacifier	78.2	93.4	154	126	74.2	105.3 (range 11.4 - 351.8)
Total Mouthing Time Excluding Pacifier						73.9 (range 11.4-136.5)
Daily Activity Time (Time Awake – Time Eating)						615 (range 415-770)

*Numbers in bold, italics represent estimates from Figure 1 of Sugita, 2003. The "Estimated" row is added to compare total times summed from the estimated values with the total mouthing values provided in the report. Estimated values generally agreed to within a few minutes except for an 8 minute difference in the higher age group.
Prescreening pilot study– N=50 age 3-12 months, 5 of each month, mothers asked to observe, mouthing time was longest in the 6-10 month age group so then focused on this age range.
Observers: Parents but researchers analysed videotapes.
Observation: Parents asked to record waking time, sleeping time, time eating and videotape activity during waking hours excluding meals for 2 days. Videotaping was for about 15 minutes in every hour. A total of 10 videotapes made for 150 minutes. Mouthing time excluding mealtime per daily activity time per infant estimated from the total mouthing time during the observation period. Pacifier defined as a silicon or natural rubber mouthpiece in the shape of a nipple fixed to a ring.

Summary:

The CPSC (2002) study presents the most detailed analysis for mouthing and migration rates specific to soft plastic toy products. The additional migration data specific to toys and greater level of differentiation on mouthing duration for soft plastic toys are both contributing factors to the refinement in exposure estimates. The observational mouthing data utilise professional observers to improve reliability in activity recordings, a relatively large sampling size and long total observation time. The greater degree of refinement of the of time mouthing soft plastic toys addresses a key area of uncertainty identified in the CHAP (2001) assessment. Further, the Table 5 summary of information from the Smith and Norris study indicates that for the 3-12 month age group, the average number of different objects mouthed per child ranges from 10 – 26 for the observation periods. This indicates that typical child mouthing activity covers multiple objects rather than a single one, supporting the use of object-specific mouthing duration rather than total mouthing time.

The CPSC (2002) mouthing observation study represents the largest sampling population of any of the mouthing surveys utilised in exposure and risk assessments. In addition, it was conducted by trained observers, typically felt to be more reliable than parents. The Smith and Norris information conducted a study to address this issue. In this study, parents and trained observers both provided comparative data, but both provided maximum estimates higher than those obtained with a video camera. It was explained that the video camera allowed closer views which enabled greater discernment of events where mouthing behavior stopped and then started again, with objects near but not actually touching the lips or mouth, resulting in lower maximum mouthing durations.

For all of the detailed mouthing observations studies (Groot et al. 1998, Kiss, 2002 and Greene, 2002a; Sugita 2003), time spent mouthing during the observation periods was adjusted to a daily mouthing time. This was done by first estimating the potential "exposure time", which is defined as the length of time that a child is awake and not engaged in eating. The time mouthing during the observation period is then adjusted to a total daily mouthing time based upon the potential exposure time. Mouthing frequency can be greater for time spent in indoor locations as compared to outdoors (USEPA, 2008), but total awake-and-not-eating time regardless of location has been used this adjustment. In general, as the majority of time is spent indoors this is not likely to affect the estimate significantly, but is an additional source of conservatism.

Further, there are a number of areas of conservatism related to the in vivo migration rate data. For the RIVM 1998 study, it could not be determined whether the phthalate measured in the saliva was "free" (i.e., material which was extracted from the PVC disc), or whether it was associated with PVC as microparticles which may have been chipped from the disc. It was assumed that all of the phthalate was bioavailable and therefore absorbed; however, it could not be confirmed how much of the phthalate in the saliva was actually absorbed. Similarly, all studies assume that 100% of the saliva generated is swallowed, although during child mouthing activity some may be lost from the mouth and/or remain on the object.

VI. Exposure Assessment Reported by the Scientific Committee on Health and the Environmental Risks (SCHER)

In 2008, the Scientific Committee on Health and Environmental Risks (SCHER) was asked to evaluate the overall scientific quality of the report on phthalates analysed (i.e. di 2-ethylhexyl phthalate (DEHP) and DINP) in a Danish EPA study which investigated the exposure and possible risk of chemicals in consumer products and articles. Specifically, a survey, as well as, a health assessment of chemical substances in school bags, toy bags, pencil cases and erasers was performed. The migration of substances to artificial sweat and saliva was also studied for some of the samples.

The results indicated that 9 of the erasers contained phthalates; 3 of them contained 22-44% of DEHP and six contained 32-70% DINP. Migration results were only reported for di-isobutyl phthalate (maximum 88 μg/g/h) and DEHP (maximum 6 μg/g/h) from 11 and 5 products, respectively.

SCHER reported that in general, the study design and the report contained several weaknesses, which hampered its evaluation and the ability to come to conclusions based on the results. The report was confusing as not all details required for an evaluation were included; some information is given in the section on exposure, some in the section on risk assessment. Furthermore, only limited and sometimes diverging information on quality assurance of the analyses is provided. However, SCHER agreed that the presence of phthalates in school supplies other than erasers is of low concern since dermal contact may be the only reasonable exposure pathways for children. Exposure from this pathway is expected to be very limited due to limited skin contact and inefficient dermal uptake. Erasers containing phthalates were noted as a potential cause for concern; however, based on the report, a science-based risk assessment of this potential exposure could not be performed due to a number of deficiencies outlined in the report.

SCHER considered that, due to the many weaknesses in the Danish EPA study and its reporting, firm conclusions on release of DEHP and DINP from the erasers as a basis for an assessment of potential health risk could not be made. However, SCHER did use some of the data presented in the Danish EPA report for a screening risk assessment of the exposures to phthalates from erasers to indicate the magnitude of a possible problem and information needs. In addition to using this data, the SCHER provided phthalate toxicity data as has been reported in the literature and recent EU risk assessments. For DINP, a group tolerated daily intake (TDI) of 0.15 mg/kg bw/day was previously reported and was based on peroxisome proliferation in rodent liver; peroxisome proliferation has been shown to not be relevant for human risk assessment. The pivotal toxicological effects for DINP were noted as hepatic changes (i.e. spongiosis hepatis). Please refer to pages 10 – 12 and page 51.

The SCHER concluded that “the phthalates in the articles tested do not significantly contribute to the body burden of phthalates in children. Analysis of exposure data on phthalates based on biomonitoring showed that exposures to DEHP and other phthalates in the general population, except di-n-butyl phthalate, are below the TDIs based on the comprehensive database on the toxicology of these compounds”.

VII Exposure Assessment for other applications

The EU Risk Assessment Report (ECB 2003) on DINP includes several exposure scenarios. These are:

Scenario 1: Toys and baby equipment
Scenario 2: Food and food related uses
Scenario 3: Building materials and furniture
Scenario 4: Car and public transport interior
Scenario 5: Clothing, gloves and footwear

These scenarios were very conservative; for example the scenarios for "building materials and furniture" and "car and public transport interior" imply that an adult of child is either in a building or in a car and never outdoors. Even with these conservative assumptions the risk Characterisation led to the conclusion for all of these scenarios that there is "no need for further information or testing or risk reduction measures beyond those which are being applied already".

VIII. Risk Characterisation

Hazard Characterisation Summary

Toxicokinetic and metabolism data in rats are available by the oral and dermal routes. DINP is significantly absorbed from the gastrointestinal tract (at least 50%), but dermal absorption is very limited. Human dermal absorption, based on *in vitro* and *in vivo* skin penetration studies, is less than that of rodents.

DINP is seen to have a low order of acute toxicity by the oral, dermal and inhalation routes and is not considered a skin or eye irritant or skin sensitiser. DINP is not mutagenic or clastogenic as evidenced by both *in vitro* and *in vivo* assays.

The primary findings in the repeated dose studies are effects in the liver and kidney. In rats, the effects in the liver, besides some minor and probably adaptive effects, are indicative of peroxisomal proliferation, including 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 (Bility *et al*., 2004).

In monkey studies, the data obtained following repeated oral administration of DINP provided no evidence that the compound caused induction of peroxisome proliferation. The NOAEL of 500 mg/kg/d from the marmoset and cynomolgus monkey studies clearly indicates that monkeys and subsequently humans are far less sensitive than rodents to peroxisome proliferation and its relative liver effects.

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. Nevertheless, for liver effects observed with DINP, a NOAEL of 88 mg/kg/d was assumed from a well-conducted chronic / carcinogenicity rat study according to GLP (Moore, 1998), based on liver toxicity at higher doses consisting of hepatic biochemical changes (increased ALT, AST), of liver weight increase in both sexes concurrently with histopathological findings. Two main chronic studies have been conducted on DINP, the above referenced study by Moore, 1998 and a study by Lington et al, 1997. The NOAEL in the Moore study is 88 mg/kg/day. The NOAEL in the Lington et al study is 15 mg/kg/day. The difference in these NOAELs is the result of the use of different dose levels:

Lington et al 1997	**Moore 1998**
Dose levels mg/kg/day	
Male rats (doses slightly lower than females and hence used for NOAEL determination)	
0	0
15 No observed adverse effect level	29.2
	88.3 No observed adverse effect level
152 Effect Level	359 Effect level
307	733

The Lington et al study shows a NOAEL of 15 mg/kg/day. However the next dose level in this study is at 152 mg/kg/day where effects on liver and kidney were observed. The true NOAEL therefore lies somewhere between 15 and 152 mg/kg/day. The Moore study includes doses between 15 and 152 mg/kg/day and in doing so comes closer to identifying the real NOAEL. The doses in the Moore study are 29.2 mg/kg/day and 88.3 mg/kg/day, 359 mg/kg/day and 733 mg/kg/day. Effects on the liver and kidney are seen at 359 and 733 mg/kg/day but not at 29.2 or 88.3 mg/kg/day. Therefore by using the data from both of these studies it can be concluded that the effect level for liver and kidney effects is 152 mg/kg/day and the NOAEL is 88.3 mg/kg/day. SCHER in their most recent opinion on school supplies have indicated their choice of 15 mg/kg/day based on the Lington et al study. However, based on the Moore study, this is not the true NOAEL. The Moore study more correctly identifies the NOAEL at 88.3 mg/kg/day.

For kidney effects, a NOAEL of 88 mg/kg/d is also derived from 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, a common aging effect in rodents, at low doses, specifically in males. There were also 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 hyaline droplets occurs rapidly, whereas continued chemical treatment results in additional histological changes in male rats: papillary mineralization and atypical hyperplasia, leading to renal adenomas or carcinomas on prolonged exposure. Moreover exposure to DINP results in a dose-dependent alpha 2u-globulin accumulation in male rat kidneys (Caldwell *et al*. 1999) and is likely the mechanism for kidney tumors seen only in male rats administered high dietary levels (1.2%) of DINP.

Structural and functional reproductive effects were examined in one- and two-generation feeding studies in rats that included an *in utero* exposure during the entire duration of pregnancy. The one-generation dose range finding study indicates that male and female rat fertility and structure of reproductive organs are unaffected by exposure to DINP at a maternal dose of 555–1,129 mg/kg bw/day during gestation and lactation, respectively, and adult exposure to concentrations as high as 1,676 mg/kg bw/day in males and 1,694 mg/kg bw/day in females. The data are sufficient to indicate that DINP exposures are not associated with detectable effects on reproductive function.

Data from Waterman *et al*., 1999 and Hellwig *et al*., 1997 indicated an increased incidence of cervical ribs and accessory 14th (lumbar) ribs which are considered to be evidence of developmental delays and reversible changes in rodents. An increase in cervical ribs and lumbar ribs was observed at the common dose of 1,000 mg/kg bw/day in the two studies. Thus a NOAEL of 500 mg/kg-bw/day was derived.

The two-generation reproductive study by Waterman *et al*., 2000 suggests a reduction in 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). 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 remained approximate since pups switched diet from milk to solid food between PND 14 and 21 but may be estimated to be 159 mg/kg/d, the lowest dose of the maternal estimated range (159 - 395 mg/kg/d) during post-partum

Based on the toxicokinetic data in rodents and as defined in the EU Risk Assessment (2003), adult human interal exposures were calculated taking into account a 50% bioavailability for adults and children aged 3 – 15 and 100% bioavailability for infants and newborns (0 – 3 years). This approach was based on a study on DEHP (Sjoberg et al, 1985. Absorption, estimated by cumulative excretion of the administered dose, was approximately 44% in the younger animals and 26% in the older animals. Application of a scaling factor of two across age groups is not appropriate for DINP due to the differences in percent absorbed at lower dose levels (see pages 5- 8 for further information on toxicokinetics.

In conclusion, based on effects on the liver and kidneys, a NOAEL of 88 mg/kg/d is recommended as the key effect for calculating a margin of safety (MOS) for both adults, children aged 3-15. infants (aged 6 months to 3 years) and newborns (0 – 6 months) exposed to DINP In order to assess risk based on pup bodyweight effects, a MOS is calculated using the LOAEL of 159 mg/kg/day derived from the 2-generation study with DINP where decreased mean male and female pup body weights were observed.

Table 9 Key Studies from which MOS is Calculated for Adults and Infants

End Point	Study	Effects observed at LOAEL	NOAEL	Reference
Repeated Dose Toxicity	2 years, diet, rat	358-442 mg/kg/day (6,000ppm) increased ALT, AST increased liver and kidney weights histopathological findings in liver	88 mg/kg/day (1,500ppm)	Moore, 1998
Pup bodyweight effects	2-generation, diet, rat	159 mg/kg/day (0.2%) decreased body weight in offspring		Waterman *et al*., 2000

Exposure Characterisation Summary

For the purposes of calculating margins of safety (MOS) exposure data from the various studies is used.

Table 10 Key studies showing exposure estimates to DINP from toys for children

Study	**Groot et al 1998/RIVM 1998**	**CPSC 2002/Babich et al 2004**	**Sugita et al 2003**	**Sugita et al 2003**
Scope	"Soft PVC toys" excluding pacifiers and based upon total mouthing time	Soft plastic toys – mouthing times specific to these	"Toys" – based upon total mouthing time excluding pacifiers	"Toys" – based upon total mouthing time including pacifiers
Age range months	3 – 36	3 – 36	3 – 10 months	3 – 10 months
Exposure from toys/ childcare articles ug/kg/day for the age group with the highest exposure	Mean 14.4 95th percentile) 39.7	Mean 0.08 95th percentile 0.53	Mean 16.6 95th percentile 43.1	Mean 23.6 95th percentile 68.7

These estimates can be compared with those used by the EU Risk Assessment and the CSTEE of 200 ug/kg/day.

Available biomonitoring data sets including the CDC, 2005 report, Wittasek *et al*., 2007, and Wittasek and Angerer, 2008 (Table 3) show that for the general population (US and EU populations aged six years and over) exposures are very low, with the highest median value reported being 0.6 ug/kg/day and the highest 95th percentile exposure reported being 1.5 ug/kg/day. Compared to the EU Risk Assessment estimates of exposure for consumers these biomonitoring data indicate that the EU Risk Assessments are overestimating exposure by one to

two orders of magnitude. This data is consistent with the more recent studies for exposure estimates to DINP from toys and therefore supports the view that the original EU Risk Assessment exposure estimates were conservative. For the purposes of calculating risk to children and deriving MOS from exposure to DINP from all sources including toys, the 95th percentile estimated mean exposure (*i.e.* 1.5 µg/kg/day) was added to the estimated intakes from the key toy and childcare exposure studies summarized in Table 2.

Table 11 Key Biomonitoring Data

Biomonitoring Data Set	**Population**	**Metabolite**	Calculated Daily Intake (ug/kg/d)	
			Median	**95th Percentile**
CDC, 2005	NHANES 1999-2000 Sub-sample Ages 6-11	MINP	<LOD	0.94
CDC, 2005	NHANES 1999-2000 Sub-sample Ages 6+	MINP	< LOD	0.73
Wittasek *et al*., 2007	Sampling Year 2001/2003 119 Germans Ages 20-29	Oxo-MINP, OH-MINP	0.37	1.50
Wittasek and Angerer, 2008	Sampling Year Unknown 102 Germans Ages 6-80	Oxo-MINP, OH-MINP, cx-MINP	0.60	Not provided

Risk Calculations and Conclusions

Table 12 Margin Of Safety (MOS) values based on NOAELs identified in this report and estimated exposure from toys, childcare articles and other sources for children aged 0 – 36 months. MOS values represent the factor by which the no observed effect level exceeds the estimated exposure.

Study	**Groot et al 1998/RIVM 1998**	**CPSC 2002/Babich et al 2004**	**Sugita et al 2003**	**Sugita et al 2003**
Scope	"Soft PVC toys" based upon total mouthing time excluding pacifiers	Soft plastic toys- mouthing time specific to these	"Toys" based upon total mouthing time excluding pacifiers	"Toys" based upon total mouthing time including pacifiers
Age range months	3-36 months; exposures greatest for 3-6 month group	3-36 months, exposures greatest for 12 – 23 month group	3-10	3 – 10
Exposure from toys/ childcare articles ug/kg/day	3-6 months: means 14.4 ug/kg/day; 95th ptiles 39.7 ug/kg/day:	12-23 months: 0.08 (mean) 0.53 (95th percentile)	16.6 (mean) 43.1 (95th percentile)	23.6 (mean) 68.7 (95th percentile)
Mouthing Duration (min/day)	Mean 36.9 minutes	12-23 months, soft plastic toys: mean 1.8 minutes/day, 95th percentile 9 min/day	mean: 74 minutes	mean : 105 minutes
Exposure from Biomonitoring Wittasek et al 2007 – (ug/kg-bw/day)	1.5 (95th percentile)	1.5 (95th percentile)	1.5 (95th percentile)	1.5 (95th percentile)
Total Exposure (ug/kg/day) 95th percentile from exposure study plus 95th percentile from Biomonitoring	**upper: 41.2**	**upper: 2.03**	**upper 44.6**	**upper 70.2**

NOAEL for Chronic toxicity ug/kg/day Moore (1998)	88000	88000	88000	88000
Margin of Safety – NOAEL/Total Exposure	**2135**	**46649**	**1973**	**1253**
LOAEL for pup body weight effects ug/kg/day Waterman et al (2000)	159000	159000	159000	159000
Margin of safety – NOAEL/Total Exposure	**3859**	**78325**	**3565**	**2264**

The above margins of safety can be compared with those calculated in the EU Risk Assessment (2003) for children as follows
3 – 15 year olds MOS 4000
0.5 – 3 year olds with toys MOS 176
Newborns with toys MOS 176

The margins of safety from the EU Risk Assessment are lower in view of the more conservative estimates of exposure. In all case margins of safety exceed the minimum requirement of 100 confirming the safe use of DINP in toys and childcare articles.

IX. Conclusions

Recent hazard data has confirmed the appropriateness of the rat no observed adverse effect level (NOAEL) used in the EU Risk Assessment of 88 mg/kg/day. This NOAEL is conservative in view of the fact that two repeat dose primate feeding studies have shown NOAELs of 500 mg/kg/day with only minor effects being seen at 2500 mg/kg/day. Recent exposure data including biomonitoring of urinary metabolites has shown that previously estimated exposures to DINP have been overestimated. This is consistent with the fact that phthalates are physically bound within the polymer matrix and with the fact that high molecular weight phthalates such as DINP migrate to a much lower degree than low molecular weight phthalates.
Based on these most recent hazard, exposure and biomonitoring data a risk Characterisation has been carried and margins of safety have been calculated for the use of DINP in toys and childcare articles. These show margins of safety of at least 1000 based on the most conservative exposure data. These data remove previous conflicts and uncertainties with regard to DINP and

lead to the conclusion that the precautionary principle no longer needs to be applied in the case of DINP. All of these data confirm the positive conclusion for the use of DINP in toys and childcare articles which was reached in both the EU Risk Assessment Report (2003) and the Consumer Product Safety Commission (2002).

Exposure Characterisations for school supplies have been reviewed by SCHER (2008) with the Conclusion that "the phthalates in the articles tested do not significantly contribute to the body burden of phthalates in children. Analysis of exposure data on phthalates based on biomonitoring showed that exposures to DEHP and other phthalates in the general population, except di-n-butyl phthalate, are below the TDIs based on the comprehensive database on the toxicology of these compounds".

Previously conducted exposure scenarios and risk Characterisations for DINP as part of the EU Risk Assessment (ECB, 2003) led to the conclusion that there is "no need for further information or testing or risk reduction measures beyond those which are being applied already".

X. References

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APPENDIX I : DINP-Induced Rodent Liver Effects

Comments on Key Chronic Studies Showing DINP-Induced Spongiosis Hepatis

With reference to Lington *et al*., 1997 and Moore, 1998, the incidence of spongiosis hepatis was seen to be dose-related and significantly elevated in rats chronically treated with DINP. However, the data presented are not evidence of serious liver injury in rats, nor does it support the conclusion that DINP can be anticipated to cause serious liver injury in humans. Reasons for this assertion are outlined below.

In both studies, deviation from the accepted practice of only diagnosing spongiosis hepatis when it is found separate from altered foci or hepatocellular neoplasia may have confounded interpretation of the incidence. However, if spongiotic lesions were proportionately associated with preneoplastic and neoplastic hepatocellular lesions, this altered evaluation system would have been unlikely to affect the final count of spongiotic lesions since the aggregate number of preneoplastic and neoplastic hepatocellular lesions for each treatment group/sex was not dramatically different and not likely to have significantly affected the total number of spongiosis hepatis lesions.

Clearly more spongiotic lesions were identified in the Lington study than the Moore study given that there were more liver sections examined. Since this method was applied to all groups in the Lington study, it should not affect the accurate comparison of groups.

The severity grade of spongiosis hepatis in DINP-treated male rats that had diagnoses of spongiosis hepatis from the Lington study was determined during a re-evaluation using a subjective scale based on the size and number of lesions in each of 4 liver lobes (Brown, 2000). A subjective scale with 1 as minimal, 2 as mild, 3 as moderate and 4 as severe was used. For rats found dead, the average scores for all groups were: control, 1.0; 30 ppm, 1.17; 300 ppm, 1.5 and 600 ppm, 1.5. For rats from the terminal sacrifice the average scores were: control, 1.10; 30 ppm, 1.28; 300ppm, 1.61 and 600 ppm, 1.57. Overall, when both groups are combined, the majority of the lesions were minimal to mild with some lesions graded as moderate. None of the lesions was considered severe. Approximately 94% of the grading in the top two doses was in the minimal and mild categories. The utility of this type of subjective review has limitations. A morphometric analysis would be likely to be more meaningful. However, it is evident from this review that there is no dramatic dose-related change in the character of the spongiosis hepatis in DINP-treated male rats and that these data do not provide evidence of severe liver injury.

Peroxisome Proliferation in Rats and Marmosets

Increases in liver weights have been cited as one of the indications for severe liver disease. In the Moore study liver weight to body weight ratios were significantly increased in male rats in the 600 ppm, 1200 ppm, and the 1200 ppm recovery dose groups and in female rats in the 600 ppm and 1200 ppm dose groups. Significant body weight reductions were observed in these dose groups for male rats and the 1200 ppm dose group in the females. This decrease in the denominator of the ratio can lead to an apparent increase in liver weight, but in all cases the liver to brain weight ratio was also increased confirming a relative increase in liver weight for these groups. In the Lington study 300 ppm and 600 ppm male and female DINP-treated rats had increased liver weight/body weight ratios starting at 6 months of treatment and continuing until

24 months of treatment, although there were some body weight decreases in the male 600 ppm group from 12 months on and in the female 300 ppm group at 24 months. The liver to brain weight ratio was not provided in this study. However, the mechanism leading to the liver weight increases in not clear. The cause for the increase in liver weight for the male rats is likely to be multifactorial. Given the presence of mononuclear cell leukemia and hepatocellular neoplasia, liver weight data is difficult to interpret. It should be noted that liver weights in male and female DINP-treated marmosets, which may be more representative of the human response, were not affected by 13 weeks of treatment with 2500 mg/kg/day of DINP (Webley, 1998).

Peroxisome proliferators have the ability to increase liver weight through stimulation of peroxisome proliferator-activated receptor (PPAR) alpha causing an increase in cell proliferation as well as expansion of cell volume through increased formation of peroxisomes (Cattley *et al*., 1998). This activity may provide an explanation for increases in liver weight. There are several publications demonstrating that DINP has peroxisome proliferator activity in mice (Kaufmann *et al*., 2002) and that it does not exhibit peroxisome proliferation activity in PPARα-null mice (Valles *et al*., 2003). DINP has also been shown to have modest peroxisomal proliferative capacity in rats (Smith *et al.,* 2000; Moore, 1998).

In the Moore study significant elevations in palmitoyl-CoA oxidase were observed in the 12,000 ppm DINP dose group male rats at all time points (weeks 1,2, 13, 104) and female rats at 6,000 ppm at 104 weeks. The main histologic finding in hepatocytes in the Moore study related to increased hepatocytes eosinophilia and increased hepatocytes size in the majority of male and female rats treated with 12,000 ppm, both of which are characteristic responses to peroxisome proliferators. These findings lead to the conclusion that peroxisome proliferation in the 12,000 ppm dose group is present. It is possible that peroxisome proliferation may play a role at the 6,000 ppm dose as well given the increase in palmitoyl-CoA oxidase levels in the liver of female rats in the Moore study. Therefore, in the high dose group (12,000 ppm) the increased liver weight may likely be attributed to peroxisome proliferation given the lack of significant vacuolization, congestion of other disturbances to account for liver mass increases. It should be noted that DINP has been shown to induce smooth endoplasmic reticulum (microsomes), which could contribute to the increased liver weight and can lead to increased cytoplasmic eosinophilia and modestly increase cell size (Bird *et al*., 1986). It is not clear that peroxisome proliferation affected the lower dose groups, as there was no evidence of peroxisome proliferation by morphological evaluation in the Lington study (maximum dose 6,000 ppm DINP) in any of the DINP-treated rats. Additional studies, provided as unpublished reports (Certa, 1993; Jansen *et al*., 1992) add some support for peroxisomal proliferation activity in rats treated with lower doses of DINP, but the changes in enzyme activity are relatively small and are not consistent between studies.

MNCL

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). 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).

The results of DINP chronic studies are consistent with these findings. MNCL was found in two studies in the F-344 rat (Lington *et al*., 1997; Moore *et al*., 1998a) but not in the B6C3F1 mouse (Moore et al., 1998b) or the Sprague-Dawley rat (Bio/dynamics, 1986). When assessing the significance of changes in MNCL incidence, points to consider include: (1) that the factors contributing to a high, variable, spontaneous incidence of MNCL in the F-344 rat are unknown; (2) that there are a number of factors which contribute to variability in MNCL frequency for unknown reasons – including the use of corn oil as a vehicle (Haseman *et al*., 1985), single vs. group housing (Haseman *et al*., 1998), splenic toxicity, lifespan, body weight and dietary fat (but not dietary restriction) (Elwell *et al*., 1996); and (3) that treatment with genotoxic agents that might logically be expected to increase the incidence of cancer in general have either no effect or actually reduce MNCL incidence (Waalkes *et al*., 1991; Lijinsky *et al*., 1993; Elwell *et al*., 1996).

A recent review of MNCL (Thomas *et al*., 2007) suggests that a weight of evidence approach be taken when statistically identified increases in MNCL occur with exposure. The authors propose similarities between F344 MNCL and human natural killer large granular cell leukemia (NK-LGL) based on functional, clinical and morphological characteristics, but emphasize that the mechanisms of leukemogenesis may be very different. Without further research to clarify the leukemic cell of origin, and define candidate molecular targets, the case for potential human relevance remains weak, particularly in light of the high, variable spontaneous incidence of MNCL in the Fischer 344 rat – the species in which MNCL was seen in conjunction with DINP administration.

PPARα-Independent Mechanisms of Tumorigenesis

The mechanism for liver tumor induction by phthalates seems most consistent with a PPARα-mediated mode of action (Klaunig *et al*., 2003). Other mechanisms for carcinogenicity in rodents are not supported by the data. Ito *et al*., 2007 have proposed an alternative mechanism for induction of liver tumors by DEHP that is independent of PPARα activation. The report compares the effects of long-term dietary exposure of up to 0.05% DEHP on liver toxicity of wild type and PPARα null (-/-) mice. The results presented must be carefully considered in light of the utility of the PPARα null mouse model used and additional reports indicating an inherent susceptibility of these mice to tumorigenesis.

Ito *et al*. reported the use of a PPARα -/- mouse strain produced according to a method published by Lee *et al*. (1995). These knockout mice had both PPARα alleles replaced using the homologous recombination technique. Four biological endpoints were assessed after 24 months of treatment; the endpoints were referred to as: macroscopic liver findings; microscopic liver findings; oxidative damage (8-OHdG levels) and proto-oncogene expression levels (mRNA and/or protein). A statistically significant increase in the number of liver tumors (i.e., hepatocellular carcinomas, hepatocellular adenomas, and chologiocellular carcinomas) from 2-8 (10-25.8%) was seen between the wild type and knockout mice fed the top dose DEHP diet ($p<0.05$). This was mostly due to a jump from 2 to 6 in hepatocellular adenoma incidence between these two

groups. It should be noted that statistical significance was reached only when the total numbers of tumors were combined. Ito *et al.* discuss the low number of tumors and report them to be a reflection on the relatively low doses of DEHP used in the study.

There was no significant effect reported on bodyweight or liver weight, though the data suggested a trend towards an increase in liver weight for the PPARα -/- animals, especially the 0.05% DEHP exposed group (+/+ mean = 1.27g ±0.18; -/- mean =1.78g±0.84). The reported 8-OHdG data suggest -/- mice suffered from an increased hepatic oxidative stress with DEHP as compared to the +/+ mice, though this was not supported by unchanged mRNA levels for 8-oxoguanine DNA-glycosylase 1, an 8-OHdG repair enzyme. As +/+ mice showed lower 8-OHdG levels than -/- mice at all DEHP exposure levels and in the controls (0% DEHP), PPARα may prevent the oxidation of DNA dG. Ito *et al*. did not address the plausibility of any biological relevance between raised 8-OHdG levels and increased incidence of liver tumors in -/- mice exposed to DEHP.

On the basis of their data, Ito *et al*., 2007 proposed an alternative mechanism for DEHP induced liver tumors, which is independent of PPARα activation in that inflammation and protooncogenes altered by 0.05% DEHP-derived oxidative stresses may be involved in the tumorigenesis found in the PPARα-null mice, but not in wild-type mice. However, the utility of these data is limited in that a number of reports have indicated that aged PPARα null mice are more vulnerable to tumorigenesis due to fundamental mechanistic differences (Mandard *et al*., 2004; Kostadinova *et al*., 2005; Balkwill and Couseens, 2005; Pikarsky *et al*., 2004; Takashima *et al*., 2008). Most recently, gene expression profiles of hepatocellular adenoma tissues as well as control livers of wild-type and PPARα null mice were examined (Takashima *et al*., 2008). The genes identified to contribute to tumorigenesis (i.e. Gadd45a and caspase 3-dependent apoptosis genes) in the null mice were unique to the null mice.

As spontaneous tumors are known to occur in the PPARα null mice at 24 months, Ito *et al.* indicate the possibility that DEHP merely promoted the formation of the spontaneous liver tumors in the aged null mice; a mechanism that is unique to the null mice and would not exist in the wild type mice. Importantly, with respect to DINP, literature searches reveal no reports that DINP induces production of reactive oxygen species in livers of rodents, humans or non-human primates, or in cultured liver cells from these species. 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.

Overall Conclusion

The primary findings in the repeated dose studies are effects in the liver and kidney. The main histologic lesion, spongiosis hepatis, should be regarded as a rat-specific lesion without a counterpart in human hepatic pathology. In rats, the effects in the liver, besides some minor and probably adaptive effects, are indicative of peroxisomal proliferation, including 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. PPARα-independent

mechanisms of tumorigenesis are not well supported by the limited studies currently available. Therefore, the available data do not support the conclusion that DINP is known to cause, or can reasonably be anticipated to cause, serious or irreversible liver toxicity in humans.