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APTCO, LLC'S COMMENTS IN OPPOSITION TO

February 27, 2015 NOTICE OF INTENT TO LIST STYRENE

UNDER THE AUTHORITATIVE BODIES LISTING MECHANISM OF PROPOSITION 65

APRIL 29, 2015

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INTRODUCTION

Because there is insufficient evidence that styrene causes cancer, California and federal law require that the Office of Environmental Health Hazard Assessment (OEHHA) withdraw its proposal to list styrene as *known* to the state to cause cancer under Proposition 65 based on the National Toxicology Program's (NTP) listing of styrene in its *Report on Carcinogens, Twelfth Edition* (2011) (12th RoC).

APTCO, LLC (APTCO) addresses in these comments the reasons OEHHA is not authorized under California law and why it would be unlawful under federal law for OEHHA to list styrene under Proposition 65 when there is insufficient evidence that it causes cancer. The primary reasons are:

1) The NTP did not consider a key animal study that concluded there was no convincing evidence that styrene causes cancer. The NTP's listing is based on two animal studies, which it concludes are *sufficient*, but the NTP changed the outcome of one of the two animal studies (NCI 1979), which was negative, by replacing the concurrent controls in the study with historical controls from a different laboratory. The NTP did this to create a second positive study to support its listing. This violated both the NTP's principles and generally accepted principles of scientific testing. In addition, the International Agency for Research on Cancer (IARC) had concluded in 2002 that the same two animal studies the NTP later relied upon in the 12th RoC to list styrene did not constitute sufficient evidence that styrene causes cancer, because the NCI study was negative. OEHHA's Authoritative Bodies listing criteria require that OEHHA consider the scientifically valid study the NTP did not consider, (NCI 1979). Neither Proposition 65 nor the California courts authorize OEHHA to list chemicals unless they have been shown to cause cancer based on sufficient evidence.

2) Because the NTP considered Mode of Action (MOA) data, OEHHA is required to consider MOA the NTP did not consider. Additional MOA data show the NTP's animal evidence on styrene is not relevant to humans, undermining the sufficiency of the NTP's laboratory animal evidence.

3) Even though OEHHA's proposal to list styrene is not based on human evidence because the NTP concluded the human evidence is limited, and Proposition 65 requires at least sufficient human or animal evidence, consideration of the new human data that has emerged since the NTP's listing shows that the human evidence the NTP considered is further limited. This new

data also reinforces the relevance of the new mode of action data showing that animal evidence on mouse metabolism of styrene is not relevant to humans.

4) If OEHHA proceeds to list styrene under Proposition 65 based on insufficient evidence that it causes cancer, i.e, only one positive animal study and one re-constructed and scientifically invalid animal study, its listing would violate the Freedom of Speech, Due Process and Interstate Commerce clauses of the United States Constitution and would also be preempted by federal law.

As APTCO shows below, the Proposition 65 statute, the California Courts of Appeal and the Authoritative Bodies listing regulation require that OEHHA independently review the scientific evidence the NTP considered to ensure that it is *in fact* based on sufficient evidence. OEHHA cannot merely adopt or defer to the NTP's opinion that there is sufficient evidence that styrene causes cancer. If it intends to proceed with this listing, OEHHA must legally and scientifically justify how styrene can be listed under Proposition 65 based solely on one animal study, particularly when new data have emerged in the four years since the NTP's listing confirming that there is insufficient data to conclude that styrene causes cancer.

APTCO MAKES A SAFE AND VALUABLE PRODUCT

APTCO is based in Delano, California and manufactures expandable polystyrene (EPS) grape boxes which are used to ship grapes worldwide. For forty years the EPS industry has been supplying boxes and packaging that have been considered safe worldwide.

The United States Food and Drug Administration considers polystyrene food packaging safe. See 21 C.F.R. § 170.1640 (2014). After the NTP's 12th RoC styrene listing, NTP Director Dr. Linda Birnbaum told the Associated Press that styrene *is certainly not an issue* in finished products (June 2011). NTP Associate Director John Bucher agreed with this statement and told the Associated Press that he believed polystyrene was *not worth being concerned about* (August 2011). Bloomberg News reported that American Cancer Chemical Society Chief Medical Officer Otis Brawley stated that *consumers don't need to worry about polystyrene cups and food containers* (June 2011).

Given these facts, OEHHA is not authorized to subject APTCO as well as tens of thousands of people and businesses nationwide and in California, to the significant and likely devastating effects of a listing that contends styrene is

known to cause cancer when scientifically valid data clearly show it is not *known* to cause cancer.

[A] wrong decision that a chemical does pose a substantial risk of causing cancer in humans may result in serious negative consequences to those who forego using a beneficial product. Baxter Healthcare Corp. v. Denton, 120 Cal.App.4th 343, 344 (2004).

The following additional facts demonstrate the significant responsibility OEHHA owes to APTCO and the public when deciding whether to proceed with a styrene listing:

- Styrene is naturally occurring. It was first discovered in the bark of the Liquidambar tree two hundred years ago. It is naturally present in many fruits, vegetables and spices, including cinnamon, strawberries and peanuts. Styrene kept at room temperature will self-polymerize into polystyrene.

- Expandable polystyrene (EPS) packaging has the advantage of being inert and does not impart a smell or taste into food, nor does it absorb water from the food. This helps keep food fresh and flavorful.

- Researchers agree that as much as 40 to 50 percent of fruits and vegetables spoil due in part to inadequate preservation, protection, storage and transportation. E.g., IFT, *Food Packaging – Roles, Materials, and Environmental Issues*, Journal of Food Science (2007) (citing Food and Agriculture Organization of the United Nations (FAO), *Prevention of post-harvest food losses: fruits, vegetables, and root crops. A training manual.* (Rome 1989)); FAO, *Global Food Losses and Food Waste: Extent, Causes and Prevention* (Rome 2011).

- The FAO has concluded that *economically avoidable food losses have a direct and negative impact on the income of both farmers and consumers*. Id. at Introduction.

- EPS packaging such as APTCO's grape boxes help prevent massive amounts of food spoilage and waste, but that food storage tool could be lost under a Proposition 65 listing. This illustrates another wrong-headed consequence of OEHHA's NOIL for styrene.

- OEHHA first attempted to list styrene in 2009 based on the IARC's 2002 styrene listing which was based on the same evidence the NTP relied upon for its 12th RoC. Responding to OEHHA's attempted listing based on IARC's listing, Sacramento Superior Court Judge Shelleyanne Chang issued a preliminary injunction to preclude OEHHA from proceeding with its proposed styrene listing until the court could determine whether the IARC's limited evidence was sufficient under Proposition 65. The court ultimately ruled it was not sufficient to support a finding that styrene is known to cause cancer. The Court of Appeal affirmed Judge Chang's ruling. Judge Chang's preliminary ruling was based on the following factors indicating potential immediate and irreparable harm if styrene had been listed under Proposition 65 *contrary to California law*:

    - The *devastating effect* OEHHA's listing would have on the $28 billion styrene industry, including on its use of food packaging employed in the transportation of California agricultural products.

    - California's $1.3 billion in strawberry sales, its $285 million in raspberry sales and its $30 million in blueberry sales would be significantly impacted by a Proposition 65 listing of styrene.

    - *The designation of a product as a carcinogen, particularly associated with food, could have a devastating effect on that product's use. Such a designation would likely have the intended* stigmatizing effect. *Citing Baxter Healthcare Corp. v. Denton*, 124 Cal.App.4th 333, 344 (2004).

Styrene Information and Research Center v. Office of Env'l Health Haz. Assessment, Sacramento Sup. Ct., Case No. 34-2009-0053089-CU-JR-GDS, Order on Preliminary Injunction at 4-5 (Aug. 2, 2009), final ruling affirmed, SIRC v. OEHHA, 210 Cal.App.4th 1082 (2012).

Notably, in the proceedings before Judge Chang, OEHHA did not dispute SIRC's showing that *recent evidence shows that styrene is not a human carcinogen*. Id. Now, OEHHA completely changes its agency position with no new scientific evidence—just NTP's reconstruction of the NCI's 1979 study, using historic controls from a different laboratory, a relatively small control group and rough estimates. That's arbitrary, inconsistent agency behavior and it's not good science.

Lacking any new scientific data, OEHHA once again is attempting to list styrene as a chemical *known* to cause cancer when that contention has twice before been undone—once through full litigation and appeal of the same facts OEHHA relies upon now.

THE PROPOSITION 65 STATUTE AND THE CALIFORNIA COURTS AUTHORIZE OEHHA TO LIST ONLY CHEMICALS THAT HAVE BEEN CLEARLY SHOWN TO CAUSE CANCER THROUGH SUFFICIENT EVIDENCE BASED ON SCIENTIFICALLY VALID TESTING ACCORDING TO GENERALLY ACCEPTED SCIENTIFIC PRINCIPLES

The Safe Drinking Water and Toxic Enforcement Act of 1986 ("Proposition 65"), Cal. Health and Safety Code § 25249.5 et seq. (2014) provides in Section 25249.6 (with emphasis added):

> *No person in the course of doing business shall knowingly and intentionally expose any individual to a chemical known to the state to cause cancer or reproductive toxicity without first giving clear and reasonable warning to such individual, except as provided in Section 25249.10.*

Section 25249.8(b) provides (with emphasis added):

> *A chemical is known to the state to cause cancer or reproductive toxicity within the meaning of this chapter if in the opinion of the state's qualified experts it has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity, or if a body considered to be authoritative by such experts has formally identified it as causing cancer or reproductive toxicity, or if an agency of the state or federal government has formally required it to be labeled or identified as causing cancer or reproductive toxicity.*

The Proposition 65 statute addresses only *known* carcinogens. It also provides that only chemicals that have been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer qualify as *known* carcinogens. These statutory requirements are confirmed by the original intent of the statute, which was stated in the preamble to the proposed statute, which provided in part:

> *The people of California find that hazardous chemicals pose a serious potential threat to their health and well-being, that state government agencies have failed to provide them with adequate protection, and that these failures have been serious enough to lead to investigations by federal agencies of the administration of California's toxic protection programs. The people therefore declare their rights: (a) To protect themselves and the water they drink against chemicals* <u>*that cause*</u> *cancer, birth defects, or other reproductive harm. (b) To be informed about exposures to chemicals* <u>*that cause*</u> *cancer, birth defects or other reproductive harm.*

Prop. 65 Ballot Pamphlet, Proposed Stats. Gen. Elec., at 53 (Nov. 4, 1986) (emphasis added) (<u>also</u> <u>quoted in</u> <u>SIRC v. OEHHA</u>, 210 Cal.App.4th at 1098, emphasizing the phrase *that cause* to support the Court's holding that OEHHA has a duty to it list only chemicals that *in fact* cause cancer).

This statute's original intent was also expressed in the Proposition 65 ballot materials: *There are certain chemicals that are* <u>*scientifically known--not merely suspected, but known--to cause*</u> *cancer and birth defects. Proposition 65 would: Warn us before we're exposed to any of these dangerous chemicals.* Ballot Pamphlet at 54 (emphasis added).

The ballot further stated: <u>*Proposition 65 singles out chemicals that are scientifically known to cause cancer*</u> *or reproductive disorders*. <u>Id.</u> (emphasis added). Finally: <u>*Proposition 65's new civil offenses focus only on chemicals that are known to the state to cause cancer or reproductive disorders. Chemicals that are only suspect are not included.*</u> <u>Id.</u> (emphasis added).

It was on the basis of this important distinction between chemicals known to cause cancer and those merely suspected of causing cancer, that California voters approved Proposition 65. OEHHA cannot unilaterally and arbitrarily change that legal compact now.

The California Courts of Appeal agree the statute authorizes OEHHA to list only *known* carcinogens - not *suspected* carcinogens. When OEHHA attempted to list styrene based on the same evidence the NTP relied upon, found in the IARC's 2002 conclusions, which explicitly provided that <u>both</u> human and animal evidence was *limited* and thus <u>insufficient to conclude that styrene is a known carcinogen</u>, the Court ruled in <u>SIRC v. OEHHA</u>, 210 Cal.App.4th 1082, that OEHHA lacked

sufficient evidence in humans or animals to list styrene as *known* to cause cancer. Id. at 1101. The Court stated the following regarding chemicals listed under Proposition 65's Labor Code listing mechanism:

> *Our analysis in Western Crop [(2000) 80 Cal.App.4th 741], like that in [Deukmejian], [(1989) 212 Cal.App.3d] was based on a recognition that chemicals may be included on the Proposition 65 list only if there is a sufficient showing that they in fact cause cancer or reproductive toxicity. This interpretation is consistent with the legislative history underlying Proposition 65 and does not conflict with the minimum requirements language of section 25249.8, subdivision (a).*

Id.

THE AUTHORITATIVE BODIES LISTING CRITERIA PRECLUDE OEHHA FROM LISTING A CHEMICAL IF THE AUTHORITATIVE BODY'S LISTING IS NOT BASED ON SUFFICIENT EVIDENCE, IF IT FAILED TO CONSIDER SCIENTIFICALLY VALID DATA SHOWING THE CHEMICAL DOES NOT CAUSE CANCER, IF THERE WAS AN INSUFFICIENT NUMBER OF POSITIVE STUDIES OR IF NEW SCIENTIFICALLY VALID DATA SHOW THE CHEMICAL DOES NOT CAUSE CANCER

The requirements under the Authoritative Bodies listing regulation reflect the Proposition 65 statute's mandate that OEHHA may list only those chemicals for which sufficient and scientifically valid data support the conclusion that they are *in fact known* to cause cancer. The regulation provides in pertinent part as follows (with emphasis added):

> *(e) For purposes of this section [chemicals formally Identified by Authoritative Bodies], "as causing cancer" means that either of the following criteria has been satisfied:*
>
> > *(1) sufficient evidence of carcinogenicity exists from studies in humans. For purposes of this paragraph, "sufficient evidence" means studies in humans indicate that there is a causal relationship between the chemical and cancer.*
> >
> > *(2) sufficient evidence of carcinogenicity exists from studies in experimental animals. For purposes of this paragraph, "sufficient evidence" means studies in experimental animals indicate that there is an increased incidence of malignant tumors or combined malignant*

and benign tumors in multiple species or strains, in multiple experiments (e.g., with different routes of administration or using different dose levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset.

(f) The lead agency shall find that a chemical does not satisfy the definition of "as causing cancer" if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (e), paragraph (1) or subsection (e), paragraph (2).

27 Cal. Code Regs § Section 25306 (*Chemicals Formally Identified by Authoritative Bodies*).

A failure to meet these standards is considered a regulatory failure to provide *substantial evidence*.

(i) . . . . Objections shall be made on the basis that there is no substantial evidence that the criteria identified in subsection (e) . . . have been satisfied.

OEHHA's predecessor, the California Health and Welfare Agency, promulgated the Authoritative Bodies listing regulation in 1990 and explained its intended understanding of the sufficiency of evidence requirement of the regulation to require at least two or more positive studies: *Where there is in fact an insufficient number of positive animal or human studies, but the authoritative body has concluded anyway that the chemical causes cancer, the Agency will be prevented by the regulation from bringing the chemical to the list.* Final Statement of Reasons, Section 12306 [now § 22506] – Chemicals Formally Identified by Authoritative Bodies (Feb. 1990).

The California Courts of Appeal agree that OEHHA may not merely defer to an authoritative body's conclusion that a chemical causes cancer and must independently examine the underlying record to ensure a chemical in fact causes cancer. For example, the SIRC v. OEHHA Court held, as quoted above, that OEHHA must determine there is sufficient evidence that a chemical *in fact* causes cancer prior to listing a chemical that has been deemed a carcinogen under the Labor Code listing mechanism. 210 Cal.App.4th at 1101.

In Western Crop Protection Ass'n v. Davis, 80 Cal.App.4th 741, the plaintiffs contended OEHHA had encroached upon the role of the state's qualified experts

to determine whether an EPA chemical listing in fact satisfied the Proposition 65 listing criteria. The Court ruled that OEHHA had not because the Authoritative Bodies listing regulation authorizes OEHHA to ensure chemicals meet the listing criteria in the regulation pursuant, in part, to the regulation's mandate that OEHHA review objections to the placement of chemicals on the Proposition 65 list made on the ground that there is not substantial evidence that the criteria identified in the regulation have been satisfied. Id. at 753-54. Thus, OEHHA must actively evaluate and determine whether the regulation's criteria have been met. See *id*. OEHHA cannot passively propose or make a Proposition 65 listing because an authorized body made a listing or announced a chemical causes cancer. That is inadequate.

The Court of Appeal in Western Crop emphasized that *[it] is a question of California law whether the EPA action satisfies the statutory criteria of "formally identified [chemicals on the list] as causing . . . . reproductive toxicity"* and not a question of what the EPA may have said or done. Id. at 756.

Finally, OEHHA does not satisfy the requirements of Proposition 65 or its own agency in attempting to list styrene because of an *exception* under the settlement it has entered in Sierra Club. v. Brown, Alameda Sup. Ct., Case No. RE07356881, Stipulation for Entry of Partial Consent Judgment and Order Thereon (2013). A settlement with the Sierra Club does not authorize OEHHA to take action it is not otherwise authorized to do. OEHHA is bound by its statutory authority and limitations and remains required to comply with its own rules concerning Proposition 65 despite the Sierra Club settlement. Entering that settlement does not *liberate* OEHHA from its statutory and regulatory limits, nor does it endow OEHHA with new regulatory powers.

OEHHA CANNOT PASSIVELY ACCEPT THE NTP'S CONCLUSION THAT STYRENE IS ANTICIPATED TO CAUSE CANCER

Here, OEHHA is bound by California law – not the NTP's 12th RoC. APTCO will discuss in more detail below that the NTP's listing of styrene cannot support the conclusion that styrene is *known* to cause cancer because the NTP's listing was not based on sufficient evidence of carcinogenicity in experimental animals, because the NTP failed to consider a scientifically valid key animal study that found no convincing evidence of styrene's carcinogenicity, and because the NTP failed to consider certain mode of action evidence that shows metabolic responses to styrene in laboratory mice are inapplicable to humans.

Proposition 65 does not authorize OEHHA to list styrene as a *known* carcinogen based solely on the NTP's opinion that there is sufficient and valid scientific evidence for styrene as a carcinogen. In deciding whether styrene meets the Proposition 65 listing criteria, OEHHA must consider the scientifically valid animal study the NTP did not consider. It must also consider the animal and human evidence that has emerged during the four years since the NTP's listing, including new Mode of Action (MOA) data that further undermine the sufficiency of the NTP's animal evidence as it relates to humans. Consideration of this additional evidence shows the NTP's styrene listing does not meet California's Proposition 65 standard.

With respect to MOA data, although the NTP's listing was based on its allegedly *sufficient* animal data, the NTP reviewed mode of action data for styrene and concluded the data do not contradict the relevance of cancer studies in mice for evaluation of human hazard. Under the Authoritative Bodies listing criteria, OEHHA must therefore consider MOA data the NTP did not consider that show the NTP lacked substantial evidence that this data is relevant to humans.

In Baxter HealthCare Corp. v. Denton, 120 Cal.App.4th at 372-73, the court held that substantial evidence showing that the biological mechanism through which a chemical causes cancer in rodents does not exist in humans establishes that a chemical poses no significant risk of causing cancer in humans. Likewise, OEHHA cannot proceed to list styrene as a *known* human carcinogen if there is new mode of action data the NTP did not consider showing that styrene does not pose a risk of cancer in humans due to metabolic or physiologic differences between laboratory mice and humans.

BECAUSE THE NTP DID NOT CONSIDER THE SCIENTIFICALLY VALID NCI ANIMAL STUDY ON STYRENE WHICH CONCLUDED THERE WAS NO CONVINCING EVIDENCE OF INCREASED INCIDENCE OF MALIGNANT TUMORS, THERE IS *INSUFFICIENT* EVIDENCE OF CARCINOGENICITY IN EXPERIMENTAL ANIMALS FOR OEHHA TO LIST STYRENE

The NTP erroneously concluded that styrene was *reasonably anticipated to be a human carcinogen*. In order to make this conclusion, because of insufficient epidemiological evidence, the NTP had to meet the following definition of *reasonably anticipated to be a human carcinogen* as set forth in the 12th RoC:

> *There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor or age at onset.*

NTP, Report on Carcinogens, Twelfth Edition, *Introduction* at 4 (2011) (emphasis added).

The NTP summarized its finding of *sufficient evidence* in its introduction to its 12th RoC Styrene Assessment: *Styrene caused lung tumors in several strains of mice and by two different routes of exposure. The most robust studies are two-year studies of inhalation exposure in CD-1 mice (Cruzan et al. 2001) and oral exposure (by stomach tube) in B6C3F1 mice (NCI 1979). . . . In male B6C3F$_1$ mice, oral/gavage exposure to styrene increased the combined incidence of benign and malignant tumors (alveolar/ bronchiolar adenoma and carcinoma), and a positive dose-response trend was observed (NCI 1979)*. 12th RoC, Styrene Substance Profile, id. (emphasis added).[1]

OEHHA cites to and quotes this finding and the two animal studies supporting it as the sole basis for its Notice of Intent to List Styrene (NOIL):

> *Formal identification and sufficiency of evidence for styrene: . . . .*
>
> *Styrene caused lung tumors in several strains of mice and by two different routes of exposure. The most robust studies are two-year-studies of inhalation exposure in CD-1 mice (Cruzan et al., 2001) and oral exposure (by stomach tube) in B6C3F1 mice (NCI, 1979). Inhalation exposure caused benign lung tumors (alveolar/bronchiolar adenoma) and increased the combined incidence of benign and*

[1] The NTP also claimed in its summary that the 2001 and 1979 studies *are supported by findings* in other studies. It left out the fact that within its report, it in fact concluded that this "supporting" evidence was limited. Id. The Styrene Expert Panel for the 12th RoC concluded in 2008 that *[m]ost of the rodent cancer bioassays summarized [in the Draft Report on Carcinogens Background Document for Styrene] had design flaws and other limitations*. NTP Styrene Expert Panel Report, Part A, p. 14 (2008). The Expert Panel emphasized in its report that the additional *supporting* studies (e.g., Ponomarkov and Tomatis 1978) were *limited in their ability to detect carcinogenic effects because of study design (low doses, short treatment, short study duration, small group size), high early mortality, or limited reporting (tumor diagnosis).* Id., Part B at 3. The NTP included a similar explanation in its 2008 Final Background Document: *Many of the studies were severely limited in their ability to detect carcinogenic effects*. 2008 Final Background Document at 214.

> *malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma) in CD-1 mice of both sexes; in females it also increased the separate incidence of malignant lung tumors. In male B6C3F1 mice, oral exposure to styrene increased the combined incidence of benign and malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma), and a positive dose-response trend was observed (NCI 1979).*

OEHHA, Notice of Intent to List: Styrene (Feb. 27, 2015) (Citing and quoting 12th RoC).

The NTP contended that it met its criteria for sufficient evidence in animals with positive test results for multiple routes of exposure (oral and inhalation) as a basis for its listing of styrene. But the NTP had no new evidence of styrene causing cancer beyond what IARC had in 2002. A review of the IARC monograph confirms that other than multiple routes of exposure, the other two criteria were not met. Tumors have only been reported in one species (mice) not rats or other species and for only one tissue site (the lung). All studies by oral or inhalation routes in rats have been negative (no increase in tumor incidence). In mice, lung tumors were only clearly elevated over controls by inhalation, but were largely benign, occurred late in life, and did not show a treatment-related dose-response pattern (i.e., increase in tumor incidence with increase in dose). A screening study involving intra-peritoneal administration (injection into the body cavity) of styrene in mice found no increase in tumor incidence. Of the four studies conducted by the oral route of exposure, two were negative (no increase in tumors) and the other two were considered inadequate for evaluation of carcinogenicity by IARC (2002). One of these oral gavage (bolus dosing) studies involved an extremely high dose that resulted in severe toxicity and early mortality and had to be terminated early. In the other study by NCI (1979), tumor incidence, although elevated over controls in males (not in females), was relatively low (arguably within the range of historical controls), more than half were benign, and the tumors occurred late in life.

The NCI's 1979 study, which NTP reversed from negative to positive by picking historic controls from a different laboratory actually demonstrated no convincing evidence for carcinogenicity, and eliminates one of the two routes of exposure. Therefore, only *one species*, one tissue site, and *one route of exposure* support

NTP's listing. The NTP criteria for sufficient evidence of carcinogenicity in animals have not been met.

The Proposition 65 listing criteria also are not satisfied by considering the NCI's 1979 conclusion regarding no evidence for carcinogenicity because it results in only one strain or one route of exposure with a positive finding. See Cal. Code Regs. § 23506(e)(2). This is not sufficient for a Proposition 65 listing. See id.

THE NTP'S RECONSTRUCTION OF NCI'S 1979 STUDY USED ROUGH ESTIMATION AND SMALL SAMPLE SIZES

The NTP revealed how it reconstructed the 1979 NCI study by replacing the control incidence of tumors with a low estimate that was considered to accurately represent the true control rate for the 1979 NCI study. Nevertheless, the true background *control* rate of spontaneous tumors for comparing the tumor incidence in the treated male mice cannot be determined with such precision. At issue here is the relatively low incidence of tumors in the treated animals and the lack of any tumors in the concurrent controls (i.e., control animals administered the corn oil vehicle but not styrene at the time of the study) versus the range in spontaneous lung tumor rates reported in historical controls in other past studies conducted by the same laboratory (0 to 20%). This bordered on guess-work.

Another contributor to uncertainty is that the 1979 NCI study used only 20 male and 20 female control mice instead of 50 of each sex for the treatment groups. With a small sample size, the odds of zero tumors in the controls is more likely relative to groups with more animals. Because the study had only two treatment groups, the odds of the high dose group having a higher incidence of tumors than the low dose group is 50% even with no treatment effect of styrene. Thus, given the low tumor rates in the styrene-treated male mice, the appearance of an increasing dose-response pattern could easily have resulted by chance.

THE NTP'S RE-CONSTRUCTED CONTROL GROUP ESTIMATES WERE INAPPROPRIATELY DRAWN FROM A DIFFERENT LABORATORY

In its *Final Report on Carcinogens Background Document for Styrene, September 29, 2008* ("2008 Final Background Document"), the NTP explained that it re-constructed a new historical control group for the original study by using vehicle control mice from different studies, different calendar years and different laboratories with a lung tumor incidence of two to three times less than the

historical incidence in control groups at the relevant NCI testing laboratory. The NTP offered the following analysis of the original study it chose to ignore and indicated in brackets how it changed the results of the study:

> *The Cochran-Armitage exact trend analysis also indicated a significant dose-response relationship for combined alveolar/bronchiolar neoplasms in male mice. This was supported by increased incidence of alveolar/bronchiolar neoplasms (adenoma and carcinoma combined) in male mice in the high-dose group compared with controls (Table 4-1). Because the incidence of lung tumors in the male vehicle-treated controls (0%) in this study was unusually low compared with historical untreated controls (32 of 271, 12%), there was some uncertainty regarding the significance of the lung tumors. NCI (1979a) reported that the historical incidence of these tumors in vehicle control male mice was 0 of 40 (2 studies from Litton Bionetics, including the styrene study); however, this was considered by NCI to be too small a number of animals for meaningful use as historical controls. [The NTP reviewed for the purpose of this document lung tumor incidences in historical vehicle controls from NCI studies conducted at other laboratories. However, although the studies were performed at different laboratories, the historical vehicle control animals were from the same source and same study protocol, and the tests were performed in the same chronological window. The selection criteria included data from corn oil vehicle controls for gavage studies in male B6C3F1 mice conducted prior to 1979 with a similar duration (total of 91 weeks) and from the same source as the styrene study. In addition to the two studies from Litton Bionetics (NTP 1979a, 1979b), there were 12 applicable studies conducted by Hazelton Laboratories (NTP 1976a, 1976b, 1977, 1978a, 1978b, 1978c, 1978d, 1979e, 1979f, 1979g, 1979h, 1979i). The incidence of combined lung tumors in historical vehicle controls from these 14 studies was 11 of 273 (4%). <u>Therefore, the incidence of lung tumors in control male mice in the NCI (1979a) study was not unusually low and support the finding that lung tumors as a result of styrene exposure is significant.</u>] . . . . <u>NCI (1979a) concluded that there was suggestive evidence for the carcinogenicity of styrene in male B6C3F1 mice, but no convincing evidence was obtained for either sex.</u>*

2008 Final Background Document at 196-87 (brackets in original; emphasis added). See also NCI*, Bioassay of Styrene for Possible Carcinogenicity*, *Technical Report Series No. 185* (1979), http://ntp.niehs.nih.gov/?objectid=07059C3F-BEA9-487F-F6B78FB485DFE06F.

None of the controls from the Litton Bionetics study or laboratory suited the NTP's purpose, but employing historical controls from 12 studies from another laboratory allowed the NTP to create a new study with a positive conclusion of carcinogenicity to support its styrene listing. When the NTP did this, it departed not only from its own accepted rules regarding the use of historical controls, but also from generally accepted rules of scientific practice.

In response to comments made by a member of the NTP's Board of Scientific Counselors during the 2009 Expert Panel review of the Styrene Assessment that *the evidence from the gavage bioassay studies in mice was suggestive but not conclusive* and that he or she was concerned about the NTP's use of historical controls in the NCI study from a different laboratory, the NTP defended its use of historical controls. The Styrene Expert Panel recommended that the NTP refer to the historical control guidelines used in the NTP's current reports and by the IARC. Styrene Expert Panel Report, Part A at 15 (quoting http://monographs.iarc.fr/ENG/Preamble/currentb3studiesanimals0706.php; NTP Technical Report on the Toxicology and Carcinogenesis Studies of Methylene Blue Trihydrate (CAS No. 7220-79-3) in F344/N Rats and B6C3F1 Mice, p. 32 (Gavage) (2008)).

THE NTP FAILED TO CONSIDER THE ORIGINAL SCIENTIFICALLY VALID NCI 1979 STUDY

Estimating a control incidence based on use of historical controls from different laboratories is scientifically inappropriate because laboratories can differ in control mouse lung tumor incidence due to differences in diets, caging regimens and other environmental factors that can affect the outcome of a study. Haseman et al. (1984). Moreover, in the case of the 1979 NCI study, the low incidence of tumors observed even in the high dose group relative to historical control ranges for the same laboratory, in combination with a smaller number of control mice, result in an inability to determine whether the results are treatment related. Replacement of the control incidence by a point estimate based on data from other laboratories does not change the relatively low incidence of tumors observed in the study and the other uncertainties regarding control incidence.

Thus, the fundamental issue with the 1979 NCI study resulting in inconclusive results for carcinogenicity is the low incidence in the treated animals.

The NTP applies a general policy of using historical data only from the same laboratories. A 1984 study regarding statistical issues related to the use of historical control data by the NTP revealed significant laboratory-to-laboratory variability, with the most significant for lung tumors in male B6C3F1 mice. Haseman *et al.*, *Use of Historical Control Data*, 12 Toxicologic Pathology at 132-33 (1984). The authors of this study reviewed the NTP's historical data on these mice from various laboratories and found, for example, that *the incidence of lung alveolar/bronchiolar adenoma or carcinoma [was] nearly three times as great in Lab C as in Lab D.* Id. at 131 (emphasis added).

The authors of this study found that when the NTP uses historical control data in certain cases, the reason it limited the data to studies conducted at the same laboratory was to eliminate much, but not all, variability such as was found in the NTP's historical data and also to be *consistent with the recommendations of other investigators who have considered the control issue.* Id. at 133. See also Joseph K. Haseman, *et al.*, *Neoplasms Observed in Untreated and Corn Oil Gavage Control Groups of F_{344}/N Rats and C_{57}BL/6NxC_3H/HeN)F_1 (B6C_3F_1) Mice*, 75 Journal of the National Cancer Institute, 975 at 981 (1985), http://jnci.oxfordjournals.org/content/75/5/975.abstract) (describing and analyzing the NTP toxicology and carcinogenesis program and finding inter laboratory variability in corn oil gavage studies).

The NTP maintains a data base of historic control data, and the studies and data entered into the data base undergo a rigorous peer-review process that includes three independent pathology reviews and a final working group review process for all studies. Charlotte Keenan *et al. Best Practices for Use of Historical Data of Proliferative Rodent Lesions*, 37 Toxicologic Pathology 679, 698 (2009), http://tpx.sagepub.com/content/37/5/679.full.pdf+html. Keenan notes that *the peer-review process is a pivotal procedure completed prior to incorporation of data into the NTP . . . historical control data base.* Id. at 687.

The NTP claimed in the 12th RoC that it relied on peer-reviewed studies for its Styrene Assessment: *The data and findings from the publicly available, peer-reviewed carcinogenicity studies of styrene in experimental animals are summarized in this section.* 2008 Final Background Document at 195. Although the original NCI study was peer-reviewed in 1979 prior to publication (see

Bioassay of Styrene at 45), the NTP reconstructed the original study with estimates and small control groups drawn from other laboratories, and thereby changed the study's outcome to provide the purportedly *sufficient* evidence it needed.

The NTP's commitment to use only peer-reviewed studies to support its listings in the RoC is rendered meaningless if it can create a *new* reconstructed study from a peer-reviewed study and use its *new* reconstructed study as the critical support necessary to support its finding sufficient evidence of styrene's purportedly carcinogenicity in animals.

Michael F.W. Festing and Douglas G. Altman stated in *Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals*, 43 ILAR Journal 244, 256 (2002), that because so many factors can influence individual results of a study, *in nearly all studies, contemporary controls are almost essential, and historical data, particularly from another laboratory, should be treated with considerable caution. . . . When similar experiments are performed repeatedly in the same laboratory, there will often be scope for using historical data.* (http://iacuc.ucsd.edu/PDF_References/Guidelines.pdf) (emphasis added).

Charlotte Keenen et al. found that *HCD [historical control data] from the laboratory that conducted the study under review will likely be more comparable than HCD compiled from several laboratories.* Keenan, *Best Practices*, 37 Toxicologic Pathology at 679, http://tpx.sagepub.com/content/37/5/679.full.pdf+html. The NTP not only broke its own rules and generally accepted rules, but it also failed to follow the best procedures for use of historical control data, if such use would have been acceptable practice in this case.

The NTP also violated the NCI's procedures in place at the time of the original NCI study. In 1976, the NCI published its *Guidelines for Carcinogen Bioassay in Small Rodents, No. 1.* Nowhere in these guidelines did the NCI recommend concocting a new control data point using historical control data from different laboratories for statistical analyses. See NCI *Guidelines for Carcinogen Bioassay in Small Rodents* (1976), http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr001.pdf.

Significantly, the NCI states in the Introduction to its 1976 Guidelines that because of the *great social and economic impact* of many chemical agents, *it is essential that the procedures used to determine their carcinogenicity be established on the*

best scientific bases as are practically possible. . . . certain features are common to all well designed and properly conducted long-term animal studies. Id. at 1.

Even though the NTP's listings significantly affect public policy and the economy, the NTP violated generally accepted standards of scientific inquiry, and departed from the NCI's standards pursuant to which it conducted the 1979 study, by reconstructing that study. The rules it broke were meant to protect the public from policies that are based on insufficiently rigorous scientific analysis. The NTP ignored those protections.

OEEHA is authorized under Proposition 65 to list chemicals only if it finds that listing them would in fact be based on at least two positive studies which were conducted according to scientifically valid testing. In order to stay within the bounds of its listing authority, OEHHA must consider the scientifically valid data the NTP did not consider – that is, the *original* 1979 NCI study in which the NCI concluded there was no convincing evidence of increased incidence of tumors. The NTP's reconstructed NCI study was not based on scientifically appropriate procedures but it also was a schematic for NTP to ignore the negative results of the NCI's 1979 study. OEHHA cannot ignore the outcome of NEI's 1979 work that found no causal link between styrene and cancer.

Consideration of the original 1979 NCI study shows that the NTP's styrene assessment was based on insufficient evidence of carcinogenicity in experimental animals. The NTP assessment therefore lacks the sufficient evidence required to list styrene under Proposition 65 as known to the state to cause cancer. It also shows the NTP failed to consider a key, scientifically valid study that OEHHA is obligated to consider under Proposition 65.

THE CALIFORNIA COURT OF APPEAL RULED IN 2012 THAT OEHHA COULD NOT LIST STYRENE BASED ON THE IARC'S LISTING WHICH WAS BASED ON THE IARC'S CONCLUSION REGARDING THE NCI 1979 STUDY

As previously discussed, in 2002, the IARC reviewed the same styrene evidence the NTP reviewed, appropriately considered the results and conclusions of the NCI 1979 study and the overall weight of the scientific evidence, and concluded that styrene did not meet the criteria for its category of *carcinogenic to humans*, nor even for its *probably carcinogenic to humans* category. The IARC found there was merely *limited* evidence of carcinogenicity in humans and in experimental animals. International Agency for Research on Cancer, IARC Monographs on the

Evaluation of Risks of Carcinogenic Risks to Humans, Vol. 82, *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene, and Styrene* (2002).

OEHHA proposes to list styrene as *known* to cause cancer even though the NTP, like the IARC, also had insufficient evidence of carcinogenicity from studies in experimental animals. The NTP's claim in the 12th RoC that its styrene listing was based on sufficient animal evidence could not have been accurate because no new animal evidence had surfaced since the IARC's styrene listing. The only thing *new* was the NTP's manipulation of the existing NCI 1979 mouse study which failed to demonstrate a linkage between styrene and cancer in mice. In violation of generally accepted scientific principles, the NTP reconstructed the 1979 study into a positive study in order to provide the *sufficient* animal evidence it needed to support its listing of styrene.

OEHHA is bound by California law – not the NTP's 12th RoC. When OEHHA attempted to list styrene based on the same evidence underlying the IARC's styrene conclusions, which explicitly provided that the human and animal evidence was *limited*, the California Court of Appeal ruled in SIRC v. OEHHA, 210 Cal.App.4th at 1090-91, 1101 that it is unlawful for OEHHA to list chemicals under Proposition 65 unless there is *sufficient evidence that the chemicals it proposes to list in fact cause cancer* (emphasis added).

Nothing changed during the time between the IARC's and the NTP's reports, yet OEHHA again is proposing to list styrene based on the same evidence the Court of Appeal held cannot support a Proposition 65 styrene listing.

NEW MODE OF ACTION DATA THE NTP DID NOT CONSIDER SHOW THAT MOUSE LUNG STUDIES ARE NOT RELEVANT TO HUMAN CANCER RISK

In addition to the lack of sufficient scientifically valid animal evidence, recent Mode of Action (MOA) studies show that existing animal evidence on styrene is irrelevant to human cancer risk. These MOA studies provide strong evidence that mouse lung tumors are not relevant to humans because mouse lung tumors are caused by reactive compounds that result from the metabolism of styrene by the CYP2F2 enzyme, which is specific to mice. The analogous enzyme in humans is CYP2F1, which has little capacity to metabolize styrene. When the human CYP2F1 gene is inserted into the mouse genome in lieu of the mouse CYP2F2 or if expression of the CYP2F2 gene is *knocked out*, the toxic effects that lead to lung tumors seen in mice do not occur with styrene exposure. In addition to these

differences in enzymatic metabolism of styrene, mice and humans anatomically differ in the location, number, and morphology of the specific lung cell types with high styrene metabolic activity and thus sensitivity to lung tumor formation.

With prior permission from the Styrene Information and Research Center (SIRC), APTCO concurs with and incorporates herein the following MOA analysis by SIRC on pages 8 through 13 of SIRC's March 25, 2015 Comments on Notice of Intent to List Styrene:

The second animal study upon which NTP relied was conducted by Cruzan et al., in CD-1 mice and reported in 2001. These mice were exposed by inhalation and developed lung tumors. However, the tumors resulted from a mechanism of action that does not occur in humans or even in other animal species, including rats.

- *NTP hypothesized that mouse lung tumors developed as a consequence of styrene metabolizing to styrene oxide. Studies, available but not considered by NTP, demonstrate that styrene oxide does not cause lung tumors in mice or rats.*

- *The mechanism of action causing lung tumors is unique to mice. Styrene is metabolized in mouse lungs by an enzyme, CYP2F2, causing cytotoxicity, that is, cell damage. The resulting regeneration of cells to repair the damage causes increased cell replication (hyperplasia) and eventually lung tumors.*

- *Laboratories have bred mice with the CYP2F2 enzyme "knocked out." These knockout mice do not experience cytotoxicity when they are exposed to styrene.*

- *Rats do not have the CYP2F2 enzyme. The rat counterpart is CYP2F4. Styrene does not cause cytotoxicity or lung tumors in rats. Humans do not have the CYP2F2 enzyme. The human counterpart is CYP2F1, and it is present at a much lower level even than the CYP2F4 enzyme in rats.*

- *Laboratories have developed mice with the human CYP2F1 enzyme rather than the CYP2F2 mouse enzyme. These "humanized" mice do not experience cytotoxicity when exposed to styrene.*

These conclusions are bolstered by four studies not considered by NTP that were published after the Report on Carcinogens. They are:

1. *Carlson, G.P. 2012. Modification of the metabolism and toxicity of styrene and styrene oxide in hepatic cytochrome P450 reductase deficient mice and CYP2F2 deficient mice. Toxicology 294(2-3):104-108.*

2. *Cruzan, G; Bus, J; Hotchkiss, J; Harkema, J; Banton, M; Sarang, S. (2012). CYP2F2-generated metabolites, not styrene oxide, are a key event mediating the mode of action of styrene-induced mouse lung tumors. Regul Toxicol Pharmacol 62: 214-220. http://dx.doi.org/10.1016/j.yrtph.2011.10.007*

3. *Cruzan, G; Bus, J; Hotchkiss, J; Sura, R; Moore, C; Yost, G; Banton, M; Sarang, S. (2013). Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. Regul Toxicol Pharmacol 66: 24-29. http://dx.doi.org/10.1016/j.yrtph.2013.02.008*

4. *Shen, S; Li, L; Ding, X; Zheng, J. (2014). Metabolism of styrene to styrene oxide and vinylphenols in cytochrome P450 2F2- and P450 2E1-knockout mouse liver and lung microsomes. Chem Res Toxicol 27: 27-33. http://dx.doi.org/10.1021/tx400305w*

Further elaboration of these points follows, but because NTP did not consider these data, an authoritative bodies listing may not proceed.

Mode of Action of Mouse Lung Tumors Requires CYP2F2 Metabolism

Do the mouse lung tumors provide evidence of human cancer? This can be examined by determining the Mode of Action (MOA) by which styrene induces lung tumors in mice. Facets examined include toxic effects, cells affected, metabolic considerations, and gene mutations. Cruzan et al., (2015) examined mouse lung genomic responses in styrene treated wild-type CYP2F2 knockout and CYP2F1 humanized mice. The evidence supports the conclusion that the mouse-specific lung toxicity and tumorigenicity are not relevant to humans. The metabolism of styrene by mouse lung CYP2F2 to cytotoxic metabolite(s) has been postulated as an essential step for mouse lung toxicity and mouse lung specific tumorigenicity (Cruzan et al., RTP, 2012, 2013). The purpose of this study was to use whole-lung genomic analysis to further investigate potential MOAs of styrene

in C57BL/6 wild-type, CYP2F2 knockout (-/-; KO) and CYP2F21 humanized (2F2-KO + 2F1,2A13,2B6-transgenic, TG) male mice. Mice were exposed to 0, 40, or 120 ppm styrene for 6 hours a day, 5 days a week, for either 1 or 4 weeks. Five biological replicates for each treatment group were analyzed for relative gene expression using Affymetrix whole genome HT_MG430_PM Titan arrays. 287 genes were significantly differentially expressed in wild-type mice at both styrene concentrations. Gene ontology enrichment showed a strong dominance of cell cycle regulatory pathways consistent with cell proliferation. No genes were significantly differentially expressed in knockout mice. Only a single gene was significantly differentially expressed at 120 ppm in transgenic mice after 1 week and a different single gene at 40 ppm after 4 weeks. This study supports the conclusion that the MOA of styrene mouse lung toxicity requires CYP2F2 metabolism (but not by human CYP2F1) as a key gateway event, and also evidences that alternative MOAs mediated by either parent styrene or non-CYP2F2 generated styrene metabolites (e.g., styrene oxide) are unlikely.

Toxic Effects Are Limited to Lung Bronchiolar Epithelium in Mice Only

Toxic effects in the mouse lung from styrene have been demonstrated following inhalation, oral, or intraperitoneal (IP) administration. In studies with up to 2 weeks of exposure, increased cells and protein is found in broncho-alveolar lavage fluid (BALF), cell replication is increased (as measured by bromodeoxyuridine (BrdU) incorporation) in lung bronchiolar epithelium but not in alveolar cells, the intensity of staining of the endoplasmic reticulum in bronchiolar cells is diminished, and hyperplasia is observed in terminal bronchioles. Longer exposures indicate continued increased cell replication causing hyperplasia, which eventually extends into alveolar ducts.

No toxic effects are seen in alveolar cells at any time point from styrene exposure, even up to 2 years.

Using whole lung homogenates from C57BL/6 mice exposed to 40 or 120 ppm styrene by inhalation for 1 or 4 weeks (6 hrs/day for 5 days/week), full genomic evaluation indicated that styrene dramatically increases the expression of genes controlling cell cycle and replication.

In rat lungs, no cells are affected even at inhalation concentrations that are 8-fold higher – up to 1,000 ppm styrene 6 hrs/day 5 days/week – for 2 years. (Cruzan et al., 1998) In mice, Club (formerly called Clara) cells are affected.

Increased BrdU labeling occurs only in bronchiolar tissue. Hyperplasia is found only in terminal bronchiolar tissues. Using enriched cell fractions from mouse lungs, Carlson reported that metabolism of styrene occurred only in Club (Clara) cells (Hynes et al., 1999).

Some have suggested that styrene is metabolized in Club cells and the metabolites cause events in nearby alveolar cells that lead to tumors. There is no evidence of toxicity or mutagenicity in alveolar cells in mouse lung. In fact using RNA from whole lung of C57BL/6 mice exposed to styrene at 40 or 160 ppm 5 days/week for 1 or 4 weeks, there was no indication of mutagenic events in the lung.

While Styrene Has Some Genotoxic Potential In Vitro, There Is No Convincing Evidence That Styrene Is Genotoxic In Vivo

The overall picture presented by the available in vitro assay results available is that at least in some test systems (including tests from in vitro chromosome aberration studies in mammalian cells), styrene has some genotoxic potential in vitro. However, based on standard in vivo regulatory tests, arguably the more relevant testing environment, there is no convincing evidence that styrene is mutagenic/clastogenic.

In vitro mutagenicity assays (Ames) of styrene are negative (IARC, 1994). Micronucleus and chromosomal aberration assays in rats and mice are negative (IARC, 1994, 2002). Since styrene causes increased tumors only in mouse lung, assessment of genotoxicity in mouse lung is most relevant. Limited assays of genotoxic potential have been conducted in the lungs of mice exposed to styrene. There were no increases in chromosomal aberrations in the lungs of B6C3F1 mice exposed to 125, 250 or 500 ppm styrene for 2 weeks (Kligerman et al. 1993). Using A/J mice, a strain very susceptible to lung tumor formation, in an initiation/promotion assay, styrene administered for 7 weeks by IP injection did not initiate lung tumor formation (Brunnemann et al., 1992).

Other data are often cited to support a genotoxic MOA for styrene. In vitro mutagenicity assays of styrene-7,8-oxide (SO) are generally positive when epoxide hydrolase is inhibited (IARC, 1994). In vivo assays of micronucleus formation and chromosomal aberrations following exposure to SO were of mixed results, about half positive and half negative. However as described later, lung toxicity and

tumor formation in mice from styrene exposure does not appear to be related to SO.

Studies in workers exposed to styrene and in vitro studies using human cells provide conflicting results. Most in vitro studies of micronucleus formation and chromosomal aberrations using human lymphocytes are positive (IARC, 1994, 2002). (This is in contrast to the in vivo studies in rats and mice, which are uniformly negative.) About 30 studies of workers in industries where workers are exposed to styrene have exhibited micronucleus and/or chromosomal aberrations. There does not seem to be any correlation between styrene exposure and micronucleus formation. However, about half of the chromosomal aberration studies are positive.

The one assay that seems to be consistently positive across in vitro, animal, and human studies is sister chromatid exchange (SCE). However, the Organisation for Economic Cooperation and Development (OECD 2014) recently removed SCE from the list of acceptable assays for genotoxicity because there is no functional connection between SCE and tumor formation.

DNA Adducts from Styrene Are Not Sufficient to Cause Tumors

Exposure to styrene by mice, rats and humans results in N-7-SO DNA adducts, as well as other adducts. Some authors have taken the findings of DNA adducts in mouse lung as an indication of a mutagenic MOA for styrene-induced mouse lung tumors and SO as the tumorigenic metabolite. Exposure of mice to 40 ppm styrene vapors for 2 years results in increased lung tumors; short-term exposures to this level of styrene in mice results in DNA adducts in lung. The lung DNA adducts are not an indication of a mutagenic MOA in lung since there are a greater number of adducts/gram tissue in liver, but no increase in liver tumors. I.e., DNA adducts from styrene are not sufficient to cause tumors. Furthermore, rats exposed to 500 ppm styrene vapor develop more DNA adducts ducts from styrene are not sufficient to cause tumors.

The role of DNA adducts, in general, for causing mutations that lead to tumors as a linear, non-threshold MOA is questioned by recent research on aflatoxin. Johnson et al., (2014) recently published a set of experiments on the MOA of aflatoxin-induced liver cancer and chemoprevention. Aflatoxin B1 (AFB1) forms N-7-guanine adducts, produces GST-P-positive foci and eventually hepatocellular carcinoma. A specific gene signature is produced by AFB1

exposure. Administration of 200 μg/kg AFB1 via daily gavage for 4 weeks to F344 rats resulted in 100 pmol N-7AF-Guanine adducts/mg creatinine. Lifetime exposure to this dose resulted in a 96% incidence of hepatocellular carcinoma. Simultaneous exposure to 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) reduced the DNA adducts by 66% (i.e., DNA adducts in the AFB1/CDDO-Im treated rats was 34 pmol N-7AF-Guanine adducts/mg creatinine). The genomic signature was also altered. The incidence of hepatocellular carcinoma was 0.

This study demonstrates that even for a genotoxic carcinogen such as aflatoxin, there is a threshold of DNA damage before cancer occurs.

A commentary by Drs. Olden and Vulimiri (2014) noted:

> *They showed that AFB1 is a classic genotoxic substance in that it binds covalently to DNA and induces mutations. In fact, DNA adduct formation exhibits a characteristic linear dose–response curve over a wide range. But, further analysis demonstrated a threshold mode of action, with respect to internal dose of active metabolite and hepatocarcinogenesis. That is, there was substantial adduct formation and DNA damage without having any affect on development of hepatocellular carcinoma.*

Although a genotoxicity MOA is the NTP's default assumption and limited genotoxicity data are found for styrene, there is no evidence that styrene induces mouse lung tumors through a genotoxic MOA, and these are the only tumors found in animals.

Metabolic Activation is Essential to Mouse Lung Toxicity

Metabolism by CYP2F2 is absolutely essential for toxic effects in mouse lung from styrene exposure. Although CYP2E1 readily metabolizes styrene to styrene-7,8-oxide (SO), elimination of CYP2E1 (CYP2E1-null mice) had no impact on styrene-induced lung toxicity. Styrene lung toxicity is reduced in CYP2E1-null mice.

Preliminary studies demonstrated that inhibition of CYP2F2 by 5-phenyl-1-pentyne reduced the lung toxicity of styrene (Green et al., 2001). More recent studies using CYP2F2-null mice demonstrated a complete loss of lung toxicity from styrene in the absence of CYP2F2 metabolism (Cruzan et al, 2012 and Carlson 2012). These studies also indicate that SO is not the toxic agent from styrene,

because there was no toxicity from SO in the absence of further metabolism by CYP2F2 (Cruzan et al., 2012).

Genomics analysis demonstrated altered expression of genes related to control of cell cycle in the lungs of mice exposed to styrene. There was no altered expression in the lungs of CYP2F2-null mice (Cruzan et al., 2015).[2]

The fourth study that NTP did not have an opportunity to consider is Shen et al., (2014), which demonstrated that toxic naphthalene metabolites are generated in the lung by CYP2F2 and not CYP2A5, while in the nose it is CYP2A5, not CYP2F2, that generates toxic naphthalene metabolites.

MOA Conclusions

Metabolism of styrene or styrene oxide in the mouse lung by CYP2F2 is required for toxicity. In the absence of CYP2F2, there is no increase in cells, lactate dehydrogenase (LDH), or protein in broncho-alveolar lavage fluid (BALF), no increase in bromodeoxyuridine (BrdU) incorporation, and no differential expression of cell cycle genes in lung. Based on the lung tumor initiation assay, lung chromosomal aberration assay and lung genomic analysis, there is no indication of a genotoxic MOA. Using mice with the human CYP2F1 gene in place of the normal mouse CYP2F2 indicates that humans are incapable of producing sufficient metabolites to cause the lung effects seen in mice.

Mode of Action (MOA) research evidences that the mouse lung tumors are caused by mouse-specific metabolism by CYP2F2, which does not occur in humans. Thus, the animal data in Cruzan et al., (2001) are irrelevant to human cancer risk. Thus, the animal data are less than sufficient and does not support the listing of styrene under Proposition 65.

Comments of the Styrene Information Research Center on Notice of Intent to List Styrene, pp. 8-13 (March 25, 2015).

APTCO submits the following additional comment:

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[2] *Bolstering this conclusion is a SIRC-sponsored 4-week styrene inhalation lung toxicity study in KO, WT, and TG mice that supported the conclusion that CYP2F2 metabolism was a key event in the production of lung toxicity in mice following short-term exposure of inhaled styrene vapor. The final report from this study is provided as an attachment to these comments.* Please refer to SIRC's Comments for this attachment.

Club cells in mouse lungs are much more abundant than in human lungs and have morphological differences that result in higher activity of the type of P-450 enzymes (e.g., CYP2F enzymes) that would metabolize chemicals such as styrene. Green T. 2000. Pulmonary toxicity and carcinogencity of trichloroethylene: species differences and modes of action. Environ. Health Perspect. 108(Suppl2): 261-264. Rhomberg, LR, JE Goodman, and RL Prueitt. 2013. The weight of evidence does not support the listing of styrene as *reasonably anticipated to be a human carcinogen* in NTP's Twelfth Report on Carcinogens. Human and Ecol. Risk Assess. 19:4-27.

DNA adducts are formed by covalent bonds between reactive molecules and DNA. Although DNA adducts are thought to potentially lead to DNA damage and mutagenic changes, the body has mechanisms for repairing such lesions given that DNA adducts form endogenously in the body through normal metabolic processes, even in the absence of exposure to mutagenic or carcinogenic substances. The current state of knowledge indicates that DNA adducts involving chemicals may reflect chemical exposure, but are not of a measure of mutagenic changes or cancer risk. DNA adduct formation in isolation thus cannot serve as an assessment of cancer risk but must be considered within the context of the available scientific evidence regarding the mode of action of tumor formation. As explained below, the evidence for styrene likewise indicates that DNA adducts are not related to risk of tumor formation. Jarabek AM[1], Pottenger LH, Andrews LS, Casciano D, Embry MR, Kim JH, Preston RJ, Reddy MV, Schoeny R, Shuker D, Skare J, Swenberg J, Williams GM, Zeiger E. 2009. Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Crit Rev Toxicol. 39(8):659-678.; Swenberg, JA, K Lu, BC Moeller, L Gao, PB Upton, J Nakamura, and TB Starr. 2011. Endogenous versus exogenous DNA adducts: Their role in carcinogenesis, epidemiology, and risk assessment. Toxicol. Sci. 120(S1):S130-S145; Pottenger LH[1], Andrews LS, Bachman AN, Boogaard PJ, Cadet J, Embry MR, Farmer PB, Himmelstein MW, Jarabek AM, Martin EA, Mauthe RJ, Persaud R, Preston RJ, Schoeny R, Skare J, Swenberg JA, Williams GM, Zeiger E, Zhang F, Kim JH. 2014. An organizational approach for the assessment of DNA adduct data in risk assessment: case studies for aflatoxin B1, tamoxifen and vinyl chloride. Crit Rev Toxicol. 2014 Apr;44(4):348-391.

NEW HUMAN DATA THE NTP DID NOT CONSIDER SHOW STYRENE CANNOT BE LISTED ON PROPOSITION 65

New human data have emerged that further limit the NTP's already limited human data. The new data provides substantial evidence that the Authoritative Bodies' Proposition 65 listing criteria are not met.

With SIRC's prior permission, APTCO also concurs in and incorporates herein the following human data analysis by SIRC on pages 4 through 5 of SIRC's March 25, 2015 Comments:

Human Data

NTP's RoC found the human data on the carcinogenicity of styrene to be limited. This does not support listing under Proposition 65.[3] *Further, the RoC's evaluation was based on studies that have since been updated. These data were not available to NTP and so, reasonably, they were not considered. These new data demonstrate, however, the inadequacy of the human data that NTP considered on the carcinogenicity of styrene. This includes studies of human data based on a cohort of US reinforced plastics and composite (RPC) workers by Wong et al., (1994); a cohort of Washington state RPC workers by Ruder et al., (2004); and a combination of 8 cohorts of RPC workers in the EU by Kogevinas et al., 1994). Wong and Ruder found no styrene-related increases in cancer. Kogevinas reported no styrene-related increases in cancer based on cumulative exposure or duration of exposure, but an increase in total lymphomas based on average exposure. Part of the Kogevinas cohort was taken from a cohort of Danish workers that may have been exposed to styrene in RPC operations (Kolstad et al, 1994). Workers were divided based on companies where less than 50% of the workers were thought to be involved in RPC operations and companies where more than 50% were thought to be involved. Kolstad reported increased leukemia in the overall cohort among workers hired before 1960 and among those employed for less than 1 year. Workers from companies where more than 50% were thought to have been involved in RPC operations were included in the Kogevinas study.*

[3] *Styrene Information Research Center v. Office of Environmental Health Hazard Assessment*, 210 Cal.App.4th 1082, 1101 (2012).

After the NTP concluded its review, the Wong, Kogevinas, Ruder, and Kolstad studies have been updated. Each update shows a lack of styrene carcinogenicity, vitiating prior assessments to the contrary.

- *Collins et al., (2013) updated the Wong cohort of US RPC workers. More than 85% of the cohort has been followed for more than 30 years. There were no increased incidences of leukemias or lymphomas based on cumulative exposure, average exposure, duration of exposure or peak exposures. Increased lung cancer followed an inverted dose-response pattern; i.e., lowest incidence among highest exposed workers, and was attributed to smoking.*

- *Coggon et al., (2014) have updated their cohort of the Kogevinas study. They found no styrene-related increase in cancer.*

- *Ruder et al., (2014) presented an update of Washington state RPC cohort in June 2014 at the conference "Challenges for Occupational Epidemiology in the 21st Century." No styrene-related cancers were reported.*

- *Kolstad et al., (2014) presented update of Danish RPC workers in June 2014 at the conference "Challenges for Occupational Epidemiology in the 21st Century." No styrene-related cancers were reported.*

Collectively, these new studies demonstrate the proposition that styrene is not carcinogenic in humans. The prior limited evidence characterization of the human data by NTP in the RoC is, then, undermined by this human data that were not available to the NTP and thus could not have been – and was not – considered by NTP. Given the number of human studies, the number of workers followed and the length of follow up, a conclusion that styrene is not a human carcinogen is well-founded.

Comments of the Styrene Information Research Center at 4-5.

APTCO submits the following additional comment:

A published review in 2009 of the state of the evidence by five distinguished epidemiologists reported, *We found no consistent increased risk of any cancer among workers exposed to styrene. A study of reinforced plastic workers reported an association between average estimated styrene exposure and non-Hodgkin lymphoma (NHL, P = 0.05) but no trend with increasing duration of exposure.*

Other studies of styrene exposure and NHL found no increased risk. In two US studies of reinforced plastic workers, esophageal cancer mortality was increased, but these findings were generated in a background of multiple comparisons. Results for other cancers were unremarkable. Conclusions: The available epidemiologic evidence does not support a causal relationship between styrene exposure and any type of human cancer. Boffetta, P, Adami HO, Cole P, Trichopoulos D, Mandel JS. 2009. Epidemiologic studies of styrene and cancer:

A review of the literature. J Occup Environ Med. 2009;51:1275–1287.

THE UNITED STATES CONSTITUTION AND FEDERAL LAW PRECLUDE A STYRENE PROPOSITION 65 LISTING

No matter how it is framed, the question a court faces when confronted with an agency's interpretation of a statute it administers is always, simply, whether the agency has stayed within the bounds of its statutory authority.

City of Arlington v. Federal Communications Commission, 599 U.S. ___, 133 S. Ct. 1863 (2013).

Proposition 65 does not authorize OEHHA to list styrene based on the NTP's Styrene Assessment because Proposition 65 does not authorize OEHHA to list chemicals without sufficient and valid scientific evidence that styrene is known to cause cancer.

If OEHHA proceeds to list styrene under Proposition 65, not only will it exceed the scope of its statutory authority, but it will exceed the scope of what it is permitted to do under the United States Constitution. The Freedom of Speech, Due Process and Interstate Commerce clauses of the Constitution preclude OEHHA from subjecting in-state and out-of-state persons and businesses to the significant effects of listing a chemical as a *known* carcinogen when the chemical is not a known carcinogen.

A styrene Proposition 65 listing would also be preempted by federal laws and regulations, such as those of the Food and Drug Administration and the Occupational Safety and Health Administration under which styrene is not deemed a carcinogen. The FDA, for example, considers polystyrene safe in food packaging and as a food additive for over thirty years.

OEHHA is bound under California, federal law and the United States Constitution to ensure that it actions are in fact based on accurate, scientifically valid and sufficient data.

CONCLUSION

OEHHA must withdraw the NOIL for styrene because it is not authorized to list styrene under California or federal law. APTCO has presented evidence that:

1. The NTP did not consider a key animal study NCI (1979) that concluded that there was no convincing evidence that styrene causes cancer. Instead the NTP reconstructed that study and, contrary to sound scientific principles and practices, substituted the control group with estimates creating a different, small set of controls from another laboratory, to change the outcome from a negative to a positive result.

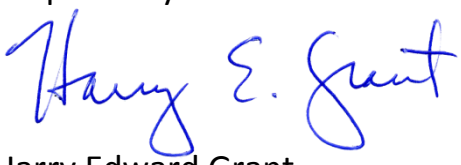
2. Because the NTP considered Mode of Action (MOA) data, OEHHA is required to consider MOA the NTP did not consider. Additional MOA data show the NTP's animal evidence on styrene is not relevant to humans, undermining the sufficiency of the NTP's laboratory animal evidence.

3. Even though OEHHA's proposal to list styrene is not based on human evidence because the NTP concluded the human evidence is limited, and Proposition 65 requires at least sufficient human or animal evidence, consideration of the new human data that has emerged since the NTP's listing shows that the human evidence the NTP considered is further limited. This new data also reinforces the relevance of the new mode of action data showing that animal evidence on mouse metabolism of styrene is not relevant to humans.

4. If OEHHA proceeds to list styrene under Proposition 65 based on insufficient evidence that it causes cancer, i.e, only one positive animal study and one re-constructed and scientifically invalid animal study, its listing would violate the Freedom of Speech, Due Process and Interstate Commerce clauses of the United States Constitution and would also be preempted by federal law.

APTCO acknowledges that OEHHA has been willing to take into consideration and act upon APTCO's objections to other actions OEHHA has proposed over the past several years. Now, APTCO urges OEHHA to withdraw this Notice of Intent to List Styrene.

Respectfully submitted on behalf of APTCO, LLC,



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cc: Scott Hakl, APTCO, LLC

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