Comments on the Diisononyl Phthalate (DINP) No Significant Risk Level (NSRL) Derived by the Office of Environmental Health Hazard Assessment (OEHHA)

Comments Submitted to:

Office of Environmental Health Hazard Assessment
California EPA

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INTRODUCTION

On December 20, 2013, the State of California added diisononyl phthalate (DINP) to the list of chemicals known to the state to cause cancer (“Proposition 65 List”) (OEHHA 2015a). This listing was primarily due to evidence of carcinogenicity from multiple long-term studies in rodents exposed to DINP in the diet. No epidemiology studies were identified that investigated the risk of cancer associated with documented exposure to DINP.

As laid out by the California Office of Environmental Health Hazard Assessment (OEHHA), a No Significant Risk Level (NSRL) for a carcinogen or potential carcinogen is considered a safe harbor level and is equivalent to an exposure level that results in one (1) excess cancer in an exposed human population of 100,000 persons, assuming a lifetime exposure at the specified level (OEHHA 2011). Under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (“Proposition 65”), if an exposure to a carcinogen or potential carcinogen can be shown to be less than the specific NSRL or, in the case of reproductive toxicants, a Maximum Allowable Daily Level, the responsible person or organization is said to have “safe harbor” from the Proposition 65 warning requirement and drinking water discharge prohibition.

On January 2, 2015 the Office of Environmental Health Hazard Assessment (OEHHA) proposed a NSRL of 146 μg/day DINP based on the increased incidence of mononuclear cell leukemia (MNCL) and liver neoplasms in Fischer 344 rats (Lington et al., 1997; Moore 1998a) (OEHHA 2015b). After review of the occurrence and etiology of MNCL in Fischer 344 rats, as well as evaluation of the studies conducted by Lington et al. (1997) and Moore (1998a), ToxServices concludes that the increased incidence of MNCL in Fischer 344 rats following DINP treatment is not relevant for the assessment of human cancer risk. In this report, ToxServices reviews relevant DINP carcinogenicity data, with particular focus on the relevance of DINP-induced MNCL to human risk assessment, and recommends basing the NSRL on increased incidence of hepatic neoplasms in mice following chronic oral exposure to DINP.

OVERVIEW OF DINP CANCER STUDIES

The OEHHA report on the carcinogenicity of DINP cited six studies in male and female rats and mice (OEHHA 2013) consisting of: three dietary studies in Fischer 344 rats (Lington et al., 1997; Moore 1998a), one dietary study in Sprague-Dawley rats (Bio
dynamics 1986), and two dietary studies in B6C3F1 mice (Moore 1998b). These studies report statistically significant increases in multiple tumor types including hepatocellular tumors (adenomas, carcinomas, and combined adenomas and carcinomas), mononuclear cell leukemia (MNCL) of the spleen, and renal tubule cell carcinomas (See Table 1). They also report a number of rare tumor types including: renal transitional cell carcinoma, renal tubular cell carcinoma, pancreatic acinar cell carcinoma, testicular interstitial (Leydig) cell carcinoma, and uterine adenocarcinoma (OEHHA 2013).
Table 1: Summary of DINP Carcinogenicity Studies in Rodents

<table>
<thead>
<tr>
<th>Authors</th>
<th>Species/Strain</th>
<th>Design</th>
<th>Concentrations (ppm)</th>
<th>Mononuclear Cell Leukemia</th>
<th>Liver Tumors</th>
<th>Kidney Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lington et al. 1997</td>
<td>Rat/Fischer 344</td>
<td>Two-year dietary exposure with male and female (110/sex/dose) rats</td>
<td>0, 300, 3,000, and 6,000 ppm DINP</td>
<td>Significant increase in both sexes compared to controls (see Table 2)</td>
<td>Slight increase in both sexes compared to controls (not significant)</td>
<td>Renal tumors were observed in males (not significant). No renal tumors in females.</td>
</tr>
<tr>
<td>Moore 1998a</td>
<td>Rat/Fischer 344</td>
<td>Two-year dietary exposure with male and female (70-85/sex/dose) rats</td>
<td>0, 500, 1,500, 6,000 and 12,000 ppm DINP</td>
<td>Significant increase in both sexes compared to controls (see Table 2)</td>
<td>Significant increase in high dose males and females.</td>
<td>Renal tumors were observed in males (not significant). No renal tumors in females.</td>
</tr>
<tr>
<td>Moore 1998a</td>
<td>Rat/Fischer 344</td>
<td>78-week dietary exposure followed by a 26-week recovery with male and female (55/sex/dose) rats</td>
<td>0 and 12,000 ppm DINP</td>
<td>Significant increase in both sexes compared to controls (see Table 2)</td>
<td>No significant differences in males or females.</td>
<td>Significant increase in males compared to controls. No renal tumors in females.</td>
</tr>
<tr>
<td>Bioodynamics 1986</td>
<td>Rat/Sprague-Dawley</td>
<td>Two-year dietary exposure with male and female (70/sex/dose) rats</td>
<td>0, 500, 5,000 and 10,000 ppm DINP</td>
<td>None reported</td>
<td>Significant increase in female rats compared to controls.</td>
<td>None reported</td>
</tr>
<tr>
<td>Moore 1998b</td>
<td>Mouse/B6C3F₁</td>
<td>Two-year dietary exposure with male and female (70/sex/dose) mice</td>
<td>0, 500, 1,500, 4,000 and 8,000 ppm DINP</td>
<td>None reported</td>
<td>Significant increase in males and females compared to controls.</td>
<td>None reported</td>
</tr>
<tr>
<td>Moore 1998b</td>
<td>Mouse/B6C3F₁</td>
<td>78-week dietary exposure followed by a 26-week recovery with male and female (55/sex/dose) mice</td>
<td>0 and 8,000 ppm DINP</td>
<td>None reported</td>
<td>Significant increase in males and females compared to controls.</td>
<td>None reported</td>
</tr>
</tbody>
</table>
MNCL was significantly increased in male and female Fischer 344 rats in two studies (Lington et al. 1997; Moore 1998a). Lington et al. (1997) fed male and female Fischer 344 rats (110/sex/concentration) diets containing 0, 300, 3,000 or 6,000 ppm DINP (CAS# 68515-48-0, purity 99%) for 2 years. MNCL occurred in male rats (p<0.01) treated with 3,000 and 6,000 ppm and female rats (p<0.001) treated with 6,000 mg/kg/day at an incidence significantly higher than control animals (Fisher pairwise comparison) following a 2 year treatment (See Table 2). No MNCL was reported in male or female rats (10/concentration) at interim sacrifices at 6, 12, or 18 months (Lington et al. 1997). Moore (1998a) fed male and female Fischer 344 rats (70-85/sex/concentration) diets containing 0, 500, 1,500, 6,000 or 12,000 ppm DINP (CAS# 68515-48-0, purity 99%) for at least 2 years. MNCL occurred in male and female rats treated with 6,000 and 12,000 ppm at an incidence significantly (p<0.05) higher than control animals after treatment for 2 years (See Table 1). In a recovery study, rats (55/sex/concentration) were fed diets containing 0 or 12,000 ppm DINP (CAS# 68515-48-0, purity 99%) for 78-weeks followed by a 26-week recovery period during which animals received the control diet. MNCL occurred at an incidence in treated males (p<0.001) and females (p<0.01) significantly greater than the controls after the recovery period (Moore 1998a) (See Table 2).

<table>
<thead>
<tr>
<th>Authors</th>
<th>DINP dietary concentrations (ppm)</th>
<th>Incidence in Males</th>
<th>Incidence in Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lington et al. 1997</td>
<td>0</td>
<td>33/81 (41%)</td>
<td>22/81 (27%)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>28/80 (35%)</td>
<td>20/81 (25%)</td>
</tr>
<tr>
<td></td>
<td>3,000</td>
<td>48/80** (60%)</td>
<td>30/80 (38%)</td>
</tr>
<tr>
<td></td>
<td>6,000</td>
<td>51/80** (64%)</td>
<td>43/80*** (54%)</td>
</tr>
<tr>
<td>Moore 1998a</td>
<td>0</td>
<td>22/65 (34%)</td>
<td>17/65 (26%)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>23/55 (42%)</td>
<td>16/49 (33%)</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>21/55 (38%)</td>
<td>9/50 (18%)</td>
</tr>
<tr>
<td></td>
<td>6,000</td>
<td>32/65* (49%)</td>
<td>29/65* (45%)</td>
</tr>
<tr>
<td></td>
<td>12,000</td>
<td>30/65* (46%)</td>
<td>30/65* (46%)</td>
</tr>
<tr>
<td>Moore 1998a</td>
<td>0</td>
<td>22/65 (34%)</td>
<td>17/65 (26%)</td>
</tr>
<tr>
<td></td>
<td>12,000</td>
<td>31/50* (62%)</td>
<td>24/50* (48%)</td>
</tr>
</tbody>
</table>

Fisher pairwise comparison with controls: * p < 0.05, ** p < 0.01, *** p<0.001

OEHHA DINP NSRL

On January 2, 2015 OEHHA proposed a NSRL of 146 μg/day for DINP based on the increased incidence of MNCL and liver neoplasms in Fischer 344 rats following chronic dietary exposure to DINP (OEHHA 2015b).

RELEVANCE OF MONONUCLEAR CELL LEUKEMIA TO HUMAN RISK ASSESSMENT

Mononuclear cell leukemia (MNCL) is a spontaneous tumor type which originates in the spleen (Caldwell 1999). The mechanism for induction of MNCL is not currently understood. However, the available evidence indicates MNCL has an age-related genetic cause and the cellular origin of MNCL is hypothesized to be the natural killer (NK) cell (Thomas et al. 2007). Factors such as the vehicle used for administration, diet, housing, and splenic toxicity contribute to the
variability of MNCL in Fisher 344 rats (Elwell et al. 1996; Ishmael and Dugard 2006). In 1990, the International Agency for Research on Cancer (IARC) categorized MNCL as “an unclassified leukemia with no human counterpart” and chemicals which induce MNCL as “not classifiable as to carcinogenicity in humans” (IARC 1990). More recently, Thomas et al. (2007) suggested that MNCL is similar to the rare human tumor NK-large granular lymphocyte leukemia (NK-LGLL), and indicated that while there are similarities between MNCL in Fisher 344 rats and NK-LGLL in humans, the mechanisms may be different.

MNCL is among the most common causes of natural death in Fischer 344 rats (Losco and Ward 1984). It does not occur in mice or hamsters and it is uncommon in other rat strains (Caldwell 1999). Dinse et al. (2010) found a statistically significant difference in the incidence of MNCL between control female Fischer 344 rats and control female Sprague-Dawley rats (16.7% vs 0.97%). In 1998, Haseman et al. reported MNCL as the second most frequently occurring spontaneous neoplasm in untreated male Fischer 344 rats in 2-year carcinogenicity studies carried out by the National Toxicology Program (NTP). MNCL was present in 50.5% of untreated male Fischer 344 rats (range = 32 – 72%) and 28.1% of untreated female Fischer 344 rats (range = 14 – 52%) (Haseman et al. 1998). These rates have doubled from those reported by in 1985 (Haseman et al. 1985) and they continue to rise (Haseman et al. 1990; Haseman et al. 1998; Haseman 2003). Because of the high background rate and variability of MNCL in Fischer 344 rats, NTP decided to discontinue the use of Fischer 344 rats and instead use Sprague-Dawley rats in their bioassays (Dinse et al. 2010). As high background rates contribute to false positive findings in long-term studies with Fischer 344 rats, Thomas et al. (2007) suggested increasing the statistical stringency (i.e., p < 0.01 instead of p < 0.05) when performing analyses. While increased statistical stringency can be used as an indicator of chemical-induced MNCL, Thomas et al. (2007) emphasized that a “weight-of-evidence” approach must be taken when evaluating possible chemically-induced MNCL in Fischer 344 rats.

Another important factor to consider is the reproducibility of chemical-induced MNCL; there have been reported inconsistencies in chemical-induced MNCL between separate studies using the same or similar exposure concentrations (NTP 1997; NTP 1982; Moore 1996; Wood et al. 1991) and between male and female rats within the same study (NTP 1993). For example, inconsistencies were found in two NTP studies using butyl benzyl phthalate (BBP). In the first study, BBP treatment produced a significant increase in the incidence of MNCL in female Fischer 344 rats, while no significant differences were found in the second study which used the same concentrations of BBP with Fischer 344 rats (NTP 1997). Similar findings were reported in long-term feeding studies using di(2-ethylhexyl) phthalate (DEHP). Treatment with concentrations up to 12,000 ppm DEHP produced no significant changes in the incidence of MNCL in Fischer 344 rats of either sex (NTP 1982); whereas treatment with concentrations of 0.25% (2,500 ppm) and higher produced an increase of MNCL in male Fischer 344 rats (Moore 1996).

Due to the high background rate and variability of MNCL in Fischer 344 rats, many researchers have concluded that it is not relevant to humans (Caldwell 1999; CPSC 2010; NICNAS 2012; Ishmael and Dugard 2006). Caldwell (1999) reviewed the literature of alkyl phthalate-induced MNCL in Fischer 344 rats and concluded that “the increased incidence of MNCL in Fischer 344 rats is likely strain-specific with little or no relevance in humans”, even when the increase is
statistically significant. Following a review of the incidence of MNCL in Fischer 344 rats, Ishmael and Dugard (2006) came to a similar conclusion as Caldwell (1999) and reported that MNCL in Fischer 344 rats is “unsuitable for the calculation of cancer potency values for man”. CPSC (2010) and NICNAS (2012) reviewed the DINP cancer studies and concluded the increased incidence of MNCL in Fischer 344 rats is not relevant to humans. ECHA (2013) and Caldwell (1999) suggest that MNCL follows a threshold mode of action which is likely above the No-Observed-Adverse-Effect-Level (NOAEL) for repeated dose toxicity; indicating a margin of safety approach should be taken when assessing risk. ECHA has used published oral and dermal Derived-No-Effect-Level (DNEL) values (ECHA 2013), while NICNAS has taken a margin of exposure (MOE) approach to characterize risk associated with DINP exposure (NICNAS 2012).

EVALUATION OF DINP-INDUCED MNCL IN CARCINOGENICITY STUDIES CITED BY OEHHA

The carcinogenicity studies conducted with DINP demonstrate that the increase of MNCL is confined to studies using Fischer 344 rats (See Table 1; Lington et al. 2007; Moore 1998a). The absence of an increased incidence of MNCL in Sprague-Dawley rats (Bio
dynamics 1986) at concentrations similar to those which produced an increase in Fischer 344 rats reduces the significance of DINP-induced MNCL for human risk assessment.

Although DINP appeared to induce a concentration-dependent increase of MNCL in Fisher rats of both sexes in the Lington et al. (1997) study, the study authors concluded that the increased incidence of MNCL is not relevant to humans. The study authors indicated that while the incidence of MNCL is increased in DINP-treated animals, the increase may not be chemical-dependent. Lington et al. (1997) report that the incidence of MNCL in DINP-treated Fischer 344 rats (males = 35 – 64%, females = 25 – 54%; See Table 2) is within (males) or similar to (females) the historical range of MNCL for untreated Fischer 344 rats in NTP studies (males = 32 – 72%, females = 14 – 52%; Haseman et al. 1998).

In the Moore (1998a) 2-year treatment study, DINP-treatment produced a significant increase in the incidence of MNCL in male and female rats treated with 6,000 and 12,000 ppm DINP; however, the increased incidence of MNCL does not appear to be concentration-dependent (see Table 2). Furthermore, the reported incidences of MNCL in male and females rats in the Moore 1998a studies (2-year treatment: males = 42-49%, females = 18 – 46%; recovery study: males = 62%, females = 48%; See Table 2) are within the historical range for untreated Fischer 344 rats in NTP studies (Haseman et al. 1998).

Finally, evaluation of the Fischer 344 rat studies with increased statistical stringency (i.e., p < 0.01 instead of p < 0.05) as suggested by Thomas et al. (2007) (discussed above), found that the DINP-induced increase in MNCL in the Moore (1998a) studies does not meet this criterion of statistical significance (see Table 2). Therefore, the study performed by Lington et al. (1997) remains the only study which has a significant increase (p < 0.01) in MNCL following DINP treatment in Fischer 344 rats.
Critical evaluation of the Lington et al. (1997) and Moore (1998a) studies has identified numerous sources of uncertainty which reduce the relevance of DINP-induced MNCL in Fischer 344 rats for human risk assessment. The relevance of DINP-induced MNCL in Fischer 344 rats to human risk assessment is questioned because (1) the DINP-induced increase in MNCL is confined to Fischer 344 rats; (2) DINP-induced effects in the Moore (1998a) 2-year study do not appear to occur in a concentration-dependent manner and they do not withstand increased statistical stringency (p < 0.01); and (3) reported incidences of MNCL in DINP-treated Fischer 344 rats are within or are very similar to the historical range of MNCL in untreated Fischer 344 rats in NTP studies.

SUMMARY

The relevance of DINP-induced MNCL to humans is questionable due to its high and variable background rate in Fischer 344 rats. In 1990, IARC classified MNCL as a leukemia with no human counterpart and concluded that chemicals which induce MNCL should not be classified as human carcinogens. Since the classification by IARC in 1990, MNCL has been found to have similarities to NK-LGLL in humans, although the mechanisms may be different.

Due to the high background rate of MNCL in Fischer 344 rats, evaluation of the data with increased statistical stringency (i.e., p < 0.01 instead of p < 0.05) may reduce the probability of false positive findings. Increased statistical stringency together with a weight of evidence approach has been recommended when evaluating chemical-induced MNCL in Fischer 344 rats.

In addition to the high background rate and variability of MNCL, data interpretation is complicated by the lack of reproducibility of chemical-induced MNCL in Fischer 344 rats. Long-term studies using phthalates (BBP and DEHP) have reported inconsistencies in phthalate-induced MNCL. Two long-term feeding studies using the same concentrations of BBP found a significant increase in MNCL in female Fischer 344 rats in one study, but not the other. Similar results were reported in long-term studies using DEHP.

Following review of the incidence of alkyl phthalate-induced MNCL in Fischer 344 rats, Caldwell (1999) concluded that an increased incidence of MNCL in Fischer 344 rats is not relevant to humans. Ishmael and Dugard (2006) concluded that due to the

Evaluation of DINP-induced MNCL in carcinogenicity studies cited by OEHHA found several sources of uncertainty in the interpretation of MNCL data. Long-term dietary treatment with DINP produced an increase in MNCL which was confined to two studies (i.e., Lington et al. 1997 and Moore 1998a) using Fischer 344 rats; no MNCL was reported in studies using Sprague-Dawley rats. The increase in MNCL appeared to be dose-dependent in the Lington et al. (1997) study; however, no dose-dependent effects were seen in the Moore (1998a) study. Application of increased statistical stringency (i.e., p < 0.01 instead of p < 0.05) found that only the Lington et al. (1997) study meets this criterion. Furthermore, the reported incidences of DINP-induced MNCL in the Lington et al. (1997) and Moore (1998a) are within or similar to the historical range for untreated Fischer 344 rats in NTP studies around the time the DINP studies took place.
CONCLUSIONS

ToxServices reviewed the occurrence and etiology of MNCL in Fischer 344 rats, and critically evaluated the DINP studies conducted by Lington et al. (1997) and Moore (1998a) in Fischer 344 rats. We found that the reported incidences of DINP-induced MNCL were within or were similar to the historical range for untreated Fischer 344 rats in NTP studies. In the Moore (1998a) 2-year study, DINP treatment was reported to induce a statistically significant (p < 0.05) increase in the incidence of MNCL; however, the effects did not appear to occur in a dose-dependent manner and they did not withstand increased statistical stringency (p < 0.01). Based on the uncertainties identified in the DINP studies using Fischer 344 rats, ToxServices concludes that the reported increase in MNCL incidence does not provide an adequate basis for human risk assessment.

ToxServices suggests that OEHHA consider reevaluating the proposed DINP NSRL of 146 μg/day which is based on the increased incidence of MNCL and hepatic neoplasms in Fischer 344 rats. If OEHHA must derive a NSRL, we suggest that the NSRL be based on the increased incidence of hepatic neoplasms in mice following chronic oral exposure to DINP. We performed a cancer risk assessment for DINP according to steps outlined in Title 27, Division 4, Chapter 1: Safe Drinking Water and Toxic Enforcement Act of 1986, Article 7. No Significant Risk Levels, Proposition 65, Section 25703 (Quantitative Risk Assessment) (OEHHA 2011) and calculated a NSRL of 280 μg/day based on the increased incidence of combined hepatocellular carcinoma and adenoma in male mice (Ciotti et al. 2015). We suggest that OEHHA allow industries to use a NSRL of 280 μg/day in cancer risk assessments in order to comply with Proposition 65 in the State of California.
REFERENCES

Bio


