Comments on the Proposed No Significant Risk Level Documentation for DINP

Recently the State of California proposed a regulatory amendment to adopt a No Significant Risk Level (NSRL) for diisononyl phthalate (DINP) of 146 micrograms per day (µg/day) (OEHHA, 2015). This value was based on liver tumor incidence data from studies in rats and mice as well as increased frequencies of mononuclear cell leukemia (MNCL) from studies in Fischer 344 (F-344) rats and calculated following a linearized multistage model.

In this letter ExxonMobil Chemical Company has comments on four specific points:

(1) We do not consider the types of tumors associated with DINP treatment to be relevant to humans;
(2) We are particularly concerned about the use of MNCL data for this purpose as we do not consider the data appropriate for this specific application;
(3) We do not agree that the underlying assumptions required by the linearized multistage model have been satisfied by the data; and
(4) We are concerned that the approach taken to calculate an NSRL for DINP deviates so much from an earlier calculation of an NSRL for DEHP (June, 2001). This suggests not only inconsistent treatment, but an inexplicable elevated level of concern regarding exposures to DINP by comparison to DEHP.

More specifically:

Underlying Tumor Response Data

As summarized by OEHHA (October, 2013), there is no epidemiological data suggesting a role for DINP in human cancer; rather all of the data from which the decision to list DINP as carcinogenic under California Proposition 65 come from studies in rats and mice, specifically a two year study in F-344 rats (Lington et al., 1997), a two year study in F-344 rats and B6C3F1 mice (Moore et al., 1998a; 1998b), and a two year study in Sprague-Dawley rats (Bio/dynamics, 1986). Lington et al. (1997) reported statistically significant increases in MNCL in male F344 rats at the mid (3000 ppm) and high (6000 ppm) dietary levels and in female F344 rats at the high (6000 ppm) dietary level. No other tumor type was significantly elevated, and the lowest dietary group (300 ppm) was a no effect level. The doses were estimated to have been approximately 15, 150 and 300 mg/kg/day. Moore et al. (1998a) reported statistically significant increases in hepatocellular adenomas and/or carcinomas in male and female F344 rats in the high (12000 ppm) dietary group as well as significant increases in MNCL in the mid (6000) and high (12000) dietary groups. Two lower levels (500 and 1500 ppm) were no effect levels. No other tumor types were significantly elevated after two years of exposure. However, renal tubular carcinoma was significantly elevated in male rats in the recovery group in which they had been given DINP in the diet for 18 months and then held for an additional 26 weeks without treatment before terminal sacrifice. These

dietary levels corresponded to approximately 30, 90, 360 and 730 mg/kg/day. In Bio/dynamics (1986), significant increases in hepatocellular carcinoma were reported in female SD rats given DINP in the diet at levels of 5000 or 10000 ppm. The no effect level in female rats was 500 ppm. There were no significant elevations in tumors in male SD rats at any dose. These treatment levels correspond to approximately 30, 300 and 600 mg/kg/day. In studies in mice (Moore et al., 1998b), there were significant increases in frequencies of hepatocellular adenoma and carcinoma in male and female mice at dietary levels of 4000 and 8000 ppm. There was also a statistically significant increase in hepatocellular adenomas and carcinomas in female mice in the 1500 ppm group. Frequencies of liver tumors were not significantly elevated in the 500 ppm group, and no other tumor type was significantly elevated in this study. These treatment levels correspond to approximately 100, 300, 800, and 1700 mg/kg/day.

In summary, liver tumors, specifically hepatocellular adenomas and/or carcinomas were significantly elevated at approximately similar dietary levels in male and female F344 and SD rats and in male and female B6C3F1 mice. MNCL was significantly elevated in male and female F344 rats but not in SD rats or B6C3F1 mice, a pattern which is consistent with the species and strain specificity of this tumor type. The increased frequency of renal tubular cell carcinoma was found only in male rats, a pattern which is consistent with the species and sex specificity of this lesion. There were other tumors reported in some other studies, but as none of these were significantly elevated, the normal scientific approach would be to assume that they were not associated with treatment.

Specific Comments:

(1) The treatment-related tumors were not relevant to humans

(i) **Liver Tumors** – Concurrent with and subsequent to the studies by Moore et al. (1998a; 1998b), studies were conducted to define the underlying mechanism for liver tumor induction. The pathological information suggested that the tumors were associated with peroxisomal proliferation (or more precisely with activation of the PPARα receptor). The studies, which are described in more detail in previous comments by ExxonMobil Chemical (2013), included investigations of the dose-response relationships of indicators of PPARα activation, an investigation of receptor binding affinity, and studies showing that these changes do not occur in PPARα-deficient mice, in primates exposed under *in vivo* condition, or in human or primate cells exposed under *in vitro* conditions. DINP has also been shown to be non-genotoxic. In short, clear and complete evidence was provided to show that the rodent liver tumors were the consequence of a PPARα-mediated process. It is the consensus view by experts in the field that substances that cause tumors through a PPARα-mediated process are highly unlikely to be relevant to humans (Klaunig et al. 2003; Corton et al., 2013).

OEHHA (2013) speculated that there could be other mechanisms to explain tumor formation including activation of AhR, PXR, CAR, or inhibition of gap

junctional intracellular communication (GJIC). In previous comments (ExxonMobil Chemical, 2013) it was explained why these alternative mechanisms were either not different from the PPARα mode of action or simply not plausible. The "not plausible" argument was strengthened by data from a recent publication by Wood et al (2014) in which it was shown that DEHP treatment does not result in sustained increases in either CAR or AhR. In fact there is no plausible mode of action other than a PPARα mediated response to account for the increased frequencies of hepatocellular tumors in rats and mice.

(ii) **Kidney Tumors** – As summarized in the background section, there was a small but statistically significant increase in the frequency of renal tubular carcinoma in male F344 rats in one study. The species and sex specificity of this finding suggested that it was an α2u-globulin-mediated tumor which is not relevant to humans (EPA, 1991; Swenberg and McKeeman, 1998). Initial investigations provided information satisfying the EPA criteria (Caldwell et al., 1998) and subsequent studies provided information satisfying the additional criteria established by IARC (Swenberg, 2001 and attached abstract from Schoonhoven et al., 2001). OEHHA (2013) did not provide any technical justification to support its decision to not dismiss these tumors as not human relevant. Rather, the focus of the OEHHA (2013) summary was on the much narrower question of whether the IARC criteria had been fulfilled, without reference to the EPA criteria. In preparation for the HID meeting, the relevant data were reviewed by Dr. Gordon Hard, one of the leading experts in this field, who confirmed that indeed the kidney tumors were the result of an α2u-globulin-mediated process (Hard, 2013).

(iii) **Mononuclear Cell Leukemia (MNCL)** – MNCL is a spontaneous, commonly occurring acute leukemia that is a prominent cause of mortality in F344 rats over 18 months of age (Stromberg, 1985; Ward et al., 1990 Thomas et al., 2007), but it is rare in other rat strains and has not been reported in mice or other species. In untreated F344 rats, MNCL frequencies are reported to vary between 32% and 74% for males and 14%-52% for females with highly variable rates between studies and laboratories (Haseman et al., 1998; 2003; Thomas, 2007). As summarized by King-Herbert and Thayer (2006), "when a statistically significant tumor effect is found in test animals relative to concurrent controls, the effect may not be considered exposure-related if it falls within the range observed in historical controls" citing Haseman et al. (1990). As the data reported in the Lington (1997) and Moore et al. (1998a) studies are compatible with the historical control ranges for both sex of F344 rats, the most reasonable interpretation is that the differences are simply fluctuations in the spontaneous incidence.

Further, we believe that F344 MNCL is not a relevant model for human disease. It had been previously proposed that F344 MNCL was a relevant model for two lymphoproliferative disorders in humans, T-cell Large

Granulocytic Leukemia (LGL) and/or aggressive NK-cell leukemia (ANKL) based on morphology of cell types (e.g., Thomas et al., 2007). However, the normal human counterpart for human T-cell LGL is a CD8-positive T lymphocyte, not an NK cell which is the presumed cell of origin for F344 MNCL. ANKL in humans is associated with Epstein-Barr virus (Ryder et al., 2006), a unique etiology which is not found in F344 rats. Thus, the most current information substantiates the previous information provided by the ExxonMobil Chemical Company (2013) on this subject, i.e., that MNCL is a high frequency spontaneous disorder in F344 rats and not relevant to humans (Irons, 2015).

(2) We are particularly concerned about the use of MNCL data for the purpose of NSRL development as we do not consider the data appropriate for this specific application.

"Presented with data from several animal studies, the risk assessor first seeks to identify the animal model that is most relevant to humans, based on the most defensible biological rationale (for instance, using comparative pharmacokinetic data). In the absence of a clearly most relevant species, the most sensitive species (i.e., the species showing a toxic effect at the lowest administered dose) is used by risk assessors at USEPA, since there is no assurance that humans are not at least as innately sensitive as the most sensitive species tested" (Barnes and Dourson, 1988). In contrast to that guidance, mononuclear cell leukemia (MNCL) which is found at a high and variable frequency in aging F344 rats but only rarely in other rat strains and not in mice and does not have a common matching human counterpart, is not relevant to humans. As discussed above, the human disorder most similar to MNCL is aggressive NK cell leukemia, but the human disease is associated with Epstein-Barr virus, and thus has a different etiology than MNCL in F344 rats. In summary, MNCL is a F344-specific disease and not relevant to humans.

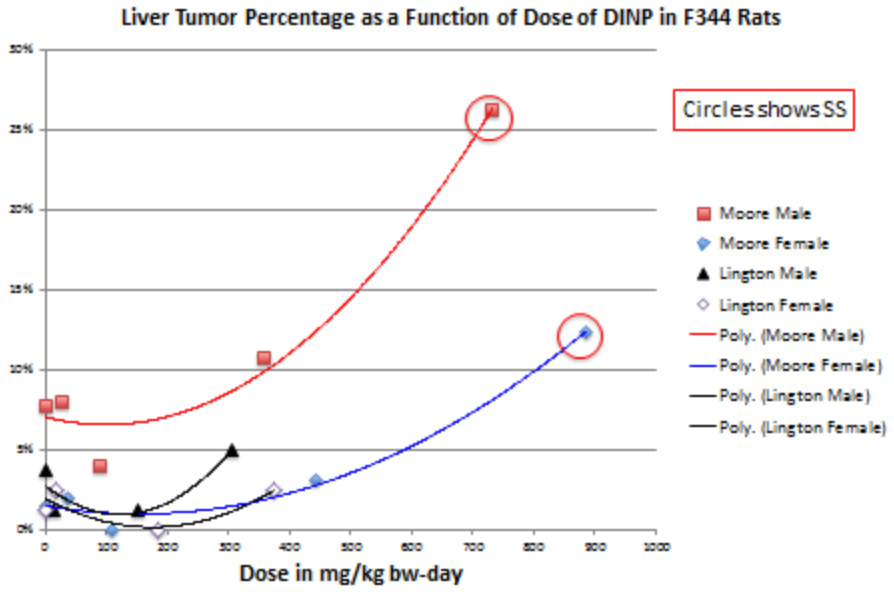
Further, there is no evidence that MNCL results from a genotoxic process and/or is the consequence of a receptor-mediated mode of action. In fact, quite the contrary, the observations that substantial differences in MNCL rate are associated with different laboratories, animal holding conditions, and methods of dose administration as discussed in more detail in Thomas et al. (2007) suggest that the differences in MNCL frequency could be related to stress. Regardless of the underlying process, because MNCL is a spontaneous aging lesion in the F344, the tumor frequency is ultimately a consequence of the lifespan of the animals, and largely unrelated, or at least not directly related to chemical treatment. It was largely a consequence of this substantial variation in MNCL frequency that led to the decision by the NTP to move away from the F344 rat. This being the case, the data are unsuited for statistical modeling.

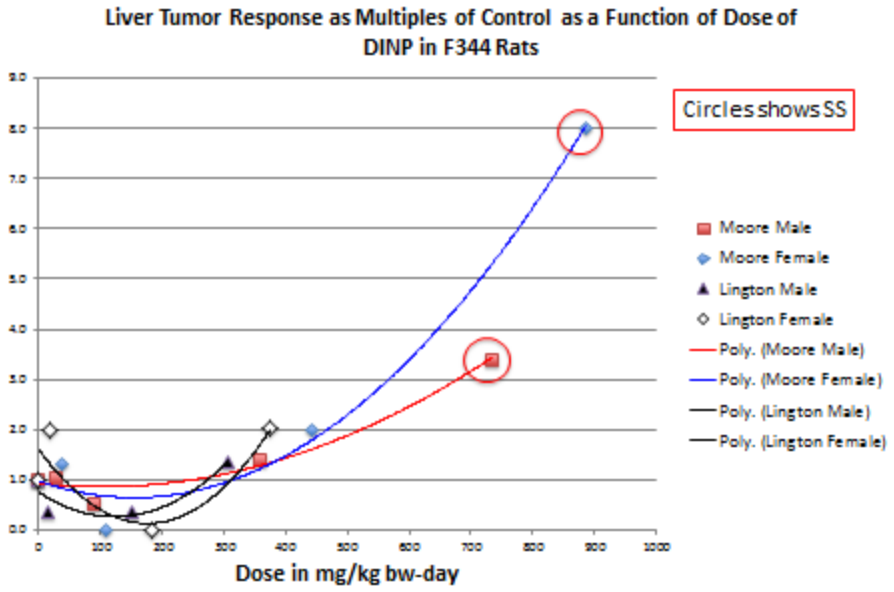
(3) We do not agree that the underlying assumptions required by the linearized multistage model have been satisfied by the data.

As summarized in the NSRL calculation for DEHP (OEHHA, 2001), there are several lines of evidence to justify the use of a linear model to extrapolate risk to rodents from low dose exposure; specifically, evidence of genotoxic potential; the absence of evidence of thresholds in cancer bioassays; and/or evidence that the tumorigenic process is receptor-mediated.

As summarized in the HID for DINP (OEHHA, 2013), DINP has been tested for gene mutation in bacteria and mammalian cells under *in vitro* conditions, for chromosome aberrations under *in vitro* conditions and for induction of micronucleus formation in mice under *in vivo* conditions. None of these studies provided evidence of genotoxic potential. In its report OEHHA (2013) acknowledges that there is no evidence that DINP is genotoxic but speculates that had other tests been performed, evidence of genotoxic potential might have been obtained. The battery of genotoxity tests that was conducted is consistent with requirements by regulatory authorities around the world to characterize the genotoxic potential of test substances, and in all of the previously conducted reviews, DINP has been judged to be non-genotoxic and no evidence of genotoxic potential was provided in the HID. In summary, as there is no evidence that DINP is genotoxic, the first requirement test for the use of a linearized-multistage model was not satisfied.

As shown in the following examples, dose response curves for the liver tumors provide empirical evidence of thresholds. Accordingly, as the data are not well fit by linear models, the second criteria is not met (note that the circles indicate the points that are different from control values by statistical criteria, i.e., ss is statistically significant).





Finally, in Table 14 of the HID, OEHHA (2013) summarizes the information on mode of action. With respect to DINP and liver tumor induction it is clear that DINP is a weak agonist for PPARα (which is the most plausible mode of action) and PPARγ (for which activation has not been plausibly associated with tumor induction) (e.g., Bility et al., 2004). Among the other categories of information related to mode of action, summarized in table 14, the first three columns indicate that there is no evidence to support a genotoxic

mechanism. The fourth column summarizes data from *in vitro* cell transformation assays and is designated as +/- based on a statistically significant result in one of nine assays. Certainly from a weight of evidence perspective, this outcome should be judged as a negative response, but even if the single positive result were judged to be evidence of *in vitro* transforming activity, it should be noted that the *in vitro* cell transformation assay is considered to detect both genotoxic and non-genotoxic carcinogens (OECD, 2007), and so, provides no support for an underlying genotoxic mechanism. The fifth column relates to activation of PPARα and γ, discussed above. The sixth column relates estrogen receptor activation. DINP was shown to be a weak agonist for the estrogen receptor under *in vitro* conditions (Harris et al., 1997) but this has no plausible relevance to a tumorigenic process as under *in vivo* conditions DINP is converted to a monoester metabolite (MINP) which does not bind to the estrogen receptor (Zacharewski et al., 1998). Similarly, with respect to the 11th column, DINP (or more precisely MINP) does not interact with the androgen receptor (McKee et al., 2004). As summarized reported in the HID, DINP does not interact with the Aromatic Hydrocarbon (AH) receptor (the 7th column). As recently shown (Wood et al., 2014), the evidence that CAR and PXR activation are not sustained in chronic administration studies with DEHP indicates that these processes are not plausibly associated with phthalate-induced tumor induction. Finally, as shown in the 10th column, Inhibition of gap junctional intracellular Communication (GJIC) is associated with DINP treatment but is not a receptor mediated phenomenon, and not independent of PPARα induction (Klaunig et al., 2003; Corton et al., 2014). In short, PPARα activation is the only plausible mode of action for rodent liver tumor induction.

There is no plausible receptor-mediated mode of action for MNCL and no plausible receptor-mediated mode of action for liver tumor induction other than PPARα induction. Thus, as DINP is (i) not genotoxic, (ii) exhibits non-linear dose response relationships for tumor induction, and (iii), other than PPARα agonism as an underlying mode of action for liver tumor induction, does not induce tumors via receptor-processes. There is no justification for a linearized multi-stage model for MNCL, and such a model could only be justified for use with the liver tumor data if OEHHA agrees that these tumors are the consequence of a PPARα-mediated process.

The conclusion by OEHHA that "[T]here are not principles or assumptions scientifically more appropriate [than the linearized multi-stage model] based on the available data" is superficial and presented with no discussion of the supporting information, presumably as, in fact, this conclusion is not scientifically justified.

(4) **We** are concerned that the approach taken to calculate an NSRL for DINP is so different from that previously taken to calculate an NSRL for di-(2ethylhexyl) phthalate (June, 2001).

In 2001 OEHHA calculated a human-extrapolated animal potency value of 0.0023 $(mg/kg\text{-}day)^{-1}$ for DEHP and calculated a no significant risk level of 300 μg/day as the oral intake level associated with a lifetime cancer risk of 10^{-5} for DEHP for a 70 kg adult. In making this calculation OEHHA assumed that the most plausible mode of action for liver tumor induction was a PPARα-mediated response. Further, OEHHA included a 10

fold scaling factor, which was justified by differences in receptor density. The liver tumor response data were modeled using a linearized multistage model which they justified with three lines of evidence: (i) there was no evidence of a threshold in the dose-response relationship; (ii) the carcinogenic process appeared to be based on a PPARα-mediated mode of action; and (iii) there was evidence from *in vitro* studies suggesting that DEHP had genotoxic potential. A significant increase in MNCL was summarized in the report but not used in the calculation.

In comparison, and as summarized in preceding sections:

(a) Exposure to DINP increased liver tumor frequencies in rats and mice, but only at levels higher than those associated with statistically significant increases in the DEHP study. For example, the DEHP calculation is based on tumor response data for female mice in which the lowest dietary level associated with a statistically significant level in tumor induction was 500 ppm whereas in the DINP studies, the lowest level was 1500 ppm. So, for liver tumor induction data, DEHP is 2-3x as potent as DINP.
(b) A PPARα-mediated role for liver tumor induction has been much more rigorously described and is the consensus of experts in the field (Klaunig et al., 2003; Corton et al., 2014) and has been shown by the most recent investigations of DEHP to be the only plausible explanation for these data (Wood et al., 2014).
(c) The evidence for qualitative differences between humans and rodents has also been more strongly supported. In addition to the differences in receptor density that were described in the report detailing the derivation of the NSRL for DEHP (OEHHA, 2001), there is now evidence for differences in receptor agonism (Bility et al., 2004). The scientific consensus is that humans are so much less sensitive to PPARα agonists than rodents that there could be no risk under any reasonable exposure situation (Corton et al., 2014). With this much greater level of knowledge, it not clear why a scaling factor was not used with the DINP data.
(d) The justification for the use of a linearized multi-stage model to estimate risks of cancer from DINP is much weaker than that for DEHP. DINP is not genotoxic, and the tumor-response data seem more likely associated with a threshold than the alternative. Following the logic in the DEHP document, a model of this type could only be justified if it were agreed that the liver tumors were the consequence of a PPARα-mediated process as no other plausible mechanism is associated with a receptor-mediated process.
(e) The MNCL data were not considered in the DEHP assessment but used in the DINP assessment. As the data are very similar in the DEHP and DINP studies, it is difficult to understand why they were used in one case but not the other. This is particularly puzzling given the recent information that the human cancer considered most likely to be similar to MNCL, aggressive NK-cell leukemia in humans has been shown to be associated with Epstein-Barr infection (Riley et al., 2006). This differentiates the human disorder from MNCL in rodents for which the etiology is entirely different (Irons, 2015).

It is difficult to understand why OEHHA used a linearized model and included the MNCL data, but did not use any scaling factors in calculating a NSRL for DINP of 146

μg/kg/day. The toxicological data provide clear evidence that although DINP does produce liver tumors in rats and mice but only at doses higher than those at which these effects are produced by DEHP. Taking the NSRL calculations at face value, one would conclude that DINP poses a greater cancer risk than DEHP, but this is completely at odds with the facts. Had OEHHA calculated a value following the procedures used for DEHP, and as recommended by the American Chemistry Council (attached herein), the outcome would have been much more compatible with the experimental data.

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Attachment 1. Comments on the Proposed California Proposition 65 NSRL Derived from Cancer Studies on DINP

Diisononyl Phthalate (DINP) Proposed Safe Harbor Level under California Proposition 65

Summary

Diisononyl phthalate (DINP) was added to the California Proposition 65 list as 'known to the State to cause cancer' on December 20th, 2013.[1] The listing becomes effective after December 20, 2014 relative to warnings and discharge discharges to drinking water sources. OEHHA has established safe harbor no significant risk levels for various chemicals.[2]

This document describes the derivation of the No Significant Risk Level (NSRL) for DINP, in accordance with California Code of Regulations Sections 25701 – 25703. This approach assumes that rodent study data were adequate basis for the listing and are such a relevant basis for this derivation. It is noted that the listing basis did not include identification of DINP as a known human carcinogen.

Using OEHHA methodology and precedent, the NSRL for DINP has been derived from the most sensitive study. Based on male mouse liver effects, the most conservative NSRL for DINP would be 2664µg/day.

[1] See: http://www.oehha.org/prop65/CRNR_notices/list_changes/122013P65list.html

[2] See: http://www.oehha.ca.gov/prop65/getNSRLs.html

Introduction

Diisononyl phthalate (DINP) was added to the California Proposition 65 list as 'known to the State to cause cancer' on December 20th, 2013.[3] The listing becomes effective after December 20, 2014 relative to warnings and discharge discharges to drinking water sources. OEHHA has established safe harbor no significant risk levels for various chemicals. [4]

This document describes the derivation of the No Significant Risk Level (NSRL) for DINP, in accordance with California Code of Regulations Sections 25701 – 25703. This approach assumes that rodent study data were adequate basis for the listing and are such a relevant basis for this derivation. It is noted that the listing basis did not include identification of DINP as a known human carcinogen.[5]

Applicable standards

The California Code of Regulations Section 25701 provides that "*The determination of whether a level of exposure . . . poses no significant risk . . . shall be based on evidence and standards of comparable scientific validity to the evidence and standards which form the scientific basis for the listing of the chemical . . .*" [6]

Further, Section 25703 provides that a "*Risk analysis shall be based on the most sensitive study deemed to be of sufficient quality.*"

DINP studies

The most sensitive study would be identified based on based on cancer-slope fit. In the case of DINP, several pertinent rodent studies exist. The rodent liver tumor response provides the most consistent response across sex, species and strains in the life time bioassays.

- Two chronic bioassays were conducted in male and female Fischer 344 rats (Lington et al. 1997, Moore 1998a).
- One bioassay was conducted in male and female Sprague-Dawley rats (Biodymanics 1986).
- Finally a chronic bioassay was conducted in male and female B6C3F1 mice (Moore 1998b).

To determine concordance of response across the bioassays, liver tumor response in male Fischer 344 rats from the Lington et al., and Moore studies was combined, and cancer slope model fit was assessed. The combination of the data provided a good fit to model, indicating the treatment related nature of the response. Given the consistency of response and the ability to model the combined data sets No Significant Risk Levels (NSRL) for were determined using rodent liver tumor data and methodologies proscribed by the State of California (2013a).

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[3] See: http://www.oehha.org/prop65/CRNR_notices/list_changes/122013P65list.html

[4] See: http://www.oehha.ca.gov/prop65/getNSRLs.html

[5] If the mechanism of action is not relevant to humans, the listing should not have occurred (CIC Criteria Document at Section I.D) and, if listed, the NSRL would be that there is no significant risk to humans.

[6] Section 25701, available at: http://oehha.ca.gov/prop65/law/pdf_zip/RegsArt7.pdf

Derivation of animal cancer potency factors

Under Section 25703, the State indicates that to conduct a quantitative risk assessment, use of a linearized multistage such as the US EPA methodology for cancer potency factor derivation is the default approach.[7] In this case, USEPA's Benchmark Dose Software was used.[8] The 95% lower-bound confidence limit on the benchmark dose (BMDL) for the liver data was determined using the multi-stage cancer model in the most current version (v 2.4) of this tool. The model was run using a polynomial term between 1 and 2; the BMDL (mg/kg-day) was selected from among the models that adequately fit the data (P-value > 0.1) by choosing the model (and associated BMDL) with the lowest Akaike's Information Criterion (AIC) score.

Table 1. Rodent liver tumor potency estimates from primary cancer studies of DINP in rodents

Study	Sex	Dose Concentration (mg/kg feed)	Calculated Intake (mg/kg day)	Combined liver tumors	q1* $(mg/kg/day)^{-1}$
Rat Studies					
Lington et al 1997	Male	0, 300, 3000, 6000	0, 15, 152, 307	3/81, 1/80, 1/80, 4/80	0.000272411
	Female	0, 300, 3000, 6000	0, 18, 184, 375	1/81, 2/81, 0/80, 2/80	N.S.
Moore 1998a	Male	0, 500, 1500, 6000, 12000	0, 29.2, 88.3, 358.7, 733.2	5/65, 4/50, 2/50, 7/65, 17/65	0.000320818
	Female	0, 500, 1500, 6000, 12000	0, 36.4, 108.6, 442.2, 885.4	1/65, 1/50, 0/50, 2/65, 8/65	0.000152717
Biodynamics 1986	Male	0, 500, 5000, 10000	0, 27, 271, 553	4/70, 7/69, 12/69, 9/70	N.S.
	Female	0, 500, 5000, 10000	0, 33, 331, 672	1/70, 1/70, 10/70, 9/70	0.000255301
Mouse Study					
Moore 1998b	Male	0, 500, 1500, 4000, 8000	0, 90.3, 276, 742, 1560	16/70, 13/67, 18/66, 28/65, 31/70	0.00037808
	Female	0, 500, 1500, 4000, 8000	0, 112, 335.6, 910, 1888	3/70, 5/68, 10/68, 11/67, 33/70	0.000328649

The most conservative cancer potency estimate (q1 = 0.00037808) was generated from the male mouse data from Moore 1998b. This identified the Moore mouse study as the most sensitive and appropriate for use in the NSRL derivation.

[7] See Section 25703 (5): http://oehha.ca.gov/prop65/law/pdf_zip/RegsArt7.pdf, '(5) …A linearized multistage model for extrapolation from high to low doses, with the upper 95 percent confidence limit of the linear term expressing the upper bound of potency shall be utilized.'

[8] See: http://www.epa.gov/ncea/bmds/index.html

Derivation of human cancer potency factors

The next step was to convert the animal data into a human potency estimate to derive the NRSL, in accordance with Section 25703. The cancer potency estimates described above are animal potency estimates. These values are converted to human cancer potency estimates by use of allometric scaling. OEHHA uses the following allometric scaling equation (California Code of Regulations Section 25701 - 25703):

$$human\ cancer\ potency\ =\ q1^{*} \times \left(\frac{bw_{human}}{bw_{animal}}\right)^{1/4}$$

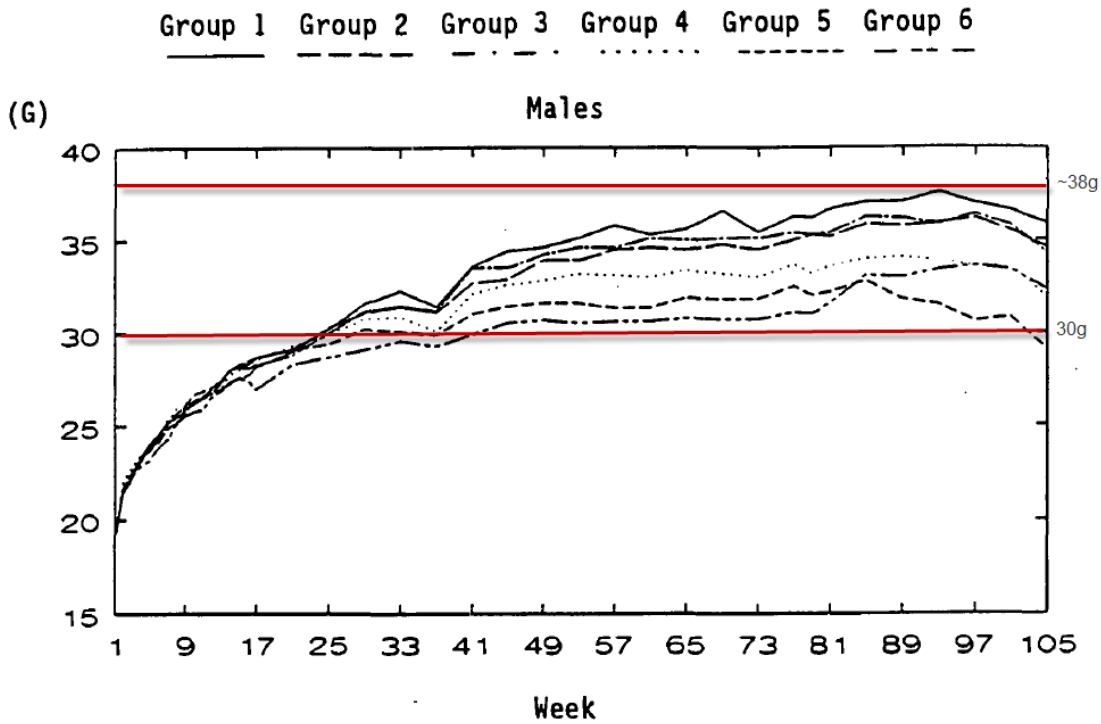
This uses a human-to-animal-to-animal bodyweight-factor scaled to the one-fourth power.

Given the lower bodyweight for mice compared to rats, the mouse to human scaling factor will be larger than the rat to human scaling factor. The combination of the most conservative tumor potency estimate, derived from the mouse data, and the use of the mouse bodyweight in the allometric scaling equations yields the highest human cancer potency estimate and is thus the value being carried forward for the NRSL development.

In order to calculate the human cancer potency value, relevant human and animal bodyweights need to be identified. Default human bodyweights for human males and females are set by the State of California for NRSL development as 70 kg and 58 kg, respectively (California, 2013a). The mouse bodyweights can be estimated using three different methodologies:

1. Data from the same strain of mice were used by the State of California to derive the NRSL value for DEHP and a bodyweight of 0.03 kg was used for that calculation (DEHP NSRL).
2. Default rodent bodyweights for laboratory male and female species used in chronic studies are supplied by TERA (http://www.tera.org/Tools/ratmousevalues.pdf), which gives a value of 0.0353 kg for male B6C3F1 mice.
3. The study specific data bodyweight data can be used to derive the allometric scaling factor. For the Moore mouse study male bodyweights were between 0.03 and 0.038 kg for the duration of the study (see Figure 1).

Figure 1: Mean Bodyweights from Moore Mouse Study



Detailed in the OEHHA justification document for the NSRL for a different chemical (DEHP) is the adoption of a reduced potency estimate for agents which produce liver tumors through the PPARalpha mode of action (OEHHA 2001). DINP has been shown to induce liver tumors through this mechanism (Klaunig et al., 2003, p. 667).
For DEHP and applicable for DINP, OEHHA notes a reduction in the number of receptors and the activity of these receptors in human liver cells compared to rodent, therefore a reduction in potency by a factor of ten has been applied (OEHAA 2002). A tenfold reduction in potency is a conservative estimate of this potency difference. Thus the equation for the calculation of human cancer potency is: animal cancer potency (q1) multiplied by the allometric scaling factor multiplied by the sensitivity scaling factor ($1/10^{th}$) resulting in the following equation.

$$human\ cancer\ potency\ = q1^{*} \times \left(\frac{bw_{human}}{bw_{mouse}}\right)^{1/4} \times \frac{1}{10}$$

Solving the equation using the three estimates of bodyweight gives the following range of human cancer potencies (Table 2). A NSRL can be derived for each of these potencies (Table 3).

Table 2. Mouse bodyweight effect on range of human cancer potencies for DINP

Study	Species	Strain	Sex	BW	Basis for BW	q1*	allometric scaling	Human Cancer Potency (mg/kg-d)-1

Moore	Mouse	B6C3F	M	0.03	DEHP NSRL default for strain, Low end of study range	0.00037808	7.0	0.000263
				0.0353	TERA default for strain	0.00037808	6.7	0.000252
				0.038	High end of study range	0.00037808	6.6	0.000248

Derivation of NSRL for DINP

The intake level posing no significant cancer risk under Proposition 65 is derived using the following equation.

$$I = \frac{R \times bw_h}{q_{human}}$$

Where I is the intake (in mg/day), R is cancer risk, bw is bodyweight and q_{human} is the theoretical human cancer potency estimate.

Daily intake levels associated with lifetime cancer risks at or below 10^{-5} are considered to pose no significant risk of cancer under Proposition 65 (Title 27, California Code of Regulations. Article 7). OEHHA assigns a bodyweight of 70 kg (highest of the values recommended), which, along with the other variables into the equation gives an NRSL range of 2664-2826 µg/day (Table 3).

Table 3. Mouse bodyweight effect on range of NSRL values for DINP

Study	Species	Strain	Sex	BW	Basis for BW	q1*	allometric scaling	Human Cancer Potency (mg/kg-d)-1	NSRL µg/day
Moore	Mouse	B6C3F	M	0.03	DEHP NSRL default for strain, Low end of study range	0.00037808	7.0	0.000263	2664
				0.0353	TERA default for strain	0.00037808	6.7	0.000252	2774
				0.038	High end of study range	0.00037808	6.6	0.000248	2826

Conclusion

Using OEHHA methodology and precedent, a proposed NSRL range for DINP has been derived. Based on the most sensitive study an NRSL in the range of 2664-2826 µg/day is scientifically justified.

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Attachment 2 – Presentation by R. McKee at Hearings on February 25, 2015

February 25, 2014

WRAP UP & PROPOSED ALTERNATIVE CALCULATION OF AN NSRL FOR DINP

R. McKee

American Chemistry Council

Perspective on DINP-Induced Rodent Tumors and NSRL Calculations

MNCL is not relevant to humans

- As documented in previous comments and further amplified by Drs. Maronpot and Irons at today's meeting

Liver Tumors in rodents treated with DINP are the consequence of a PPARα-mediated process

- As documented in previous comments and further supported by the recent publication by Wood et al. (2010).[1]

Other tumors observed in chronic rodent studies of DINP are reported at < statistically significant levels and/or are due to processes that are not relevant to humans.

- As documented in previous comments

In summary, exposure to DINP does not cause human relevant tumors and should not be listed as carcinogenic under Proposition 65

If an NSRL is to be derived, the method used for DEHP should be followed

The calculation should be based on liver tumor data

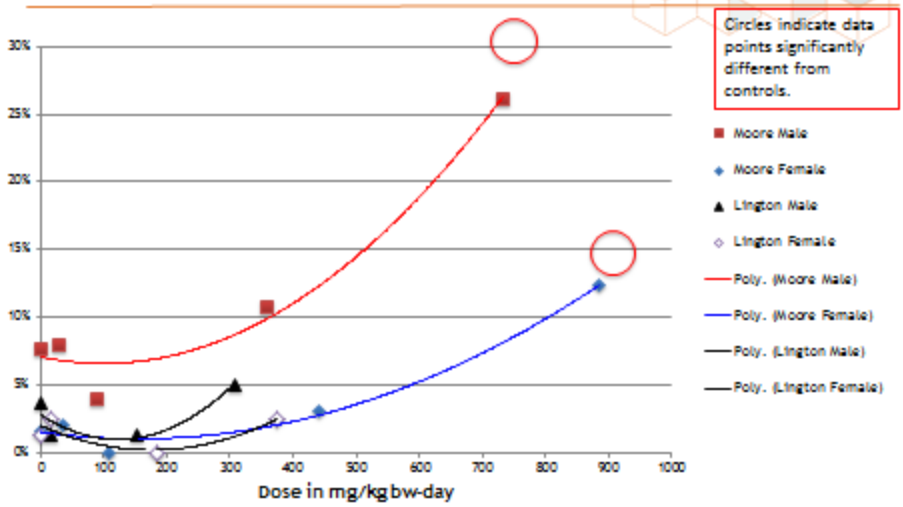
- As shown, the liver tumor data are comparable across studies and provide a good basis for statistical evaluation

A scaling factor (10x) should be applied to account for quantitative differences between rodents and humans

- Previously applied because of differences in receptor (PPARα) density,
- Further supported by evidence that MINP[2] is a more avid agonist for rodent than human receptors. [1]

1. Wood et al. (2010). Toxicological Sciences 139:21-34. 2. Bility et al. (2004). Toxicological Sciences 82:170-182.

Liver Tumor Percentage as a Function of Dose of DINP in F344 Rats



Data are most compatible with a threshold model.

Potency Estimates

- cancer potency estimates determined for liver tumor responses
  - determined using linearized multistage model from USEPA
    - first degree multistage and second degree multistage models
  - P(d) = 1 - exp[-(q0 + q1d + q2d2 + ... + qjdj)], where P is probability of cancer mortality induced at a daily dose (d)
- most conservative potency estimates from male mouse liver tumor data

Study	Species	Sex	Organ	Potency
Moore	Mouse	Male	Liver	0.000378
	Mouse	Female	Liver	0.000329
Moore	F-344 Rat	Male	Liver	0.000321
	F-344 Rat	Female	Liver	0.000153
Lington	F-344 Rat	Male	Liver	0.000264

NSRL Calculation

- animal potency estimates converted to human potency estimates by allometric scaling using Cal OEHHA method
  - The potency estimate was scaled 10x in DEHP NSRL to reflect species differences in activity of PPARα receptor concentration and activity
  - equation $$human\ cancer\ potency = q1^* \times \left(\frac{bw_{human}}{bw_{mouse}}\right)^{1/4} \times \frac{1}{10}$$
  - human potency estimate: 0.00026 (mg/kg d)-1
- potency estimate inserted into NSRL equation
  - equation $$I = \frac{R \times bw_h}{q_{human}} \qquad I = \frac{10^{-5} \times 70\,kg}{0.00026}$$
  - NSRL: 2664 µg/day

In Summary

- The rodent tumors reported in DINP studies are not relevant to humans
- If a no significant risk level is calculated, we recommend that the calculation be based on rodent liver tumors and follow the calculation method used in the previous DEHP assessment
  - There is a consistency of liver tumor response across species/genders
  - The liver tumor dose response relationships are similar across studies
  - Calculating the NSRL in this way would be consistent with historical practice
- As shown, using this method and following the methodology used previously for DEHP resulted in an NSRL of 2664 ug/day