FINAL STATEMENT OF REASONS
TITLE 27, CALIFORNIA CODE OF REGULATIONS

SECTION 25705(b) SPECIFIC REGULATORY LEVELS POSING NO SIGNIFICANT RISK

NO SIGNIFICANT RISK LEVEL: DIISONONYL PHTHALATE (DINP)

This is the Final Statement of Reasons for the adoption of a No Significant Risk Level (NSRL) for diisononyl phthalate (DINP), a chemical known to the State of California to cause cancer under Proposition 65[1]. On January 2, 2015, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Notice of Proposed Rulemaking to adopt a proposed NSRL of 146 micrograms per day (µg/day) for DINP under Title 27, California Code of Regulations, section 25705(b)[2]. The Initial Statement of Reasons sets forth the grounds for the amendment to the regulation. A public comment period was provided from January 2, 2015 to February 17, 2015. The American Chemistry Council requested a public hearing, which OEHHA held on February 25, 2015. To allow time for review of any oral or written comments presented at the hearing, the public comment period was extended to March 11, 2015. OEHHA received written public comments on the proposed rulemaking from the following organizations:

1. American Chemistry Council (ACC): The comments are comprised of ACC's comment letter and the following attachments:
    i. ACC Phthalate Esters Panel Submission of Information on DINP (Feb. 16, 2010)
    ii. Comment of Dr. Richard D. Irons regarding OEHHA proposed NSRL (Feb.12, 2015)
    iii. Presentation of Dr. Richard D. Irons at public hearing (Feb. 25, 2015)
    iv. Comment of Dr. Robert R. Maronpot regarding OEHHA proposed NSRL (March 2, 2015)
    v. Presentation of Dr. Robert R. Maronpot at public hearing (Feb. 25, 2015)
    vi. Presentation of Dr. Richard McKee at public hearing (Feb. 25, 2015).
2. Consumer Electronics Association (CEA)[3]

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986, codified at Health and Safety Code section 25249.5 et. seq., hereafter referred to as "Proposition 65" or "The Act".
[2] All further regulatory references are to sections of Title 27 of the Cal. Code of Regs., unless otherwise indicated
[3] CEA supports the comments of ACC.

3. Consumer Specialty Products Association (CSPA)
4. ExxonMobil Chemical Company (ECC): The comments are comprised of ECC's comment letter and the following attachments:
   i. ECC's Derivation of an NSRL for DINP, "Diisononyl Phthalate (DINP) Proposed Safe Harbor Level under California Proposition 65".
   ii. Presentation of Dr. Richard McKee at public hearing (Feb. 25, 2015).
5. The Chanler Group (TCG)
6. The European Council for Plasticizers and Intermediates (ECPI)
7. Toxicology Excellence for Risk Assessment (TERA): The comments are comprised of TERA's comment letter and the following attachments:
   i. Presentation of Dr. Michael Dourson at public hearing (Feb. 25, 2015).
   ii. Three revised slides received after Dr. Dourson's Feb. 25, 2015 presentation at public hearing
8. ToxServices (TS)

PEER REVIEW

On January 22, 2015, OEHHA provided the notice of proposed rulemaking and the initial statement of reasons for the proposed NSRL for DINP to the members of the Carcinogen Identification Committee for their review and comment as required by Section 25302(e). No comments were received from any committee members.

SUMMARY AND RESPONSE TO COMMENTS RECEIVED

In developing the NSRL for DINP, OEHHA relied on a 2013 OEHHA document entitled, "Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)[4]," which summarizes the available scientific data from rodent carcinogenicity studies of DINP, as well as other information relevant to the carcinogenic activity of the chemical. The NSRL is based upon the results of the most sensitive scientific studies deemed to be of sufficient quality[5].

A summary of the relevant comments received is provided below, along with OEHHA's responses to those comments. Several written and oral comments submitted throughout the regulatory process included observations about this regulation or other laws and regulations that do not constitute an objection or recommendation directed at the proposed action or the procedures followed in this rulemaking action. Accordingly,

[4] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[5] Section 25703(a)(3)

OEHHA is not required under the Administrative Procedure Act to respond to such remarks in this Final Statement of Reasons (FSOR). Since OEHHA is constrained by limitations upon its time and resources, and is not obligated by law to respond to such remarks[6], OEHHA may not necessarily provide responses to all or any of these remarks in this FSOR. However, the absence of responses to such remarks should not be construed to mean that OEHHA in any way agrees with them.

Comment 1 (ACC, CEA, CSPA, ECC, ECPI, TERA, TS): The NSRL for DINP should not be based on the increased incidence of mononuclear cell leukemia (MNCL).

Comment 1a

ACC, CEA, CSPA, ECC, ECPI, TERA and TS comment that MNCL is a strain-specific disease specific to the Fischer 344 rat, and assert that MNCL is not a relevant predictive model for human disease.

Additionally, ACC states: "The 2001 Consumer Product Safety Commission (CPSC) Chronic Hazard Advisory Panel (CHAP) concluded that "[t]he findings of mononuclear cell leukemia in the rodent bioassays for DINP are of questionable relevance to *humans*", and "the European Chemicals Bureau (ECB) found little relevance to humans, citing International Agency for Research on Cancer (IARC) conclusions that MNCL had no known human counterpart".

CSPA, ECC, and ECPI comment that MNCL occurs spontaneously in Fischer 344 rats, occurring with a high incidence in rats over 18 months of age, and TS notes that MNCL does not occur in mice or hamsters.

Response 1a

MNCL (also known as large granular lymphocyte [LgL, LGL] leukemia) is not a disease that only occurs in the Fischer rat. As noted in the International Agency for Research on Cancer's (IARC) Scientific Publication No. 99 (1990)[7], "It *[MNCL]* has been seen in other rat strains or stocks (Abbott et al., 1983; Maekawa et al., 1986, Frith, 1988) in low incidence (1.5%) and probably occurs in low incidence in many rat strains in our

[6] California Government Code section 11346.9 (a)(3)

[7] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, pp. 625-657.

experience (Table 4)". Table 4 of the IARC Scientific Publication[8] reports the naturally occurring (i.e., spontaneous) incidence of LgL leukemia in 7 strains of rats, including an incidence range of 15-22% observed in W/F [Wistar/Furth] rats, and a range of 10-50% in Fischer 344 rats. As stated in the 2013 OEHHA document entitled, "Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)[9], "The most common background tumor in F344 rats is MNCL, which is generally observed after 18 months of age."

OEHHA has been unable to verify the comment[10] regarding MNCL and the supposed lack of a human counterpart attributed by the European Chemicals Bureau (ECB)[11] to IARC, finding instead IARC statements[12] identifying a human counterpart to MNCL, and discussing the use of rat MNCL as a model for human disease. Although arguments have been made that the mononuclear cell leukemia cell type observed in the rats is unusual in humans, there is no automatic expectation of site or cell type concordance between humans and test species for risk assessment purposes[13,14,15].

[8] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, see p. 632.

[9] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, p. 34, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[10] See for example TS comments, which cite IARC, 1990, Pathology of Tumors in Laboratory Animals, 1: Tumours of the Rat. V. Turusov and U. Mohr (eds). IARC, No 99, 2nd edition, Lyon France. *[as reviewed by ECHA 2013]*.

[11] ECB (2003). European Chemicals Bureau: 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP), CAS Nos: 68515-48-0 and 28553-12-0, EINECS Nos: 271-090-9 and 249-079-5, Summary Risk Assessment Report, Special Publication I.03.101, p. 18. Available at http://echa.europa.eu/documents/10162/0645f0cb-7880-4d23-acea-27b05ed7de39.

[12] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, pp. 625-657.

[13] Maronpot RR, Flake G, Huff J (2004). Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol Pathol 32(Suppl 1): 40-48.

[14] Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors" Office of Environmental Health Hazard Assessment.(May 2009) Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html

[15] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.

OEHHA also notes that in considering DINP for listing, the Carcinogen Identification Committee (CIC)[16] evaluated the scientific evidence on the carcinogenicity of DINP, including materials prepared by OEHHA[17], materials submitted as public comments, and oral presentations made by the public at the CIC meeting. The question of human relevance of the observed rodent tumors, including MNCL, was one of the main issues presented to, discussed and considered by the CIC members[18]. In deciding to list DINP, the CIC did not dismiss the human relevance of MNCL, or any of the other rodent tumors induced by DINP. The Committee voted by a substantial majority to identify DINP as a chemical known to the state to cause cancer[19].

Thus in developing an NSRL, consistent with Section 25703, MNCL induced by DINP in rats is considered relevant for human risk assessment

A significant dose-related incidence of a tumor in rodents is considered predictive of cancer risk in humans, but the site of those human cancers may be different due to differing toxicokinetics and tissue susceptibilities in humans vs. rodents[20,21,22]. Although there is not necessarily an expectation of concordance between species with regard to histological origin and site of tumors, there are in fact a number of human lymphoid neoplasms, with a variety of different etiologies, which share features with MNCL observed in the rat. As noted by IARC in the 1990 Scientific Publication No. 99[23], "A human counterpart [to LGL leukemia] was found after characterization of the rat disease (Reynold & Foon, 1984; Reynold & Ward, 1986)." IARC[24] goes on to say, "Rats bearing LGL leukaemia have been proposed as useful models for the human disease (Stromberg et al., 1985; Bauldry et al., 1985)." As stated in the 2013 OEHHA

[16] The state's qualified experts for carcinogenicity, per Section 25302.
[17] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf
[18] Transcript for Dec. 5, 2013 CIC meeting.
[19] *Ibid.*
[20] Maronpot RR, Flake G, Huff J (2004). Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol Pathol 32(Suppl 1): 40-48.
[21] Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors" Office of Environmental Health Hazard Assessment.(May 2009) Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html
[22] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.
[23] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, see p. 633.
[24] *Ibid,* p. 639.

document[25], "Well-differentiated MNCL cells resemble normal large granular lymphocytes (LGL), but in poorly differentiated cells granules may be detected only ultrastructurally (Ward et al., 1990). Based on a morphological similarity to granular lymphocytes, MNCL is also called LGL leukemia or Tγ lymphocyte leukemia. …Caldwell et al. (1999)[26] reported no human counterpart to rat LGL leukemia. More recently, a US Environmental Protection Agency (US EPA) report (2012)[27] has noted that several authors have concluded that rat MNCL is similar to human natural killer cell (NK) LGL leukemia (Stromberg et al., 1985; Ishmael and Dugard, 2006; Thomas et al., 2007)."

US EPA (2012) goes on to state, "In humans, clonal disorders of LGLs represent a biologically heterogeneous spectrum of lymphoid malignancies thought as originating either from mature T-cell or natural killer (NK) cells (Sokol and Loughran, 2006). The indolent form of LGL leukemia is a disease of older adults, with a median age at diagnosis of 60 years. …The etiology of LGL disorders is not known (Sokol and Loughran, 2006; Rose and Berliner, 2004). Several possible etiologies have been proposed including chronic activation of T-cell by a viral antigen or autoantigen in which case LGL leukemia could be considered as an autoimmune disorder (Sokol and Loughran, 2006)."

While MNCL occurs spontaneously in Fischer rats, treatment-related increases in MNCL are also observed following chemical exposures. For example, IARC[28] notes that "the incidence of LGL leukaemia in F344 rats can be increased significantly but not greatly by a few chemicals including ethylene oxide (Snellings et al., 1984) and butylbenzylphthalate." Specifically with regard to the studies of DINP conducted in the Fischer rat, statistically significant, treatment-related increases in MNCL were observed in four two-year carcinogenesis studies (two in females and two in males)[29,30]. In

[25] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, p. 34, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[26] Caldwell DJ, Eldridge SR, Lington AW, McKee RH (1999). Retrospective evaluation of alpha 2u-globulin accumulation in male rat kidneys following high doses of diisononyl phthalate. *Toxicol Sci* **51**:153-160.

[27] US EPA (2012). Toxicological Review of Tetrachloroethylene (Perchloroethylene)(CAS No. 127-18-4) In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-08/011F. February 2012. US Environmental Protection Agency, Washington, DC.

[28] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, p. 632, also p. 636.

[29] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

addition, statistically significant, treatment-related increases in MNCL were observed in two shorter-term studies (one in females and one in males)[31] in which rats were treated with DINP for 78 weeks, followed by a 26 week recovery period. These studies were discussed in the 2013 OEHHA document[32]. The studies were also reviewed by CSPC CHAP (2001)[33], which stated that "while the lesion *[MNCL]* rarely occurs in untreated rats less than 20 months of age, DINP treated animals were first observed with this tumor at considerably younger ages. It is therefore highly unlikely that these findings were unrelated to treatment." US EPA[34], in reviewing these studies concluded that "[t]he increased mortality due to MNCL in DINP treated rats suggests that DINP is associated with the elevated incidence, progression, and severity of MNCL. The tumor findings may be biologically significant because the time to onset of tumor was shorter, and the disease was more severe in treated than in control animals. The agency *[US EPA]* believes that the data for MNCL are indicative of a carcinogenic response to DINP".

Comment 1b

ACC, CEA, CSPA, and TS comment that in addition to the 2001 CPSC CHAP report and the 2013 ECB [ECHA] report mentioned in Comment 1a, other authoritative bodies, including the US National Toxicology Program (NTP), the National Industrial Chemical Notification and Assessment Scheme (NICNAS) of the Australian Government, and a 2014 CPSC CHAP report have questioned the relevance of MNCL data for human risk assessment.

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[30] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[31] *Ibid*.

[32] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[33] CPSC (2001). Report to the US Consumer Product Safety commission by the Chronic Hazard Advisory Panel on Disononyl Phthalate (DINP), US Consumer Product Safety Commission Directorate For Health Science, Bethesda, MD

[34] US EPA (2005). Revised Technical Review of Diisononyl Phthalate. Office of Environmental Information, Environmental Analysis Division, Analytical Support Branch. March 2005.

Response 1b

As noted in response to Comment 1a, the existence of a human tumor type similar to that seen in the rodent model is not a prerequisite to using rodent tumor findings as a predictor of human cancer risk. However, there are a number of human lymphoid neoplasms, with a variety of different etiologies, which share features with MNCL observed in the Fischer 344 rat. As indicated in OEHHA (2013)[35], US EPA (2012) discusses conclusions by several authors (i.e., Stromberg et al., 1985; Ishmael and Dugard, 2006; Thomas et al., 2007) that rat MNCL is similar to human natural killer cell (NK) LGL leukemia. There are several different human leukemias that share LGL features, including T cell LGL leukemia (T-LGL), ANKL, and chronic lymphoproliferative disorders of NK cells (the latter is considered a provisional entity)[36]. OEHHA disagrees with the commenter's assertion that NTP has questioned the relevance of MNCL data for human risk assessment. As discussed in more detail in the response to Comment 1c, the NTP held a workshop in 2005 as part of its efforts to evaluate the animal models used for the NTP rodent cancer bioassay[37]. The concern expressed at the NTP workshop about the F344/N rat was based on high background incidences of testicular interstitial tumors and MNCL, and observations of declining fertility, sporadic seizure activity, and chylothorax. The concern about the high incidences of MNCL was because it decreases the ability to detect an exposure-related effect, rather than a question of the human relevance of this type of tumor[38].

DINP was listed as known to the state to cause cancer under Proposition 65 via the state's qualified experts listing mechanism, as a result of a decision to list the chemical by the CIC[39]. In making its determination, the CIC evaluated the scientific evidence on the carcinogenicity of DINP, including materials prepared by OEHHA[40], materials submitted as public comments, and oral presentations made by the public at the CIC

[35] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[36] Campo E, Swerdlow SH, Harris NL Pileri S, Stein H, Jaffe ES (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: Evolving concepts and practical applications. *Blood* 117(9):5019-5032.

[37] King-Herbert A and Thayer K (2006). NTP workshop: Animal model for the NTP rodent Cancer bioassay: Strain and Stock-Should we Switch? Toxicol Pathol, 34: 802-805

[38] *Ibid*.

[39] Section 25302

[40] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

meeting. One of the main issues presented to, discussed and considered by the CIC members[41] was whether the tumors observed in rodents exposed to DINP, including MNCL, are relevant to humans. In deciding to list DINP, the CIC did not dismiss the human relevance of MNCL, or any of the other rodent tumors induced by DINP[42]. While some entities may question the relevance of MNCL data for human risk assessment, per Section 25703, OEHHA has determined that the MNCL data are relevant and appropriate for use in developing the NSRL for DINP.

Comment 1c

ACC, CEA, ECC, TERA and TS commented that the National Toxicology Program (NTP) is moving away from using the Fischer 344 rat in its rodent cancer bioassay testing program.

ACC stated, "At an NTP workshop in 2005, in addressing whether the currently used models, the F344/N rat and B6C3F1/N mouse, continued to be appropriate to identify substances that may pose a carcinogenic hazard for humans, the rat model breakout group recommended moving away from the F344 rat".

TS stated, "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."

Response 1c

According to King-Herbert and Thayer (2006)[43], "Workshop participants advised the NTP to discontinue using the current F344/N strain due to the recent issues with fertility, seizure activity, and chylothorax and provided several options on how the program should approach identifying and selecting a new rat model." Additional concerns with the Fischer 344/N rat model were noted, including the high background incidences of

[41] Transcript for Dec. 5, 2013 CIC meeting.
[42] *Ibid*.
[43] King-Herbert A and Thayer K (2006). NTP workshop: Animal model for the NTP rodent Cancer bioassay: Strain and Stock-Should we Switch? Toxicol Pathol, 34: 802-805.

certain types of tumors including testicular interstitial cell tumors and mononuclear cell leukemia[44].

The concern about the high background incidences of MNCL and other tumors in the Fischer 344/N rat was because such high background incidences decrease the ability to detect an exposure-related effect at these sites/cell types, especially an effect that falls into the range observed in historical controls. As explained by NTP, "From a statistical perspective, high background rates of such tumors in control animals will generally decrease the ability to detect an exposure-related effect. In addition, 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 (Haseman, Arnold et al. 1990)"[45]. In other words, the concern expressed at the NTP workshop about the F344/N rat and the high incidences of MNCL was because of the decreased ability to detect an exposure-related effect in this test strain, rather than a question of the human relevance of this type of tumor.

NTP did not express concerns that the high background rates of MNCL and other tumors in the Fischer 344 rats were contributing to false positive findings in long-term studies[46]. Rather, NTP expressed concern that high background incidences of these tumors in the Fischer 344 rat decreased the ability to detect an exposure-related effect.

To date, NTP is still using substrains of Fischer 344 rats in the cancer bioassay testing program. As OEHHA's Dr. Budroe indicated during the 2013 CIC meeting[47] in response to a question from committee member Dr. Thomas, "Well, NTP hasn't exactly discontinued the use of Fischer 344 rats. They've discontinued the use of the N substrain, which is the NIH derived substrain. They are now using, for example, Han Wistar rats in some studies, but they're also using Fischer 344 NCTR substrain. And the F344/NTac substrain, which is Taconic Farms derived. So they've gone away, more or less, from using the N strain, but they are still using Fischer substrains"[48].

[44] King-Herbert A and Thayer K (2006). NTP workshop: Animal model for the NTP rodent Cancer bioassay: Strain and Stock-Should we Switch? Toxicol Pathol, 34: 802-805.
[45] *Ibid.*
[46] *Ibid.*
[47] Transcript for Dec. 5, 2013 CIC meeting, p 75.
[48] *Ibid*, pp. 75-76

Comment 1d

ACC, CEA, ECC, EPCI and TS comment that the incidence of MNCL in the Fischer 344 rat is variable and influenced by many modifying factors, including age, gender, genetics, diet, oil vehicle, housing conditions, splenic toxicity, and stress.

ECC also speculates that the differences in MNCL frequencies could be a "consequence of the lifespan of the animals, and largely unrelated, or at least not directly related to chemical treatment".

Response 1d

OEHHA agrees that the background incidence of MNCL in the Fischer 344 rat is variable, and appears to be modulated by a variety of factors. Discussing issues of tumor variability more broadly, IARC[49] notes, "The incidence of haematopoietic and other tumours in a specific rat strain or stock may vary from laboratory to laboratory or even within the same laboratory because of several factors including source of rats, animal feed, virus profile, genetic drift and binomial or nonbinomial variation (Tarone et al., 1981). Concurrent laboratory controls are always the best direct comparison with an experimental group (Chu et al. 1981)."

OEHHA disagrees with the commenter's assertion that differences in MNCL frequencies in the Fischer 344 rat are "largely unrelated, or at least not directly related to chemical treatment." As noted by IARC in 1990[50], chemical treatment-related increases in MNCL are recognized in this rat model: "Increased incidences of LGL leukaemias have been seen in F344 rats used in two-year bioassays and exposed to ethylene oxide (Snellings et al., 1984), or butylbenzylphthalate..." More recently, Dr. Maronpot, in his written comments submitted by ACC and in his presentation at the public hearing on Feb. 25, 2015, reviewed the results of the NTP's rodent cancer bioassays and summarized the number of chemicals that caused treatment-related increases in MNCL in the Fischer 344 rat. Specifically, Dr. Maronpot reviewed 570 NTP Technical Reports, and found that 24 reported positive, treatment-related increases in MNCL (NTP findings of either "clear" or "some" evidence of carcinogenicity). He reported that 8 NTP Technical Reports found positive evidence for MNCL-induction in studies of both male and female Fischer 344 rats, 8 NTP Technical Reports found positive evidence for MNCL-induction in females, but not males, and 8

[49] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, p. 638.
[50] *Ibid.,* p. 636.

NTP Technical Reports found positive evidence for MNCL-induction in males, but not females.

In summary, chemical treatment-related increases in MNCL can and have been observed in the Fischer 344 rat, despite the often variable and high background incidence of MNCL in this rat strain.

Comment 1e

ACC, CEA, ECC, ECPI, TS comment that MNCL in the Fischer 344 rat is not a relevant or suitable predictive model for human disease.

ACC, CEA, ECC, ECPI, and TS comment that the closest analogue to MNCL in humans is a natural killer (NK) cell-derived malignancy (Aggressive NK cell Leukemia (ANKL)). ANKL and MNCL share common cells of origin, immunophenotype, and certain molecular and clinical features. ANKL is rare, believed to have Epstein-Barr virus (EBV) mediated etiology, and has not been associated with exposure to chemicals. It is extremely aggressive and occurs in younger adults. MNCL has a high spontaneous incidence in Fischer 344 rats, is an aggressive and often fatal disease in older rats, and has no evidence of a viral etiology. Fischer 344 rat MNCL has also been suggested to be a model for human T-cell LGL, but while MNCL is an acute leukemia of cells likely to be of NK (natural killer) cell origin, human T-cell LGL is a chronic disease of T-cells.

CSPA comments that there is no histologically comparable tumor to MNCL in humans.

Response 1e

OEHHA disagrees with the assertion that there is no biological basis to support MNCL in Fischer 344 rats as a suitable predictive model for human disease. There is no expectation of inter-species concordance as to site or cell type when using the observation of tumors in animal tests to predict human cancer risk. A significant dose-related incidence of a tumor in rodents is considered predictive of cancer risk in humans, but the site of those human cancers may be different due to differing toxicokinetics and tissue susceptibilities in humans vs. rodents[51].

OEHHA and several of the commenters also disagree with the assertion by CSPA that there is no histologically comparable tumor to MNCL in humans. Indeed, there are a

[51] Maronpot RR, Flake G, Huff J (2004). Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol Pathol 32(Suppl 1): 40-48.

number of human lymphoid neoplasms, with a variety of different etiologies, which share features with MNCL observed in the Fischer 344 rat. As recognized by IARC in 1990[52], "A human counterpart *[to LGL leukemia (MNCL)]* was found after characterization of the rat disease (Reynold & Foon, 1984; Reynold & Ward, 1986)." IARC[53] goes on to say, "Rats bearing LGL leukaemia have been proposed as useful models for the human disease (Stromberg et al., 1985; Bauldry et al., 1985)." As noted by OEHHA (2013)[54], a 2012 US EPA report[55] discusses conclusions by several authors (i.e., Stromberg et al., 1985; Ishmael and Dugard, 2006; Thomas et al., 2007) that rat MNCL is similar to human natural killer cell (NK) LGL leukemia. US EPA (2012) also states, "In humans, clonal disorders of LGLs represent a biologically heterogeneous spectrum of lymphoid malignancies thought as originating either from mature T-cell or natural killer (NK) cells (Sokol and Loughran, 2006). …The indolent form of LGL leukemia is a disease of older adults, with a median age at diagnosis of 60 years. …The etiology of LGL disorders is not known (Sokol and Loughran, 2006; Rose and Berliner, 2004). Several possible etiologies have been proposed including chronic activation of T-cell by a viral antigen or autoantigen in which case LGL leukemia could be considered as an autoimmune disorder (Sokol and Loughran, 2006)."

While the comments of Dr. Irons[56] indicate that only human aggressive NK-cell leukemia (ANKL) shares clinical features and a presumed cell of origin with F344 MNCL, the 2008 World Health Organization (WHO)[57] classification system recognizes many distinct entities among human leukemias that share LGL features, including T cell LGL leukemia (T-LGL), ANKL, and chronic lymphoproliferative disorders of NK cells (the latter is considered a provisional entity).

In classifying these lymphoproliferative disorders, the precise identification of the cell type is difficult due to the broad heterogeneity and complexity of the T cell system, the

[52] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, see p. 633.
[53] *Ibid,* p. 639.
[54] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, p. 34, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf
[55] US EPA (2012). Toxicological Review of Tetrachloroethylene (Perchloroethylene)(CAS No. 127-18-4) In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-08/011F. February 2012. US Environmental Protection Agency, Washington, DC.
[56] ACC Attachments B and C.
[57] Campo E, Swerdlow SH, Harris NL Pileri S, Stein H, Jaffe ES (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: Evolving concepts and practical applications. *Blood* 117(9):5019-5032.

plasticity of neoplastic T lymphocytes, and lack of understanding of the tumorigenic mechanisms of transformation. LGL leukemia, also sometimes diagnosed as LGL lymphoma, can arise from T cells, NK cells, or from a common progenitor cell. In their recent classification, WHO has grouped these neoplasms under NK/T cell lymphomas[58]. Regarding NK/T cell lymphomas, up to 90% of cases are Epstein - Barr virus positive (EBV+), and most cases arise from NK cells, with a minority (20%) thought to be of T cell origin. The role of viral infection in the pathogenesis of NK/T cell lymphomas is not clear[59]. Given that over 90% percent of the world population has subclinical EBV infection[60],[61], it is no surprise that up to 90% of ANKL cases are EBV+, as the latent virus, present in the memory B cells in healthy carriers, will manifest in an immunocompromised host (e.g., individuals with leukemia).

The CIC did not dismiss the human relevance of any of the rodent tumors induced by DINP when it voted to list the chemical. While the exact cell of origin of F344 rat LGL leukemia is still not fully resolved, the available evidence indicate that most of F344 rat LGL leukemia is of the NK /T cell type. Based on morphology, LGL leukemia is quite comparable to the aggressive human NK-LGL leukemia (Reviewed in Thomas et al. (2007). In any case, as already noted, while there may be increased cancer risk in multiple species, there is not necessarily a concordance between species with regard to histological origin or site of tumors[62,63,64].

In summary, OEHHA considers DINP treatment-related increases in MNCL observed in Fischer 344 rats predictive of cancer risk in humans, and notes that although site concordance is not necessarily expected, multiple human lymphoid neoplasms share

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[58] Campo E, Swerdlow SH, Harris NL Pileri S, Stein H, Jaffe ES (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: Evolving concepts and practical applications. *Blood* 117(9):5019-5032.

[59] Inghirami G, Chan WC, Pileri S (2015). Peripheral T-cell and NK-cell lymphoproliferative disorders: cell of origin, clinical and pathological implications. Immunological Reviews:263, 124-159.

[60] Dowd JB, Palermo T, Brite J, McDade TW, Aiello A (2013). Seroprevalence of Epstein-Barr virus infection in U.S. children ages 6-19, 2003-2010. PLoS ONE 8(5): e64921. doi:10.1371/journal.pone.0064921.

[61] International Agency for Research on Cancer (IARC, 2012). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 100B. Epstein-Barr Virus. World Health Organization. Lyon, France.

[62] Maronpot RR, Flake G, Huff J (2004). Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol Pathol 32(Suppl 1): 40-48.

[63] Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors" Office of Environmental Health Hazard Assessment.(May 2009) Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html

[64] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.

features in common with MNCL observed in Fischer 344 rats. OEHHA further notes that one of the main issues presented to, discussed and considered by the CIC members[65] was whether the tumors observed in rodents exposed to DINP, including MNCL, are relevant to humans. In deciding to list DINP, the CIC did not dismiss the human relevance of MNCL, or any of the other rodent tumors induced by DINP[66].

Comment 1f

ECC and TS comment that the incidence of DINP-induced MNCL is similar to that observed in historical controls, and is not dose-dependent.

ECC states, "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."

TS "concludes that the reported increase in MNCL incidence does not provide an adequate basis for human risk assessment," noting that "the reported incidences of DINP-induced MNCL were within or were similar to the historical control range for untreated Fischer 344 rats in NTP studies", and "the effects *[seen in the Moore 1998 studies]* did not appear to occur in a dose-dependent manner".

Response 1f

The commenters' use of NTP historical control data for MNCL as a comparator for the MNCL findings in the DINP rat studies[67,68], which were conducted with animals from a different source and in other laboratories (i.e., the DINP rat studies were not NTP studies and did not use the NTP Fischer 344/N rat substrain) is not appropriate,

[65] Transcript for Dec. 5, 2013 CIC meeting.
[66] *Ibid.*
[67] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.
[68] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

particularly in this case where background incidence is variable. IARC states in the Preamble for evaluating carcinogenicity evidence[69],

> "…less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analyzing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals (Haseman et al., 2984; Fung et al., 1996; Greim et al., 2003)."

Further, in discussing hematopoietic tumors in its Pathology of Tumors in Laboratory Animals IARC[70] notes,

> "The incidence of haematopoietic and other tumours in a specific rat strain or stock may vary from laboratory to laboratory or even within the same laboratory because of several factors including source of rats, animal feed, virus profile, genetic drift and binomial or nonbinomial variation (Tarone et al., 1981). Concurrent laboratory controls are always the best direct comparison with an experimental group (Chu et al. 1981)."

Therefore, the appropriate analysis of each study is the direct comparison of the MNCL incidence in DINP treated rats with that in each study's untreated controls.

There are a number of reasons to conclude that the MNCL findings in the DINP rat bioassays are dose-dependent, repeatable, and treatment-related. First, as discussed in the 2013 OEHHA document[71], the MNCL findings in the six long-term Fischer 344 rat

[69] Preamble to the IARC (International Agency for Research on Cancer) *Monographs* (Amended January 2006). Available at: http://monographs.iarc.fr/ENG/Preamble/index.php

[70] International Agency for Research on Cancer (IARC, 1990). Pathology of Tumors in Laboratory Animals. Volume 1 – Tumours of the Rat, Eds Turusov V and Mohr, U, IARC, No 99, 2nd edition, Lyon France, p. 638.

[71] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and

carcinogenesis studies of DINP[72,73] (three in females and three in males) are remarkably consistent. Statistically significant, treatment-related increases in MNCL were observed in the four two-year carcinogenesis studies (two in females and two in males)[74,75] and in the two shorter-term studies (one in females and one in males)[76] in which rats were treated with DINP for 78 weeks, followed by a 26 week recovery period. Second, as indicated in OEHHA (2013) [77] and in Tables 1 and 2 of the Initial Statement of Reasons, statistically significant dose-dependent trends in MNCL were observed in each of the multiple dose group studies (i.e., the four two-year carcinogenesis studies)[78,79]. Third, as discussed in OEHHA (2013)[80], MNCL occurred earlier in DINP-treated animals than in untreated controls, which is indicative of a treatment-related effect. Evaluations by the CSPC CHAP and US EPA similarly

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Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[72] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[73] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[74] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[75] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[76] *Ibid*.

[77] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[78] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[79] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[80] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

interpret these data. The CSPC CHAP (2001)[81] report stated that "while the lesion *[MNCL]* rarely occurs in untreated rats less than 20 months of age, DINP treated animals were first observed with this tumor at considerably younger ages. It is therefore highly unlikely that these findings were unrelated to treatment." US EPA[82], in reviewing these studies concluded that "[t]he increased mortality due to MNCL in DINP treated rats suggests that DINP is associated with the elevated incidence, progression, and severity of MNCL. The tumor findings may be biologically significant because the time to onset of tumor was shorter, and the disease was more severe in treated than in control animals. The agency *[US EPA]* believes that the data for MNCL are indicative of a carcinogenic response to DINP." Fourth, MNCL was observed in DINP-treated Fischer 344 rats of both genders. As noted by Dr. Maronpot in his presentation at the public hearing of February 25, 2015 and in his comments submitted by ACC, a review of the treatment-related MNCL findings from the NTP Fischer 344 rat bioassays of 570 chemicals indicates that it is less common to observe treatment-related increases in MNCL in both genders (8 chemicals), than in just one (16 chemicals).

Comment 1g

TS questions the reproducibility of chemical-induced MNCL, noting inconsistencies in chemical-induced MNCL in Fischer 344 rats between separate studies using the same or similar concentrations. Examples given were NTP studies of butyl benzyl phthalate, and NTP and non-NTP studies of di(2-ethylhexyl) phthalate (DEHP), all in Fischer 344 rats.

Response 1g

As discussed in the response to comment 1f, the induction of MNCL by DINP is remarkably consistent and reproducible in the six long-term Fischer 344 rat carcinogenesis studies of DINP[83,84] (three studies in females and three studies in

[81] CPSC (2001). Report to the US Consumer Product Safety commission by the Chronic Hazard Advisory Panel on Disononyl Phthalate (DINP), US Consumer Product Safety Commission Directorate For Health Science., Bethesda, MD

[82] US EPA (2005). Revised Technical Review of Diisononyl Phthalate. Office of Environmental Information, Environmental Analysis Division, Analytical Support Branch. March 2005.

[83] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[84] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US

males). Viewed in the context of Dr. Maronpot's presentation during the public hearing of February 25, 2015 and in his comments submitted by ACC, in which he reported that just 8 chemicals of the 570 tested by NTP induced MNCL in both male and female Fischer 344/N rats, the consistency of the DINP MNCL findings across studies and genders is compelling.

Comment 1h

ACC, CEA, and TS comment that the mechanism of induction of MNCL is not currently understood.

Response 1h

Carcinogenesis is a complex process and knowing the mechanism by which a chemical causes cancer of a particular type is not a prerequisite to using the dose-response data from animal studies to estimate cancer potency and derive an NSRL.

Comment 2 (ECC, ECPI): Liver tumors from DINP are proposed not to be relevant to humans.

ECC and ECPI state that liver tumors associated with DINP treatment are caused by a PPARα- *[peroxisome proliferator-activated receptor alpha]* mediated process that is proposed not to be relevant in the human system. ECPI cited as support reviews by the European ECHA Risk Assessment Committee (RAC, 2013), the US CPSC (2010), and the US CPSC CHAP (2001) of the DINP liver tumor findings observed in Fischer 344 rats, while ECC cited reviews from the scientific literature on the mode of action of PPARα-agonists and a recent study of the effects of another phthalate, di(2-ethylhexyl) phthalate (DEHP), on CAR *[constitutive androstane receptor]* and AhR *[aryl hydrocarbon receptor]* activation.

Response 2

In considering DINP for listing, the Carcinogen Identification Committee (CIC)[85] evaluated the scientific evidence on the carcinogenicity of DINP, including materials

Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[85] The state's qualified experts for carcinogenicity, per Section 25302.

prepared by OEHHA[86], materials submitted as public comments, and oral presentations made by the public at the CIC meeting. The issue of the human relevance of the DINP liver tumor findings in rodents was discussed in the materials before the CIC, and explicitly discussed by the Committee[87]. In deciding to list DINP, the CIC did not dismiss the human relevance of the rodent liver tumors, or any of the other rodent tumors induced by DINP[88]. Consistent with Section 25703, the liver tumors induced by DINP in rodents are considered relevant for human risk assessment and are used in the development of the NSRL.

Several of the reviews mentioned by the commenters[89] were cited in and provided with OEHHA's 2013 document entitled, "Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)[90], or submitted by public commenters to the CIC. As discussed in OEHHA (2013), the scientific understanding of the mechanisms of liver tumor induction by chemicals that activate PPARα has changed considerably over time. Similarly, the evaluation of the human relevance of these liver tumors has changed over time. For example, DEHP, another phthalate that activates PPARα, was considered not classifiable as to its carcinogenicity by the International Agency for Research on Cancer (IARC) in 2000; however in 2013 IARC re-classified DEHP as a Group 2B carcinogen (i.e., "possibly carcinogenic to humans")[91]. In its 2013 reevaluation of DEHP

[86] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[87] Transcript for Dec. 5, 2013 CIC meeting.

[88] *Ibid*.

[89] CPSC (2001). Consumer Product Safety Commission. Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP); CPSC (2010). Consumer Product Safety Commission. Toxicity review of diisononyl phthalate (DINP); Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, Fenner-Crisp PA (2003). PPAR α agonist-induced rodent tumors: Modes of action and human relevance. *Crit Rev Toxicol* **33**:655-780; Corton J, Cunningham M, Hummer B, Lau C, Meek B, Peters J, Popp J, Rhomberg L, Seed J, Klaunig J (2014). Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study. Critical Reviews in Toxicology 44:1-49.

[90] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[91] As discussed in Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

carcinogenicity, IARC[92] stated, "Multiple molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to the induction of cancer in rats and mice. Thus, the relevance to human cancer of the molecular events that lead to cancer elicited by di(2-ethylhexyl) phthalate *[DEHP]* in several target tissues (e.g. the liver and testis) in rats and mice cannot be ruled out."

No change to the proposed regulation was made based on this comment.

Comment 3 (ACC, CEA, ECC, TERA): Kidney tumors associated with DINP treatment are not considered relevant to humans

The renal tubular *[tubule cell]* carcinoma in DINP-treated male rats is induced by an α2u-globulin-mediated mechanism, and such tumors are not considered relevant to humans.

Response 3

This comment is not relevant to the scientific basis for the proposed rulemaking, because DINP-induced kidney tumors were not included in the dose-response analyses that serve as the basis for the derivation of the DINP NSRL.

Nevertheless, as mentioned in the response to Comment 2, the CIC did not dismiss the human relevance of any of the rodent tumors induced by DINP. Furthermore, increases in the occurrence of two distinct types of rare or uncommon malignant kidney tumors (i.e., rare transitional cell carcinoma, uncommon renal tubule cell carcinoma) were observed in DINP-treated male rats in two separate two-year studies[93,94], and an increase in renal tubule cell carcinoma was observed in a third study in DINP-treated male rats administered DINP for 78 weeks, and then observed for an additional 26 weeks[95]. While these increases in malignant kidney tumors are considered treatment-

[92]International Agency for Research on Cancer (IARC, 2013). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 101. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-water, Di(2-ethylhexyl) Phthalate. World Health Organization. Lyon, France.
[93] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.
[94] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).
[95] *Ibid.*

related[96], the kidney tumor data were not included in the derivation of the NSRL[97] because they did not contribute significantly to the cancer potency.

Comment 4 (ACC, CEA, CSPA, ECC, TERA, TS): OEHHA should base an NSRL on the rodent liver tumor data.

ACC, CEA, and ECC comment that the rodent tumors reported in the DINP studies are not relevant to humans, but indicate that "if a no significant risk level is calculated, we recommend that the calculation be based on rodent liver tumors", noting "there is a consistency of liver tumor response across species/genders", "the liver tumor dose response relationships are similar across studies", and that using liver tumors to calculate the NSRL would be consistent with historical practice.

CSPA, TERA, and TS support basing the NSRL on rodent liver tumor data. TERA notes that "liver tumors are not as potent as the leukemias found in rats, but are more potent than the kidney tumors," and TS calculates an NSRL of 280 µg/day, based on the increased incidence of combined hepatocellular carcinoma and adenoma in male mice.

Response 4

OEHHA agrees with the commenters that the liver tumor data from the DINP studies should be used in the dose-response analyses that serve as the basis for the NSRL. However, OEHHA disagrees that the NSRL should be based on hepatic neoplasms alone. As described in the Initial Statement of Reasons, both MNCL and liver tumor data from four long-term carcinogenesis studies of DINP conducted in Fischer 344 rats[98,99] (two in females and two in males) were included in the dose-response analyses used to derive the NSRL. The tumor findings from these four rat studies are remarkably consistent. Treatment-related increases in MNCL were observed in all four studies and

[96] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[97] Initial Statement of Reasons for Diisononyl phthalate (DINP), available at: http://www.oehha.ca.gov/prop65/law/pdf_zip/121914_ISORA_25903.pdf

[98] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[99] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

increases in liver tumors were observed in three. The MNCL response is thus more consistent than the liver tumor response across these Fischer 344 rat studies of DINP.

Following Section 25703, OEHHA considers the most sensitive study or studies deemed to be of sufficient quality for risk analysis and derivation of NSRLs under Proposition 65. For purposes of deriving an NSRL for DINP, the most sensitive studies of sufficient quality for risk analysis are the four long-term carcinogenesis studies of DINP conducted in Fischer 344 rats[100,101].

Comment 5 (ACC, CEA, CSPA, ECC): OEHHA should apply a 10-fold scaling factor (i.e., divide the cancer potency by 10) to account for reduced sensitivity of humans as compared to rodents.

ACC, CEA, and CSPA comment that OEHHA should revise the proposed NSRL for DINP, basing the NSRL on rodent liver tumor responses, and should apply a factor of 10 to account for the reduced sensitivity of humans as compared to rodents to agents that cause liver tumors through a mechanism involving PPAR-α activation and peroxisome proliferation, as was done when developing the NSRL for DEHP. Specifically, a 10-fold scaling factor should be applied to account for differences in liver receptor (PPAR-α) density between rodents and humans. Using this approach, ACC and CEA propose an NSRL for DINP of 2664 µg/day, based on the liver tumor response observed in a study conducted in male mice.

ECC has similar comments, and additionally expresses concern that the approach taken to calculate the NSRL for DINP is inconsistent with that previously taken to calculate an NSRL for DEHP. ECC proposes an NSRL for DINP in the range of 2664-2826 µg/day, based on the same male mouse liver tumor data as that used by ACC.

Response 5

The approach taken by OEHHA in deriving an NSRL for DEHP in 2002 was based on the scientific information available at that time regarding the mode of action by which DEHP induced liver tumors. Specifically, the activation of liver PPARα was thought

[100] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.
[101] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

back then to be a required key event in the induction of liver tumors by DEHP. Given that understanding, OEHHA applied a ten-fold scaling factor to account for the approximately 10-fold lower PPARα density in human as compared to rodent liver, and the assumed corresponding lower level of human cellular sensitivity to DEHP-induced liver carcinogenesis. However, the approach taken in deriving the DEHP NSRL was never adopted as an approach for all agents that induce rodent liver tumors through a PPARα mode of action. Moreover, as discussed in the response to Comment 2 above, and as summarized in OEHHA (2013)[102], new scientific information published since 2002 modifies the scientific basis for the risk assessment of DEHP. Research conducted since 2002 has shown that DEHP can induce liver tumors in mice in the absence of PPARα activation. Other studies have further called into question the proposed PPARα activation-dependent mechanism of liver tumor induction[103]. We note in particular the IARC (2013)[104] conclusion that DEHP-induced liver carcinogenesis in rodents is the result of a "combination of molecular signals and multiple pathways rather than a single hallmark event". On the basis of the available mechanistic information and chemical structural similarity of DINP and DEHP, it is likely that the mechanisms of liver carcinogenesis for the two chemicals are similar.

As indicated in OEHHA (2013)[105], "The mechanisms by which DINP induces tumors are not known; however several studies provide information on a number of possible mechanisms of action." Among the possible mechanisms discussed are hypotheses that have been put forward suggesting the involvement of PPAR-agonism in DINP induced mouse and rat liver tumorigenesis, but these are not the only mechanisms or tumor sites plausibly involved in DINP carcinogenesis. Therefore, application of a 10-fold scaling factor is not warranted in the derivation of the NSRL for DINP.

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[102] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[103] As summarized in Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[104] International Agency for Research on Cancer (IARC, 2013). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 101. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-water, Di(2-ethylhexyl) Phthalate. World Health Organization. Lyon, France.

[105] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

As discussed in the response to comment 4 above, both MNCL and liver tumor data from four long-term carcinogenesis studies of DINP conducted in Fischer 344 rats[106,107] (two in females and two in males) were included in the dose-response analyses used to derive the NSRL. The DINP NSRL of 146 µg/day is based on analysis of the most sensitive studies of sufficient quality, and is developed using the best currently available science. No change to the proposed regulation was made based on this comment.

Comment 6 (ECPI): DINP did not produce treatment-related preneoplastic and neoplastic lesions in the liver.

The derived NSRL is based on four two-year diet studies conducted in male and female rats (Moore, 1998; Lington et al., 1997). Statistically significant increases in MNCL and liver tumors were observed in both sexes in the Moore (1998) studies. In the studies by Lington et al. (1997), statistically significant increases in MNCL were observed in both sexes, and a statistically significant increase in liver carcinoma was observed at the highest dose in male rats. "However, Lington et al. (1997) concluded from their overall study-results that DINP did not produce treatment-related preneoplastic and neoplastic lesions in the *[sic]* in the liver based on the data presented."

Response 6

OEHHA agrees with the commenter that (1) MNCL and liver tumors are treatment-related and significantly increased in the Moore (1998) studies[108] in male and female rats[109], and (2) MNCL is significantly increased in both sexes and liver carcinoma is significantly increased in males[110] in the Lington et al. (1997) studies[111].

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[106] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[107] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[108] *Ibid*.

[109] MNCL was statistically significantly increased ($p < 0.05$) at the two highest doses by pairwise comparison with controls in both males and females. The increased incidence of MNCL with dose was statistically significant by the exact trend test in males ($p < 0.01$) and females ($p<0.001$). Liver tumors (combined hepatocellular adenoma and carcinoma) was statistically significantly increased at the highest dose by pairwise comparison with controls in males ($p<0.001$) and females ($p<0.05$). The increased

In contrast with the commenter's interpretation of Lington et al. (1997)[112], OEHHA finds that the statistically significant positive trend in hepatocellular carcinoma of the liver, a malignant neoplastic lesion, observed in the Lington et al. (1997) male rat study is biologically significant and related to DINP treatment. Similar observations of DINP-induced hepatocellular tumors in the male rat study by Moore (1998)[113] provide additional support for this finding. In addition, OEHHA notes that increases in preneoplastic liver lesions, such as liver foci, are neither expected nor required in order to determine that an increase in neoplastic liver lesions, such as hepatocellular carcinoma, is treatment-related.

Comment 7 (ACC, ECC, TERA, TS): The use of the linearized multistage model is not consistent with the data.

ECC states, "We do not agree that the underlying assumptions required by the linearized multistage model have been satisfied by the data," and notes that DINP is not genotoxic, argues that the justification for using a linearized multistage model for DINP is much weaker than for DEHP, and claims the liver tumor incidences provide empirical evidence of a threshold. TERA presents similar concerns as ECC about the use of the linearized model.

ACC, ECC, and TERA argue that the DINP rodent liver tumor data are most compatible with a threshold model. In support, ACC and ECC provide the presentation of Dr. Richard McKee and TERA provides the presentation (and subsequently revised slides) of Dr. Michael Dourson, both of which were presented at the Feb. 25, 2015 public hearing on the NSRL, and which contain graphical representations of the DINP rat liver tumor data.

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incidence of these liver tumors with dose was statistically significant by the exact trend test ($p<0.001$) in males and females.

[110] MNCL was statistically significantly increased at the mid- and high-dose by pairwise comparison with controls in males ($p< 0.05$ and $p<0.01$, respectively), and at the high-dose by pairwise comparison with controls in females ($p<0.001$). The increased incidence of MNCL with dose was statistically significant by the exact trend test in males and females ($p<0.001$). In males the increased incidence of liver tumors (hepatocellular carcinoma) with dose was statistically significant by the exact trend test ($p<0.015$).

[111] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[112] *Ibid.*

[113] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

TS suggests that MNCL follows a threshold mode of action.

Response 7

Consistent with Section 25703, scientific practices in other OEHHA programs[114] and other scientific guidance, including US EPA's 2005 cancer risk assessment guidelines[115], OEHHA used the benchmark dose method to estimate the low dose slope of the dose response curve. The model used for fitting the data is, specifically, the multistage model, which includes the higher order term(s) in the fitted polynomial to account for non-linearity in the dose-response. This procedure explicitly avoids making any "underlying assumptions" about mechanism, and concentrates on the mathematical standards for obtaining an adequate fit to the data in the range of observation. Detailed discussion is presented below showing that this procedure does in fact fit the DINP liver tumor data very well, as shown by the statistics in Table 1 below.

In the benchmark dose method, the extrapolation from the point of departure is a separate step from the selection of a model to fit the observed data, and is governed by mechanistic considerations only when these are available and sufficiently certain. For cancer risk assessment the default approach used by OEHHA[116,117] (and US EPA[118]) is that, in the absence of compelling information indicating the existence of a threshold, the linear extrapolation will be used. In particular, there is no need to establish a genotoxic mechanism before using the linear extrapolation. This is the default approach used by OEHHA in the absence of sufficient evidence to the contrary. It has also been shown that several proposed non-genotoxic mechanisms might well produce linear dose response in the low-dose range of interest[119].

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[114] Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors" (May 2009) Office of Environmental Health Hazard Assessment. Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html

[115] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.

[116] Office of Environmental Health Hazard Assessment (OEHHA, 2011). No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen 4-Methylimidazole. Reproductive and Cancer Hazard Assessment Branch, OEHHA, California Environmental Protection Agency, October.

[117] Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors" Office of Environmental Health Hazard Assessment.(May 2009) Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html

[118] US EPA (2012). Benchmark Dose Technical Guidance. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC. Available at URL: http://www2.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

[119] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.

OEHHA's 2013 document entitled, "Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)[120]" summarizes and discusses data relevant to several possible mechanisms of action for the carcinogenesis of DINP, and, as noted in the Initial Statement of Reasons, none of the possible mechanisms of action has the level of evidence necessary to depart from the non-threshold assumption specified in Section 25703[121]. This is consistent with guidance provided by other authoritative sources, such as US EPA (2005)[122]. While the commenters supplied a hypothesis for how DINP may cause liver cancer in rodents (through PPARα activation), the hypothesis is not supported by a robust body of scientific data, as discussed at length by OEHHA in the document supporting the NSRL calculation[123]. Similarly, there are insufficient data on the mechanism by which DINP induces MNCL to depart from the non-threshold assumption specified in Section 25703. Convincing evidence for a threshold in the dose response curve is rarely, if ever, provided by inspection or analysis of the bioassay data alone, given the limitations of statistical power imposed by practical experiment size and variability of response. Based upon Section 25703 and consistent with other OEHHA and US EPA approaches to the assessment of risks from exposure to carcinogens, OEHHA finds there is not adequate information available to conclude that the tumorigenic response in rats to DINP is a threshold phenomenon.

With regard to the proposed similarity to DEHP, the approach taken by OEHHA in 2002 in the DEHP dose response document was never adopted as an approach for all agents that induce rodent liver tumors through a PPARα mode of action. Moreover, new scientific information published since 2002 modifies the scientific basis for the risk assessment of DEHP: we note in particular the IARC (2013)[124] conclusion that DEHP-induced liver carcinogenesis in rodents is the result of a "combination of molecular

[120] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[121] See for example: Office of Environmental Health Hazard Assessment (OEHHA, 2011). No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen 4-Methylimidazole. Reproductive and Cancer Hazard Assessment Branch, OEHHA, California Environmental Protection Agency, October.

[122] US EPA (2005). US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/P-03/001B. March 2005.

[123] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[124] International Agency for Research on Cancer (IARC, 2013). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 101. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-water, Di(2-ethylhexyl) Phthalate. World Health Organization. Lyon, France.

signals and multiple pathways rather than a single hallmark event". On the basis of the mechanistic information presently available and chemical structural similarity with DEHP, it appears likely that the situation with DINP is similar.

In support of their criticism of the use of a multistage model, the commenters presented a plot showing the combined incidence of hepatocellular adenoma and carcinoma observed in male and female rats in the studies of Moore (1998)[125] and the combined incidence of hepatocellular carcinoma and neoplastic nodules observed in male and female rats in the studies of Lington et al. (1997)[126]. As indicated in the comments by TERA, the plot shows tumor incidences that have been "normalized for control incidence". Not only does this non-standard approach to dose-response analysis inappropriately transform the incidences and change the shape of the dose-response curve, it also does not provide evidence showing that the multistage model is unsuitable for the data in question. Furthermore, the data associated with the Lington et al. (1997) studies[127] in the plot provided by the commenter do not accurately reflect what was used by OEHHA in the derivation of the NSRL for DINP. As stated in the Initial Statement of Reasons, the NSRL relied on analyses of tumor incidence data that were statistically significant either by pairwise comparison or by trend.

The remainder of this response will refer only to the data used by OEHHA in its calculations.

Use of the multistage model is generally accepted as the default approach to modeling lifetime cancer bioassay data. When the multistage cancer model within the US EPA's Benchmark Dose Software (BMDS) is fit to the liver tumor incidence data used by OEHHA in part to derive the NSRL for DINP, the p-values for the global goodness-of-fit tests, shown in Table 1 below, all indicate an acceptable fit. Local goodness-of-fit measurements (not shown) also indicate that the multistage model is appropriate for the liver tumor incidence data from each of these studies.

Overall, the information the commenters provided does not include substantial evidence that leads OEHHA to find that a departure from the default approach is scientifically

[125] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).
[126] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.
[127] *Ibid.*

more appropriate. The approach used in the calculation of the NSRL – a non-threshold assumption using the multistage cancer model – is consistent with Section 25703, current quantitative assessment practices for carcinogens by OEHHA[128], and current US EPA practices[129].

No change to the proposed regulation was made based on this comment.

Table 1. P-values Associated with Fit of Multistage Model to Liver Tumor Incidence Data Reported in the rat studies of Moore (1998) and Lington et al. (1997)

Study	Sex/species	Tumor sites used in estimating potency	P-value[a] of multistage model in BMDS
Moore 1998	Male rats	Hepatocellular adenoma and carcinoma combined	0.8028
	Female rats	Hepatocellular adenoma and carcinoma combined	0.7524
Lington et al. 1997	Male rats	Hepatocellular carcinoma	0.9459

[a] According to BMDS guidance and consistent with standard statistical analysis, a significance level of α = 0.05 is used as the cutoff (for models selected *a priori*) below which the null hypothesis of adequate fit is rejected.

Comment 8 (TERA): Study choice should take into account repeatability, relevance and potency.

"OEHHA seems to think that since 'Risk analysis shall be based on the most sensitive study deemed to be of sufficient quality' (California, 2011) *[Section 25703]* that it is only the most potent study or endpoint needs to be chosen. *Au contraire.* In risk

[128] See for example: Office of Environmental Health Hazard Assessment (OEHHA, 2011). No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen 4-Methylimidazole. Reproductive and Cancer Hazard Assessment Branch, OEHHA, California Environmental Protection Agency, October.
[129] US EPA (2012). Benchmark Dose Technical Guidance. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC. Available at URL: http://www2.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

assessment, the "most sensitive study" for determining the risk in humans should consider: Repeatability…; Relevance …; Potency…."

When repeatability, relevance, and potency are considered, "the 'most sensitive study/endpoint' is clearly liver tumors found in rodents."

Response 8

Section 25703 requires OEHHA to consider the most sensitive study or studies deemed to be of sufficient quality for risk analysis and derivation of NSRLs under Proposition 65. This does not mean that OEHHA ignores issues of repeatability, relevance or potency (which may be considered aspects of study quality) in its analysis. The tumor findings in the four long-term carcinogenesis studies of DINP conducted in Fischer 344 rats[130,131] (two in females and two in males) are remarkably consistent. Treatment-related increases in leukemia (MNCL) were observed in all four studies, and treatment-related increases in liver tumors were observed in three. Thus MNCL and liver tumors are repeatable, relevant and each contributes significantly to the overall cancer potency. Hence both MNCL and liver tumors were included in the analysis and derivation of the NSRL for DINP.

No change to the proposed regulation was made based on this comment.

Comment 9 (TERA): Honoring others' judgment

The findings of expert groups and individual experts should be honored.

- The most sensitive study/effect is a liver tumor.
- A threshold approach to dose response assessment is the most scientific reasonable option for DINP's dose response assessment.

[130] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.
[131] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

Response 9

In developing the NSRL for DINP OEHHA relied on the 2013 document entitled, "Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP)[132]," which summarizes the available data from rodent carcinogenicity studies of DINP, as well as other information relevant to the carcinogenic activity of the chemical. The 2013 document is a concise review of the available scientific literature on the carcinogenic activity of DINP. It includes discussion of the issues raised by the commenter regarding the human relevance of the MNCL, liver and kidney tumor findings and refers to several of the expert reports[133] mentioned by the commenter. The 2013 document was developed to assist the state's qualified experts, the Proposition 65 Carcinogen Identification Committee (CIC), in its consideration of DINP for listing under Proposition 65[134]. Also as noted above, the Notice of Proposed Rulemaking and the Initial Statement of Reasons for the proposed NSRL for DINP were provided to the members of the CIC for their review and comment, as required by Section 25302(e). Thus in the derivation of the NSRL for DINP, OEHHA considered relevant findings supported by science, including findings by the CIC, the state's qualified experts for carcinogenicity under Proposition 65.

As noted in the response to comment 8, the tumor findings in the four long-term Fischer 344 rat carcinogenesis studies of DINP[135,136] are remarkably consistent. Treatment-

[132] Office of Environmental Health Hazard Assessment (OEHHA, 2013). Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP). California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Branch, October, 2013, available at: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/DINP_HID100413.pdf

[133] E.g., CPSC (2001). Consumer Product Safety Commission. Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP); CPSC (2010). Consumer Product Safety Commission. Toxicity review of diisononyl phthalate (DINP); ECHA (2010). European Chemicals Agency. Review of new available information for Di-'isononyl' phthalate (DINP) CAS No. 28553-12-0 AND 68515-48-0. Document developed in the context of evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to regulation (EC) No. 1907/2006 (REACH); ECB (2003). European Chemicals Bureau: 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP), CAS Nos: 68515-48-0 and 28553-12-0, EINECS Nos: 271-090-9 and 249-079-5, Summary Risk Assessment Report, Special Publication I.03.101, p. 18. Available at http://echa.europa.eu/documents/10162/0645f0cb-7880-4d23-acea-27b05ed7de39.

[134] DINP was listed as known to the State to cause cancer under Proposition 65 on December 20, 2013, based on a determination by the CIC that it had been clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer.

[135] Lington AW, Bird MG, Plutnick RT, Stubblefield WA, Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Funda Appl Toxicol* **36**:79-89.

[136] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US

related increases in MNCL were observed in all four studies, and treatment-related increases in liver tumors were observed in three. Estimating the cancer potency of DINP based solely on liver tumor data is not appropriate, as it would fail to take into account treatment-related MNCL, which is observed in both sexes of the rat, is reproducible, and contributes significantly to the overall cancer potency. Moreover, for carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a given study, in deriving estimates of carcinogenic activity it is important to account for all treatment-related tumors significantly contributing to risk. Thus data for the liver and MNCL tumor sites/types are included in the dose-response analysis to derive a multisite potency estimate. Thus, multisite potencies were estimated for the three studies in which MNCL and liver tumors were observed (and a single site potency was estimated for the fourth study in which the only treatment-related cancer observed was MNCL).

As discussed in detail in the response to comment 7, the use of a linear dose-response model rather than a threshold approach is the most scientifically appropriate option for DINP's dose response assessment.

No change to the proposed regulation was made based on this comment.

Comment 10 (TCG): NSRL should be based on the male rat study of Lington et al. (1997)

The Lington et al. (1997) study of male rats supports an NSRL of 70 µg/day. While the most sensitive study is specific to male rats, we see no legal or practical basis for not utilizing this data as the basis for the NSRL.

Response 10

OEHHA identified four long-term carcinogenesis studies of DINP conducted in Fischer 344 rats[137,138], two in females and two in males. The tumor findings are remarkably consistent across each of these well-conducted studies, with treatment-related leukemia

Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

[137] *Ibid.*

[138] Moore MR (1998). Oncogenicity study in rats with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analysis. Covance laboratories, Inc., Vienna, VA. Study No. 2598-104, as reviewed by Consumer Product Safety Commission (CPSC, 2001). Report to the US Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on diisononyl phthalate (DINP).

observed in all four studies, and liver tumors observed in three. No clear differences in cancer potency were observed between the sexes, and no single study was judged to be more appropriate than another. Thus, the geometric mean of the potency estimates derived from each of the four studies was taken as the basis for the NSRL.

No change to the proposed regulation was made based on this comment.

Comment 11 (TCG): Separate NSRL for each sex

If OEHHA considers it more scientifically appropriate to establish a separate NSRL for each sex, our position supports such a methodology.

Response 11

It is not more scientifically appropriate to establish separate, sex-specific NSRLs for DINP. The tumor findings are remarkably consistent across each of these well-conducted studies, with treatment-related leukemia observed in all four studies, and liver tumors observed in three. No clear differences in cancer potency were observed between the sexes, and no single study was judged to be more appropriate than another.

No change to the proposed regulation was made based on this comment.

Comment 12 (CEA): Concern over the timing of this rulemaking process.

"As a matter of process for future Prop 65 chemical listings and NSRLs, CEA is very concerned with the timing of this rulemaking process. The Notice of Proposed Rulemaking for the DINP NSRL was issued on December 19, 2014 - only one day before the *[warning requirement associated with the December 20, 2013]* DINP listing became effective on December 20, 2014.

Response 12

There is no legal requirement for OEHHA to establish safe harbor levels for listed chemicals. OEHHA adopts them to provide guidance for affected businesses. In recent years, when feasible and appropriate, OEHHA has attempted to propose safe harbor levels for listed chemicals prior to the effective date of the listing. It is not always possible to do so, however. In this instance, the proposed rulemaking was published concurrent with the requirement for providing warnings.

ALTERNATIVES DETERMINATION

In accordance with Government Code section 11346.9(a)(4), OEHHA has, throughout the adoption process of this regulation, considered available alternatives to determine whether any alternative would be more cost effective in carrying out the purpose for which the regulation was proposed, or would be as cost effective and less burdensome to affected private persons than the proposed action. OEHHA has determined that no reasonable alternative considered by OEHHA or that has otherwise been identified or brought to the attention of OEHHA would either be more effective in carrying out the purpose for which the action is proposed, or would be as effective and less burdensome to affected private persons, or would be more cost-effective to affected private persons and equally effective in implementing the statutory policy or other provision of law than the proposed regulation.

For chemicals listed under the Act as known to cause cancer, the Act exempts discharges to sources of drinking water and exposures of people without provision of a warning if the exposure poses “no significant risk” of cancer (Health and Safety Code, section 25249.10(c)). The Act does not specify numerical levels of exposure that represent no significant risk of cancer.

The purpose of this regulation is to establish a No Significant Risk Level for DINP. At or below this level, the Act does not require a warning or prohibit discharges of the chemical to sources of drinking water. Thus, adopting this level will allow persons subject to the Act to determine whether a given discharge to sources of drinking water or a given exposure to this chemical is subject to the warning requirement or discharge prohibition provisions of the Act (Health and Safety Code, section 25249.5 and 25349.6).

Although Section 25703 describes principles and assumptions for conducting risk assessments to derive No Significant Risk Levels, some businesses subject to the Act do not have the resources to perform these assessments. Yet each business with ten or more employees must determine whether its activities or products are subject to the discharge prohibition or warning requirements of the Act. Adopting an NSRL for this chemical provides an efficient way of determining if a business is in compliance with the Act.

LOCAL MANDATE DETERMINATION

OEHHA has determined this regulatory action will not pose a mandate on local agencies or school districts nor does it require reimbursement by the State pursuant to Part 7 (commencing with Section 17500) of Division 4 of the Government Code. OEHHA has also determined that no nondiscretionary costs or savings to local agencies or school districts will result from this regulatory action. Proposition 65 provides an express exemption from the warning requirement and discharge prohibition for all state and local agencies. Thus, these regulations do not impose any mandate on local agencies or school districts.