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BEFORE THE

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

In re: Chemicals Proposed for Listing by the "Labor Code Mechanism" (Carcinogens)	)

COMMENTS SUBMITTED IN OPPOSITION TO
THE PROPOSED LISTING OF STYRENE BY THE LABOR CODE MECHANISM

July 13, 2009

These comments are submitted on behalf of the Styrene Research Information Center in opposition to the addition of styrene to the Proposition 65 list as a carcinogen.

A. Styrene Should Not Be Added to the Proposition 65 List as a Known Carcinogen.

The Office of Environmental Health Hazard Assessment should refrain from adding styrene to the Proposition 65 list as a known carcinogen for two general reasons:

1. Styrene is not a carcinogen and no scientific body has ever said it was;
2. Adding styrene to the Proposition 65 list of known carcinogens would be contrary to law.

B. Styrene Is Not a Carcinogen.

1. OEHHA Has Incorrectly Determined That Styrene "Meets the Requirements for Listing as Known to the State to Cause Cancer for the Purposes of Proposition 65."

OEHHA bases its determination to add styrene to the Proposition 65 list as a carcinogen on the reference in Health and Safety Code section 25249.8(a) to Labor Code section 6382(d). The Labor Code subdivision provides that "any substance within the scope of the federal Hazard Communication Standard (29 CFR Sec. 1910.1200) is a hazardous substance . . .."

Subdivision (d) of 29 CFR Sec. 1910.1200, entitled "Hazard Determination" states in paragraph (4) that the following sources should be treated as establishing that a chemical is a carcinogen or potential carcinogen for hazardous communication purposes.

(i) National Toxicology Program (NTP), *Annual Report on Carcinogens* (latest edition);

(ii) International Agency for Research on Cancer (IARC) *Monographs* (latest editions); or

(iii) 29 CFR part 1910, subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration.

Included within paragraph (4) is the following:

NOTE: The *Registry of Toxic Effects of Chemical Substances* published by the National Institute for Occupational Safety and Health indicates whether a chemical has been found by NTP or IARC to be a potential carcinogen.

Paragraph (3) of the same subdivision provides that the following sources should be treated as establishing that the chemicals listed in them are hazardous:

> (i) 29 CFR part 1910, subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration (OSHA); or
>
> (ii) *Threshold Limit Values for Chemical Substances and Physical Agents In the Work Environment*, American Conference of Governmental Industrial Hygienists (ACGIH) (latest edition).

The *Registry of Toxic Effects of Chemical Substances* compiled by the National Institute for Occupational Safety and Health contains reviews of the evaluation of styrene. It states that the American Conference of Governmental Industrial Hygienists characterizes styrene as "not classifiable as human carcinogen." In addition, the federal Occupational Safety and Health Administration reviewed the data that served as the basis for IARC's initial classification of styrene and concluded that "the current evidence on styrene's carcinogenicity does not support its classification in the final rule as a carcinogen." 54 F.R. 2332, 2430 (Jan. 19, 1988).

The *Registry* also notes that the International Agency for Research on Cancer most recently in 2002 concluded that there is limited animal evidence for styrene. Similarly, in 2002, it concluded that there is only limited human evidence. IARC classified styrene as "possibly carcinogenic to humans," placing it in Group 2B.

IARC defines limited evidence of carcinogenicity as:

> The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissue or organs.

It is noteworthy that IARC characterizes limited evidence as only suggestive of a carcinogenic effect. That is a far cry from known to cause cancer. In fact, IARC concluded that "the lung tumors [in mice] were caused by lung metabolism of styrene and the process does not occur to a meaningful extent in humans."

The court of appeal, in *AFL-CIO v. Deukmejian*, 212 Cal.App.2d 425 (1989) (Duke I), addressed the issue of IARC Group 1 and Group 2 chemicals. It

logically equates IARC monographs that reflect " sufficient evidence" of carcinogenicity with "known carcinogenicity" within the meaning of Proposition 65.

The evidence of carcinogenicity for styrene falls well short of "sufficient." It is a full level lower for both human and animal evidence. As such, it fails to meet the "known to cause cancer" standard of Proposition 65 -- a standard that the Duke I court held was applicable even where listing chemicals pursuant to the Labor Code references. Further, that standard was reaffirmed in Baxter Healthcare Corporation v. Denton, 120 Cal.App.4th 333 (2004). There, the court said, "only chemicals 'known to the state to cause cancer' are listed under Proposition 65, not chemicals for which there is a mere possibility or suspicion they will cause cancer." Citing Duke I.

It should be noted that while the U.S. Environmental Protection Agency has reviewed styrene, it has consistently concluded that styrene is not a carcinogen. EPA established a maximum contaminant level (MCL) for styrene under the Safe Drinking Water Act based on non-carcinogenic end points, due to evidence including the lack of a carcinogenic response in an adequately conducted drinking water study. 40 C.F.R. § 370.2. 56 *Fed. Reg.* 3526, 3541 (Jan. 30, 1991). See also 50 *Fed. Reg.* 46936, 47004-47005 (Nov. 13, 1985); 54 *Fed. Reg.* 22062 (May 22, 1989). EPA has consistently declined to treat styrene as a carcinogen with regard to other listings, such as the proposed Hazardous Waste Identification Rule (HWIR) [57 *Fed. Reg.* 21450, 21512, 21515 and 21528 (May 20, 1992)] and the Health Effects Assessment Summary Tables (HEAST) [*Health Effects Summary Tables, Annual Update*, USEPA (March 1993) (EPA 540-R-93-058 (PB93-921199)].

2. Science Developed Since IARC's 2002 Monograph Further Demonstrates That Styrene Is Not a Human Carcinogen.

Two very recent, and fundamentally important, research papers have just been published on styrene's carcinogenic potential, each explains a critical component of the styrene cancer data. Additionally, both studies provide strong evidence that styrene is not considered carcinogenic to humans. Careful consideration of these recent styrene reports suggests that action on the part of OEHHA to list styrene as a "known" carcinogen is scientifically inappropriate.

An internationally renowned peer-review panel of epidemiologists -- led by the chief of IARC's Environmental Cancer Epidemiology Unit -- has very recently published a review of all of the available epidemiological (human) data on styrene, and concluded that styrene exposure should not be considered carcinogenic to humans. The report, "*Epidemiologic Studies on Styrene and Cancer: A Review of the Literature*,"[1] has been accepted for publication by the

[1] "Epidemiological Studies of Styrene and Cancer: A Review of the Literature," December 9, 2008, Boffetta, P. (International Agency for Research on Cancer, Lyon, France; Vanderbilt University, Nashville,

Journal of Occupational and Environmental Medicine. The authors of this review clearly stated in their conclusion that the "Available epidemiologic evidence does not support a causal relationship between styrene exposure and any type of human cancer."

As agreed by IARC, the European Union, a Harvard Center for Risk Analysis Panel that assessed styrene, the U.S. National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction, and the U.S. Agency for Toxic Substances and Disease Registry, an integrated assessment of eight chronic studies provides no evidence of increased tumors in rats from styrene exposure at concentrations *exponentially higher* than any exposure levels that would be encountered in consumer products or the environment - and orders of magnitude higher than even the highest potential workplace exposures (i.e. - up to 1,000 parts per million).

Based on five chronic studies in mice, only lung tumors are increased. Outdated mode of action assumptions have focused on styrene-7,8-oxide as the causative agent for tumors in mice. An alternate mode of action was published in 2002 (Cruzan et al., 2002) suggesting that styrene is metabolized in mouse terminal bronchiolar cells by the CYP2F2 enzyme to cytotoxic metabolites. The continued cytotoxicity leads to regenerative hyperplasia, and eventually tumors. Cytotoxicity from styrene occurs in tissues high in CYP2F (2F2 in mouse lung Clara cell and nasal olfactory epithelium cells, and 2F4 in rat nasal olfactory epithelium). Inhibition of CYP2F2 inhibits the cytotoxicity from styrene. Rats have less CYP2F4 in the lung Clara cells and do not produce sufficient metabolites to cause cytotoxicity or lung tumors. Even less human CYP2F1 is present in human tissues and 2F1 metabolizes even less styrene. Therefore, the mouse lung tumors from styrene exposure are not relevant to human risk.

Additionally, several other chemicals have very similar mouse lung tumor effects, with an absence of human effects, which lends strength to this argument. A report[2] laying out a CYP2F2-mediated mode of action in multiple chemicals has just been accepted for publication in *Regulatory Toxicology and Pharmacology*.

A comprehensive update of both the human (Attachment 1) and animal (Attachment 2) cancer data are attached with this comment letter, including significant references available since IARC's 2002 styrene classification. This

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Tennessee), Adami, H. O. (Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts), Cole, P. (School of Public Health, University of Alabama, Birmingham, Alabama) Trichopoulos, D., (Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts), and Mandel, J. S. (Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario).

[2] *Regulatory Toxicology and Pharmacology*, Ms. No.: RTO-09-20R3 accepted for publication on July 2, 2009, "Mouse Specific Lung Tumors from CYP2F2-Mediated Cytotoxic Metabolism: An Endpoint Where Data from Multiple Chemicals Converge to Support a Mode of Action," Corresponding Author: Dr. George Cruzan, Authors: James Bus, PhD; Marcy Banton, PhD; Ralph Gingell, PhD; Gary Carlson, PhD.

information further confirms that IARC's 2002 monograph is not a basis for listing styrene under Proposition 65.

C. OEHHA's Determination to Add Styrene to the Proposition 65 List As a Carcinogen is Contrary to Law.

OEHHA's proposal to add styrene and 29 other chemicals to the Proposition 65 list is based on interpretations of Proposition 65, the referenced Labor Code provisions, and the federal Hazard Communication Standard regulations. Those interpretations have not been formally adopted pursuant to the California Administrative Procedure Act, and as such, are underground regulations and are invalid.

Moreover, the proposal to add styrene and other IARC 2B chemicals is contrary to specific provisions of Proposition 65 and the promise made to the voters by the proponents of Proposition 65 in the Ballot Argument. As noted above, IARC concluded that the evidence of carcinogenicity is limited for both human and animal data, merely suggestive of a carcinogenic effect. IARC formally classified styrene as only "possibly" carcinogenic to humans. The characterization of the evidence pertaining to styrene and the classification by IARC falls far short of the Proposition 65 mandate to list only chemicals that are "known to cause cancer." Further, it violates the interpretation of the very Labor Code provisions relied on by OEHHA set out in Duke I, requiring the listing only of chemicals with "sufficient" evidence of carcinogenicity in either humans or animals.

The plain meaning of Proposition 65 is that the chemicals to be included in the Proposition 65 list by the provisions of law referenced in the Labor Code provisions were the chemicals that were listed at the time Proposition 65 passed. Those chemicals were to be added to the initial list. Nothing in the language of Proposition 65 suggests that the list was to be augmented when additions were made to the list compiled by the sources referenced in the Labor Code provisions.

Moreover, OEHHA's interpretation that the Labor Code is another listing mechanism results in inconsistencies with other provisions of Proposition 65, the Ballot Argument, and the nearly decade-long interpretation given to the provision by OEHHA and its predecessor lead agencies.

1. <u>OEHHA Cannot Predicate the Listing of Styrene Or Any Other Chemical Pursuant to the Labor Code Provisions Without Formally Adopting Its Interpretations In Accordance With the Provisions of the California Administrative Procedure Act</u>.

OEHHA's determination to add styrene and 29 other chemicals to the Proposition 65 list involves substantial interpretations of Health and Safety Code section 25249.8, subdivisions (b)(1) and (d) of Labor Code section 6382, and 29

CFR Sec. 1910.1200. The interpretations OEHHA is giving to those provisions of law have regulatory effect. However, OEHHA has not formally adopted any of the interpretations pursuant to the California Administrative Procedure Act.

OEHHA recognized last year that it was obligated to adopt regulations before adding chemicals to the Proposition 65 list based on the so-called Labor Code mechanism. It admitted in May 2008, 22 years after beginning to enforce Proposition 65, that "there are currently no regulations discussing the Labor Code mechanism for listing chemicals." OEHHA even conducted a workshop on a set of draft regulations. However, it has taken no steps since that time to adopt formally those regulations.

No basis exists for OEHHA to conclude that it is not obligated to adopt regulations setting out its interpretations and proposed implementation of the many provisions involved in the Labor Code listing mechanism. Certainly, no exception in the Administrative Procedure Act applies to OEHHA's interpretation and implementation of those provisions of law. While OEHHA might assert that its interpretations "embody the only legally tenable interpretation," the discussion below demonstrates that to be far from true.

The first significant interpretation applies to the specific language of Proposition 65. Section 25249.8(a) provides that, "On or before March 1, 1987, the Governor shall cause to be published a list of those chemicals known to the state to cause cancer or reproductive toxicity within the meaning of this chapter . . .." The subdivision also provides that, "Such list shall include at a minimum those substances identified by reference in Labor Code section 6382(b)(1) and those substances identified additionally by reference in Labor Code section 6382(d)."

For nearly 20 years, OEHHA and its predecessor lead agencies construed the reference to the Labor Code provisions to apply only to the initial list. Explicit statements setting out that interpretation were included in attachments to the comments that we submitted to OEHHA in response to the draft regulations in July 2008.

Given that OEHHA and its predecessor lead agencies construed the provision as applying only to the list that the Governor was to complete by March 1, 1987 and no further implementation was contemplated, neither OEHHA nor the other lead agencies had an obligation to adopt that interpretation by regulation. However, once it changed that interpretation and concluded that it will begin implementing that provision, it becomes obligated to set out that new interpretation formally in a regulation.

While SIRC disagrees with OEHHA's new interpretation, that is not the point that is being emphasized in this argument. The point is that OEHHA cannot implement its new interpretation without adopting that interpretation after giving

notice to the public and an opportunity for the public to comment on that proposed regulation. As noted above, that has not been done. Accordingly, any further action by OEHHA to add any chemical to the Proposition 65 list based on the so-called Labor Code mechanism is invalid.

The first Labor Code reference is to subdivision (b) of section 6382. That reference includes only one of five paragraphs contained under that subdivision. The paragraph that is referenced provides, "Substances listed as human or animal carcinogens by the International Agency for Research on Cancer (IARC)." That provision in subdivision (b)(1) is inherently ambiguous when viewed in the context of what IARC does.

First, IARC does not list chemicals as carcinogens. It prepares monographs where it characterizes the evidence and classifies the hazard.

Second, IARC does not classify chemicals as animal carcinogens. It classifies chemicals in terms of their hazard to humans. The classifications are Group 1, "the agent is carcinogenic to humans," Group 2A, "the agent is probably carcinogenic to humans," Group 2B, "the agent is possibly carcinogenic to humans," Group 3, "the agent is not classifiable as to its carcinogenicity to humans," Group 4, "the agent is probably not carcinogenic to humans."

Third, IARC characterizes the evidence. It characterizes both human and animal evidence as either "sufficient evidence of carcinogenicity," "limited evidence of carcinogenicity," "inadequate evidence of carcinogenicity," or "evidence suggesting lack of carcinogenicity."

Hence, OEHHA, to implement the so-called Labor Code mechanism, is obligated to interpret the provision in subdivision (b)(1). What does IARC do that can be interpreted to mean a substance listed is a human carcinogen? Does it mean a Group 1 chemical in which there is sufficient evidence of carcinogenicity in humans could cause IARC to say that the agent is carcinogenic to humans? Does it mean a Group 2A chemical in which there is limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in animals? Or, does a Group 2A chemical constitute a listing as an animal carcinogen?

OEHHA has interpreted the Labor Code language to include Group 2B chemicals, in which there is limited evidence of carcinogenicity in humans and limited evidence in animals, resulting in a classification that the agent is only possibly carcinogenic to humans. Does OEHHA characterize such a chemical as a listed human or animal carcinogen? Again, while SIRC disagrees with that interpretation, that 2B chemicals are listed as either human or animal carcinogens, the point here is that the interpretation to be implemented has to be adopted formally pursuant to the Administrative Procedure Act.

OEHHA has also apparently concluded that IARC's classification of Group 3 chemicals, agents not classifiable as to its carcinogenicity to humans does not constitute a listing of human or animal carcinogens. This, despite the fact that a Group 3 chemical can be based on limited evidence in animals. Accordingly, on one hand, OEHHA concludes that limited evidence in animals constitutes the listing of either a human or animal carcinogen, but in the instance of Group 3 chemicals, it does not, While that interpretation may be logical, it is an interpretation of the Labor Code provision that should be set out in a regulation.

Similarly, subdivision (d) of Labor Code section 6382 references substances "within the scope of the federal Hazard Communication Standard (29 CFR Sec. 1910.1200) . . .." Subdivision (d)(4) of section 1910.1200 identifies three sources as establishing that a chemical is a carcinogen.

The first of those sources is the National Toxicology Program (NTP) Annual Report on Carcinogens. The second is IARC monographs. The third refers to subpart Z of 29 CFR part 1910.

The reference to NTP's Annual Report on Carcinogens is specific and does not require an interpretation. However, that is definitely not true with respect to the other two sources.

The first of those is IARC's monographs. When IARC considers the carcinogenicity of a chemical, it prepare a monograph. The monograph can result, as noted above, in a finding that the agent is carcinogenic to humans, the agent is probably carcinogenic to humans, the agent is possibly carcinogenic to humans, the agent is not classifiable as to its carcinogenicity to humans, and the agent is probably not carcinogenic to humans.

Does the reference to IARC monographs mean that every chemical for which a chemical is prepared is intended to be included? Such an interpretation would result in an absurd result, in particular with respect to its application to Proposition 65. Such an interpretation would mean that chemicals for which the agent is not classifiable or is probably not carcinogenic to humans would have to be added to the list.

Accordingly, it is clear that OEHHA has made an interpretation that chemicals referenced in only some of the monographs should be added to the Proposition 65 list. It has obviously concluded that chemicals in Group 1, "carcinogenic to humans", and Group 2A "probably carcinogenic to humans" are to be included. OEHHA has also concluded that chemicals that are only possibly carcinogenic to humans are to be included as well.

SIRC disagrees strongly with OEHHA's interpretation to include chemicals that are only possibly carcinogenic. Nonetheless, the point here is that

OEHHA has made an interpretation from five possible outcomes set out in IARC's monographs. OEHHA has drawn the line between the third and fourth. The line could just as easily have been drawn between the second and third, or the first and second. That is an interpretation requiring the adoption of a regulation before that interpretation can be lawfully implemented.

While styrene is not affected by the third source listed in subdivision (d)(4) of 29 CFR Sec. 1910.1200, that is subpart Z, other chemicals proposed for listing are. As noted in Duke I, the Hazard Communication Standard referenced in Labor Code section 6382 subdivision (d) "includes thousands of substances that are not carcinogens or reproductive toxicants." The court noted that, "it is true that 'any substance within the scope of the federal [HCS](§ 3682, subd. (d)) includes chemicals other than known carcinogens.'" The court went on to say that "the list need not include all substances under HCS, but only known carcinogens . . . listed there." How does OEHHA determine, from among the thousands of chemicals listed in the Hazard Communication Standard, those chemicals that are "known to cause cancer"? That interpretation is unknown. However, whatever that interpretation is, for it to be implemented, it has to be adopted formally as a regulation pursuant to the Administrative Procedure Act.

2. Adding Styrene to the Proposition 65 List As a Carcinogen Is Inconsistent With the Statutory Standards.

As noted above, section 25249.8(a) of Proposition 65 called for the Governor to publish a list of chemicals known to the state to cause cancer on or before March 1, 1987. That subdivision also provided that such list shall include at a minimum those substances identified by reference in Labor Code section 6382(b)(1) and (d).

Styrene was not included in any list referenced in those Labor Code provisions when the Governor prepared a list by March 1, 1987. In fact, the Ballot Argument states, "At a minimum, the Governor must include the chemicals already listed as known carcinogens by two organizations of the most highly regarded national and international scientists: the U.S.'s National Toxicology Program and the U.N.'s International Agency for Research on Cancer." The critical phrase in that provision is "already listed." Styrene was not already listed by either of those entities when the Governor was required to prepare a list by March 1, 1987.

The court, in Duke I, in interpreting the Labor Code provisions concluded that the lead agency was to add to the initial list only those chemicals for which there is "sufficient" evidence of carcinogenicity. IARC has classified both the human and animal data for styrene as limited. Neither set of data has been determined to be "sufficient."

While SIRC disagrees strongly that the so-called Labor Code mechanism is to be used for augmenting the Proposition 65 list, OEHHA cannot ignore the conclusion of the court in Duke I as set out above. There, the court, making an effort to construe the Labor Code references consistently with the statutory standards in Proposition 65, equated "sufficient evidence" with "known to cause" cancer. Nothing in the language of Proposition 65, the Ballot Argument, or Duke I provides a basis for OEHHA to add to the Proposition 65 list any chemical with only limited evidence of carcinogenicity.

Moreover, regulations adopted by the lead agency define what is sufficient human and animal evidence:

> (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 does levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age onset. [27 CCR Section 25306 (3)]

These definitions are based on IARC's interpretations of the phrase "sufficient evidence." *See Hooper*, et al., *Regulation of Priority Carcinogens and Reproductive or Developmental Toxicants,* American Journal of Industrial Medicine 22: 783-808 (1992). IARC's characterization of the evidence pertaining to styrene as limited demonstrates that it is not sufficient under the very regulations implementing Proposition 65.

Further, as noted above, IARC has classified styrene as only possibly carcinogenic to humans. Again, the Ballot Argument states that "Proposition 65 . . . focus[es] only on chemicals that are known to the state to cause cancer. Chemicals that are only suspect are not included." The Duke I court confirms that interpretation for Proposition 65. It states that, "The trial court found, and we agree, that only those chemicals that are known and not merely suspected of causing cancer . . . must be on the list." Also, the court in the *Baxter* case stated, "First, only chemicals 'known to the state to cause cancer' are listed under Proposition 65, not chemicals for which there is a mere possibility or suspicion they will cause cancer."

Despite the specific language of Proposition 65, the explanatory language in the Ballot Argument, and the interpretation made by two courts, OEHHA proposes to add styrene and other chemicals to the Proposition 65 list that are only possibly carcinogenic to humans. Such a proposal is unlawful and contrary to the specific language and intent of Proposition 65.

3. Proposition 65 Does Not Authorize OEHHA to Implement the Labor Code Provisions By Adding Styrene to the List As a Carcinogen.

a. Section 25249.8's Plain Language Applies Labor Code Listings Only to the Initial list.

"The content of the initial list is governed by Section 25249.8(a)." Duke I. That subdivision states:

> On or before March 1, 1987, the Governor shall cause to be published *a list* of those chemicals known to the state to cause cancer or reproductive toxicity within the meaning of this chapter, and he shall cause *such list* to be revised and republished in light of additional knowledge at least once per year thereafter. *Such list* shall include at a minimum those substances identified by reference in Labor Code Section 6382(b)(1) and those substances identified additionally by reference in Labor Code Section 6382(d). [emphasis added]

As a matter of statutory construction, "a word or phrase repeated in a statute should be given the same meaning throughout." (*People v. Acosta* (2002) 29 Cal.4th 105, 114.) Here, in the first sentence of subdivision (a), the phrase "such list" refers to the initial list only. Under the above-cited authority, the phrase "such list" must be given the same meaning in the second sentence (i.e., it must refer to the initial list only). Thus, the second sentence of subdivision (a) requires the initial list (i.e., "such list") include the substances identified through the Labor Code. There is no requirement, or even authorization, for subsequent lists to incorporate chemicals identified by reference in the Labor Code.

Subsequent lists are governed not by subsection (a) but by subsection (b), which provides the "means for supplementing" the initial list "in consultation with the state's experts." Duke I. The court explained:

> Proposition 65 was not intended to produce a one-time list of known carcinogenic chemicals, but rather requires revision of the list annually. ... Section 25249.8, subdivision (a), insures the minimum content of the initial list, and section 25249.8, subdivision (b), directs both [the Governor] and the Panel to engage in a ... continuing search for additional chemicals which

evolving scientific knowledge demonstrates are subject to the Act. (*Id.*, at 440.)

The Court thus contemplated two stages of listing: first an "initial list" governed by § 25249.8(a) that includes the Labor Code substances "already listed" as of March 1, 1987; and next "a means for supplementing the initial list" in consultation with the state's qualified experts governed by section 25249.8(b).

With regard to the "initial list," the Court held that subdivision (a) created a mandatory duty for the Governor to include chemicals that, as of March 1, 1987, were "already listed" by the organizations mentioned in the Labor Code. However, elsewhere in the opinion, the Court specifically distinguishes this mandatory duty from the discretionary "role of the [state's qualified experts]," which "comes into play through section 25249.8, subdivision (b), which provides a means for supplementing the initial list."

b. Proposition 65 Incorporates the 1986 Version of the Labor Code.

This reading also comports with California law governing the rules of statutory incorporation by reference. In *Palmero v. Stockton Theaters, Inc.* (1948) 32 Cal.2d 53, 58, the California Supreme Court held that "It is a well-established principle of statutory law that, where a statute adopts by a specific reference the provisions of another statute, regulation, or ordinance, such provisions are incorporated in the form in which they exist at the time of the reference and not as subsequently modified." In contrast, "where the reference is general instead of specific, such as a reference to a system or body of laws or to the general law relating to the subject in hand, the referring statute takes the law or laws referred to not only in their contemporary form, but also as they may be changed from time to time. . . ." (*Id.*, at 59.)

Applying the *Palmero* rule, the court in *People v. Domaglowski* (1989) 214 Cal.App.3d 1380 construed the incorporation to be specific because the incorporating statute referred specifically to subdivisions (a) through (f), omitting the balance of the section, that is, subdivisions (g) through (i). In contrast, the court in *Fireman's Benevolent Assn. v. City Council* (1959) 168 Cal.App.2d 765, held an incorporation to be general because it referred to "all provisions of the State Employees' Retirement Law.

Here, section 25249.8(a) incorporates two specific Labor Code subsections, omitting other subsections within the same statute. Section 25249.8 makes no reference to the Hazardous Substances Communication and Training Law generally. Nor does the balance of Proposition 65 include general reference to that law or to the Labor Code. Therefore, the specific subsections must be given the meaning they had in 1986, when Proposition 65 was enacted –that is, they refer only to the substances then described in those subsections, and not to any substances that may have been added in the past 22 years.

c. The Ballot Argument in Favor of Proposition 65 Applies Section 25249.8(a) Only to the Initial List.

The *Palmero* rule also comports with the electorate's intent as revealed in the Ballot Arguments in favor of Proposition 65. As noted above, the Ballot Argument stresses that Proposition 65 lists only those "certain chemicals known –not merely suspected but known—to cause cancer and birth defects." The Ballot Argument continues:

> 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 suspected are not included. The Governor must list these chemicals, after full consultation with the state's qualified experts. At a minimum, the Governor must include the chemicals already listed as known carcinogens by two organizations of the most highly regarded national and international scientists: the U.S.'s National Toxicology Program and the U.N.'s International Agency for Research on Cancer. [original emphasis]

The Ballot Argument supports the general rule of statutory construction, which also requires that the Labor Code reference apply only to chemicals "already listed" when the voters enacted Proposition 65.

d. Under OEHHA's Long-Standing Interpretation of Section 25249.8, Only Subdivision (b) Authorizes Subsequent Listings.

For many years, OEHHA acknowledged that subdivision (b) provides the only authority to "supplement the initial list." This is important because courts give great weight and respect to an administrative agency's interpretation of the statute that governs its powers and responsibilities.[3] (*County of Santa Barbara v. Connell* (1999) 72 Cal.App.4th 175, 185.) As the court explained in *Mason v. Retirement Bd. of City and County of San Francisco* (2003) 111 Cal.App.4th 1221, 1228:

> Such deference is particularly warranted when an agency's interpretation is of long standing. (*Yamaha Corp. of America v. State Bd. of Equalization* (1998) 19 Cal.4th 1, 13.) This rule is supported by practical considerations. "When an administrative interpretation is of long standing and has remained uniform, it is likely that numerous transactions have been entered into in reliance thereon, and it could be invalidated only at the cost of major readjustments and extensive litigation." (*Whitcomb Hotel, Inc. v. Cal. Emp. Com.* (1944) 24 Cal.2d 753, 757.)

[3] OEHHA's recent change of position does not affect this rule of statutory construction. "An agency interpretation which conflicts with the agency's earlier interpretation is 'entitled to considerably less deference' than a consistently held agency view." (*INS v. Cardoza-Fonseca* (1987) 480 U.S. 421, 446, fn. 30.)

In OEHHA's 1999 status report, Director Dr. Joan Denton correctly reviews the "three mechanisms ... available for adding chemicals to the Proposition 65 list," and distinguishes the "qualified experts" mechanism based on the experts' chemical-specific findings from the "administrative" mechanisms used by OEHHA to list chemicals without specific findings by the experts. Conspicuously absent is any reference to a fourth mechanism authorizing OEHHA to add chemicals to the Proposition 65 list pursuant to Labor Code section 6382:

> Three mechanisms are available for adding chemicals to the Proposition 65 list. *These listing mechanisms provide a comprehensive approach to identify chemicals which may qualify for listing.* One listing mechanism results from findings of the "state's qualified experts" (panels of scientists from outside of state government) following their consideration of evidence compiled and analyzed by the OEHHA scientists. The other two listing mechanisms are administrative in nature. ... Under one administrative listing mechanism, a chemical may be added to the Proposition 65 list if another body, one considered to be an "authoritative body" by the state's qualified experts, has formally identified the chemical as causes cancer of reproductive toxicity. . . . Under the other administrative listing mechanism, chemicals are listed under Proposition 65 if a state of federal agency has "formally required" that the chemical be labeled or identified as causing cancer or reproductive toxicity. [emphasis added.]

Dr. Denton's 1999 report echoes other OEHHA documents that, for many years, referred to only "the three listing mechanisms" set forth in section 25249.8(b). One such document, "Mechanisms for Listing Chemicals Under Proposition 65," was posted on OEHHA's Policy and Procedure website for 11 years, from May 22, 1996, through May 15, 2007. The document states:

> [Proposition 65] ... defines *three mechanisms* by which carcinogens and reproductive toxicants are listed. First, the state's qualified experts may render an opinion that the chemical has been clearly shown to cause cancer or reproductive toxicity "through scientifically valid testing according to generally accepted principles." Second, the statute requires the listing of chemicals formally identified as carcinogens or reproductive toxicants by a body considered authoritative under Proposition 65. Third, the statute requires chemicals to be listed that are required by a state or federal agency to be ... labeled as carcinogens or reproductive toxicants. [emphasis added]

Similar explanations of the "three mechanisms" for supplementing the Proposition 65 list were included in numerous other OEHHA public notices, including both Notices of Intent to List chemicals under the various mechanisms, and regulatory notices announcing changes to the listing regulations.

In 2006, OEHHA used and posted a document entitled "Proposition 65 in Plain Language." In this document, OEHHA not only describes the "three principal ways" for listing chemicals, but also mentions the Labor Code reference, explaining, "This method was used to establish the initial chemical list following voter approval of Proposition 65 in 1986." As with the other documents, this document does not indicate that OEHHA may continue to list chemicals pursuant to the Labor Code reference.

OEHHA's new interpretation of section 25249.8 conflicts with the statute's plain meaning, with standard rules of statutory construction, with the intent revealed by the Ballot Argument, and with the long-standing OEHHA interpretation on which the public has relied for more than 20 years. Implementing this new interpretation by adding styrene to the Proposition 65 list as a carcinogen is unlawful.

D. Conclusion

OEHHA should refrain from adding styrene to the Proposition 65 list as a carcinogen. Styrene is a not a carcinogen. No entity has concluded that styrene is a carcinogen. Scientific evidence developed since IARC prepared its 2002 monographs further confirms that styrene does not cause cancer.

Moreover, adding styrene to the Proposition 65 list as a carcinogen would be unlawful. OEHHA has made numerous interpretations of the provisions of Proposition 65, the Labor Code provisions referenced in Proposition 65, and the federal regulations referenced in the Labor Code provisions. OEHHA cannot act validly without formally adopting these interpretations as regulations pursuant to the California Administrative Procedure Act. Any step to implement those interpretations prior to their formal adoption would constitute an invalid underground regulation. Moreover, the proposal to add styrene to the Proposition 65 list as a carcinogen is inconsistent with the statutory language in Proposition 65, the Ballot Argument explaining that measure, and court decisions interpreting it. Finally, OEHHA's new interpretation that the so-called Labor Code mechanism can be used to augment the Proposition 65 list is contrary to the plain meaning of the statute, the rules of statutory construction, and OEHHA and its predecessor lead agencies' 20-year interpretation.

For all the reasons set out above, OEHHA should not add styrene to the Proposition 65 list as a carcinogen.

Respectfully submitted,

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SIRC COMMENTS – Attachment 1

UPDATE OF STYRENE HUMAN EPIDEMIOLOGICAL DATA PUBLISHED SINCE THE 2002 EVALUATION OF STYRENE BY THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

SUMMARY CONCLUSIONS:

Based on a thorough evaluation of the effects of styrene in humans and experimental animals, it would not be scientifically valid for the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) to list styrene as "known to the state to cause cancer" under Proposition 65. IARC's 2002 classification of styrene as a "possibly carcinogenic to humans" is not an appropriate ministerial basis for the listing of styrene under Proposition 65.

HUMAN DATA

When reviewing the existing epidemiology data for styrene, it is important to understand how styrene is used in the various industries that have been studied as part of the existing epidemiology studies. A brief description follows:

The Reinforced Plastics Composites (RPC) industry has variable exposures among its workers. Simply being employed in the industry does not provide a good estimate of exposure. Some employees are defined as "laminators." Their job is to spray styrene-containing resin and chopped fiberglass onto an open mold and roll the mixture with paint rollers to imbed all the fiberglass into the resin. This potentially results in higher exposures during application and lower exposures when not laminating. Other workers in these facilities perform finishing work (sanding, assembling, etc.) on pieces of cured resin, where there is minimal styrene exposure. Others are maintenance workers, supervisors, or office personnel. Past exposures for laminators have exceeded 100 ppm and often approached 200 ppm. During the periods that the available epidemiology studies estimated exposures (the 1940's to 1980), exposure to laminators was above 30 ppm. Potential confounding exposures include peroxides used to initiate the reaction between styrene and the resins, and solvents used for cleaning equipment. Many other applications of styrene are closed systems and have lower exposures. Without understanding individual job history, one cannot *estimate* exposure.

Styrene-butadiene rubber (SBR) production involves reaction of styrene and butadiene in essentially closed systems. Exposures are below 5 ppm. Recent re-

evaluations have estimated styrene exposure in SBR manufacture to be less than 1 ppm. SBR workers were also exposed to butadiene and dimethyldithiocarbamate. Butadiene has been associated with increased leukemia in other studies.

Styrene exposure in monomer and polymer manufacturing has also been below 5 ppm. Styrene is manufactured by reacting benzene and ethylene (gas) to produce ethylbenzene, which is dehydrogenated. The system has to be closed to contain the ethylene. Exposures to styrene in this process come from fugitive emissions, and are very low. Workers in this industry segment are also exposed to benzene and to a number of pigments used to color polystyrene.

Based on the principle of dose-response, workers in the RPC industries, especially laminators, have been consistently exposed to 10 to 100 fold higher styrene levels than workers in other styrene-related industries. There is also less potential for confounding exposures in the RPC industry. Therefore, if styrene exposure affects cancer, the effects should be most obvious among RPC workers. Effects in SBR or monomer/polymer workers that are not found in RPC workers are not likely to represent effects from styrene exposure.

Since 2002, several studies have been published that relate to styrene epidemiology. A review of these studies, as well as those included in the 2002 IARC review, concludes that there is not adequate evidence that styrene exposure is linked to cancer incidence in the workplace.
A simplified listing and summary of these studies follows below:

2009 International Styrene Epidemiology Review

The *Journal of Occupational and Environmental Medicine* has accepted for publication a comprehensive review of the available styrene epidemiology data titled "Epidemiological Studies of Styrene and Cancer: A review of the Literature" by Boffetta *et al.* (Supported by The Styrene Information & Research Center as a grant.). The lead author, Dr. Boffetta, is currently head of epidemiology at the International Agency for Research on Cancer. Boffetta *et al.*'s conclusion is that there is *no causal relationship* between styrene exposure and human cancer is based on consideration of the full body of styrene epidemiological data.

Other studies

Seidler, A., *et al.*, 2007, "Solvent exposure and malignant lymphoma: a population-based case-control study in Germany." *J. Occup. Med. Toxicol.* 2: 2. (Supported by the Federal Office for Radiation Protection, the European Community and the German Research Foundation.)

Burns, C., et al., 2006, "Styrene and breast cancer incidence in Texas: a comment on an ecological association" *Breast Cancer Res. Treat.* 97(3): 339-340. (Supported by the Styrene Information Research Center). This comment on the Coyle, *et al.* 2006 paper noted that Coyle's reported results are likely to be an example of an ecological fallacy and also noted that the rate of breast cancer in Texas is *low* compared to the rest of the United States. Ambient styrene exposures in the Houston, TX area average 0.018 parts-per-billion. Industrial exposures are about 3 million times greater, but no excess risk of breast cancer has been found in these populations.

Delzell, E., *et al.*, 2006, "An updated study of mortality among North American synthetic rubber industry workers." *Res. Rep. Health Eff. Inst.* (132): 1-63; discussion 65-74. (Supported by U.S. EPA.)

Coyle, Y., *et al.*, 2005, "An ecological study of the association of environmental chemicals on breast cancer incidence in Texas." *Breast Cancer Res Treat* 92(2): 107-14. (Supported by the Natalie Ornish Fund, the Clay Weed Memorial Trust Fund, and CREW, Dallas, TX). This study suggested that styrene might increase breast cancer because the rate of breast cancer in Texas counties correlated with Toxics Release Inventory (TRI) emissions from those counties.

Delzell, E., *et al.*, 2005, "Styrene and ischemic heart disease mortality among synthetic rubber industry workers." *J. Occup. Environ. Med.* 47(12): 1235-43. (Supported by the Styrene Information Research Center).

Graff, J., *et al.*, J, 2005, "Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality." *J. Occup. Environ. Med.* 47(9): 916-932. (Supported by the Health Effects Institute, Massachusetts.) In this study, after controlling for butadiene exposure, there were no increases related to styrene exposure.

Sathiakumar, N. *et al.*, 2005, "An updated study of mortality among North American synthetic rubber industry workers." *Occup. Environ. Med.* 62(12): 822-829. (Support not reported.)

Ruder, A., *et al.*, 2004, "Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: an update." *Am. J. Ind. Med.* 45(2): 165-176. (Support not reported. Authors affiliated with NIOSH.)

Scélo, G., *et al.*, 2004, "Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe)." *Cancer Causes Control* 15(5): 445-452. (Supported by the European Commisson's INCO-COPERNICUS Programme, the Polish State Committee for Scientific Research and IARC.)

Guenel, P., *et al.*, 2002. "Leukemia in relation to occupational exposures to benzene and other agents: a case-control study nested in a cohort of gas and electric utility workers." *Am. J. Ind. Med.* 42(2): 87-97. (Supported by Électricité de France,-Gaz de France.)

SIRC COMMENTS – Attachment 2

REVIEW OF STYRENE ANIMAL CARCINOGENICITY AND MODE OF ACTION STUDIES, INCLUDING STUDIES PUBLISHED SINCE THE 2002 EVALUATION OF STYRENE BY THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

Animal Cancer Studies

1. Rats

There are 8 chronic studies conducted in rats. The only question of increased tumors in rats centers on mammary tumors in females. Four gavage studies were negative. One drinking water study (although at low doses because of limited solubility of styrene in water) was reported as negative by the authors, but Huff (1984) reported a significant increased trend for combined benign and malignant mammary tumors. McConnell *et al.* (1986) reported that normally fibroadenomas (the vast majority of mammary tumors in rats) should not be combined with adenomas or adenocarcinomas. Thus, this study does not provide evidence of increased mammary tumors.

An inhalation study by Jersey *et al.* (1978) reported increased adenocarcinomas at the low dose (600 ppm), but not the high dose (1000 ppm) compared to the concurrent control group. They pointed out that control group incidence (0%) was low and the low dose incidence was within the historical control range. The authors concluded that styrene did not increase mammary tumors. Conti *et al.* (1988) reported increased mammary tumors at all exposure concentrations (25-300 ppm) in an inhalation study. It should be noted that the control incidence was below the control incidence in the Charles River database for S-D rats, and the incidences in the exposed groups were within the historical control range. Furthermore, there was no dose response among the treated groups. Cruzan *et al.* (1998) reported no increase at 50 or 200 ppm (within the range tested by Conti *et al.*) and significant dose-related decreases at 500 and 1000 ppm. Table 1 below from Cruzan *et al.* (1998) compares the results from all dose groups of all eight studies.

Conclusion: A Harvard Expert Panel, IARC, NTP CERHR, ATSDR and European Union all concluded that styrene does not cause increased tumors in rats. SIRC agrees with these assessments.

Table 1. Mammary tumors results in rats exposed to styrene

Strain	Route of Exposure	Administered Daily Dose	Lifetime dose (g/kg)	Reported Response	Reference
SD	Inhalation	25 ppm	1.9	⇑	Conti *et al.*, 1988
SD	Inhalation	50 ppm	3.9	⇑	Conti *et al.*, 1988
SD	Inhalation	100 ppm	7.7	⇑	Conti *et al.*, 1988
SD	Water	125 ppm	9.9	=	Beliles *et al.*, 1985
SD	Inhalation	50 ppm	11.6	=	Cruzan *et al.*, 1998
SD	Gavage	50 mg/kg/day	13.2	=	Conti *et al.*, 1988
SD	Water	250 ppm	14.9	=	Beliles *et al.*, 1985
SD	Inhalation	200 ppm	15.3	⇑	Conti *et al.*, 1988
SD	Inhalation	300 ppm	23	⇑	Conti *et al.*, 1988
F344	Gavage (m)	175 mg/kg/3x	42	=	NCI, 1979b
SD	Inhalation	200 ppm	45	=	Cruzan *et al.*, 1998
BDIV	Gavage	500 mg/kg/wk	53	=	Ponomarkov, 1978
SD	Gavage	250 mg/kg/day	66	=	Conti *et al.*, 1988
F344	Gavage	350 mg/kg/3x	84	=	NCI, 1979b
SD	Inhalation	500 ppm	115	⇓	Cruzan *et al.*, 1998
SD	Inhalation	600 ppm	115	?	Jersey *et al.*, 1978
SD	Inhalation	1000 ppm	192	=	Jersey et al., 1978
SD	Inhalation	1000 ppm	230	⇓	Cruzan et al., 1998
F344	Gavage	500 mg/kg/day	264	=	NCI, 1979a
F344	Gavage	1000 mg/kg/day	396	=	NCI, 1979a
F344	Gavage	2000 mg/kg/day	792	=	NCI, 1979a

Conti studies dosed for 12 months; Gavage (m) was 30% b-nitrostyrene; 70% styrene – dose is styrene only; dosed 3 x/week.

2. Mice

Mice exposed to styrene develop lung tumors late in life. The scientific literature includes three hypotheses for the mode of action (MOA) related to mouse lung tumors.

Two of these hypotheses are based on styrene 7,8-oxide (SO)and its proposed properties and effects on the mouse lung.

a. Cohen *et. al.*, (2002) concluded that differences in lung concentrations of SO do not explain why mice get lung tumors from styrene exposure and rats do not.
b. Gavage administration of styrene oxide (550 mg/kg/day) to mice did not cause increased lung tumors, despite generating styrene oxide levels in the lungs at least equivalent to the level of SO from the metabolism of styrene in the mouse chronic inhalation study.
c. SO-DNA adducts from styrene exposure are very low (<1 adduct per 10^7 nucleotides) and are not greater in mouse lung than in rat lung.
d. Dr. Filser (Hofmann *et al.*, 2006) concluded that SO is not the causative agent in mouse lung tumors from styrene inhalation exposure. Isolated perfused lungs of rats exposed to 1000 ppm (non-tumorigenic in chronic study) in inspired air

produced 8 times as much SO as mouse lungs exposed to 40 ppm (lung tumors in chronic study).

Conclusions:

The toxic agent for styrene's MOA in the above-referenced studies has not been identified, but there is good evidence that it is not SO.

Several governmental bodies and independent organizations have reviewed the body of science related to styrene carcinogenicity. For example:

- **IARC (2002) concluded that "the lung tumors were caused by lung metabolism of styrene and the process does not occur to a meaningful extent in humans."**
- **The European Union (2007) said "it is reasonable to conclude that the lung tumours seen in mice are unlikely to be of any relevance for human health."**
- **The current ATSDR (2007) draft states "Thus, mice appear to be very sensitive to the induction of lung tumors and the mechanism of inducing lung tumors is not likely to be relevant to humans."**

SIRC agrees with the conclusions of these organizations.

3. Mode Of Action (MOA) for Mouse Lung Tumors

The third mode of action for styrene mouse lung tumors has been proposed, and is described more fully below. SIRC is including this discussion because SIRC finds this hypothesis to be the *only* credible suggestion for tumor formation in mice from styrene exposure, given the absence of cancer effects in rat and human data, and because the significant amount of available data addressing this particular MOA hypothesis helps to distinguish the mouse effects as being *unrelated* to a human health concern.

Metabolism of styrene, and several related compounds, by CYP2F2 causes unique metabolites that cause cytotoxicity in the terminal bronchioles and lung tumors (Cruzan *et al.*, 2002, 2005). The proposed MOA described below is supported by similar effects from at least 7 other structurally similar chemicals, some of which cannot form a vinyl epoxide, such as styene 7,8-oxide.

The key characteristics for this MOA hypothesis are:

a. Target organs for cytotoxicity are consistent with location of CYP2F (mouse lung Clara cells and nasal olfactory epithelium, and rat nasal olfactory epithelium).

b. Inhibition of CYP2F2 (5P1P) inhibits cytotoxicity.

c. Inhibition of CYP2E1 (or CYP2E1-knockout mice) does not reduce cytotoxicity from styrene or 4VP (4-hydroxystyrene).

d. Ring-oxidized metabolites probably responsible; 4VP is toxic at 5 fold lower dose than SO.

e. Ring-oxidized metabolites similar in structure to toxic metabolite from coumarin.

f. Similar toxic and lung tumor response from ethylbenzene and cumene, which are not converted to vinyl epoxide.

g. Methyl group at 3 or 4 position of benzene ring (p-methylstyrene, 3-vinyltoluene) prohibit increased mouse lung tumors.

A detailed description of the data supporting this MOA for styrene-induced mouse lung tumors, which is based on evidence from styrene and other structurally-related chemicals (Cruzan *et al.*(2009), in press), is as follows:

A. Postulated MOA for Mouse-Specific Lung Toxicity and Tumorigenicity

CYP2F2 metabolism of several chemicals in terminal bronchiolar Clara cells in mice results in the generation of cytotoxic metabolites. Initial exposures lead to cytotoxicity in terminal bronchioles, followed by reparative cell replication. On continued exposure, the increased cell replication continues, leading to cellular crowding and then to hyperplasia in the terminal bronchioles. As the hyperplasia continues, it expands into the alveolar ducts. Some of this hyperplasia proceeds to form adenomas in the mouse lung, which has a high spontaneous incidence of adenomas in control mice. A few of the adenomas may progress to carcinomas. The analogous CYP2F4 in rats may be as capable of forming these cytotoxic metabolites; however, rats have much lower levels of CYP2F4 in terminal bronchioles and do not produce sufficient levels of these metabolites to cause cytotoxicity, hyperplasia, or lung tumors. Tissues that are high in CYP2F enzymes (CYP2F2 in mouse lung terminal bronchioles and nasal olfactory epithelium; CYP2F4 in rat nasal olfactory epithelium) develop cytotoxicity from these chemicals, which may or may not progress to tumors. Humans have very small amounts of the orthologous isozyme CYP2F1 in lungs or nasal turbinates. CYP2F1 appears to be much less active, if at all, in metabolizing these compounds. Therefore, no cytotoxicity or lung tumors are expected from human exposures to these chemicals. The key element of the hypothesis is that the lung-specific toxicity of this series of compounds converges on their metabolism to cytotoxic metabolites by mouse CYP2F2, which may differ in both specificity and rate of metabolism compared to rats and humans.

Examples of chemicals that are proposed to cause mouse lung tumors by this MOA: Coumarin, naphthalene, styrene, ethylbenzene, a-methylstyrene, cumene, divinylbenzene, benzofuran.

B. Key events

Key events in this MOA are: delivery of the chemical to the respiratory system, metabolism in lung, cytotoxicity in terminal bronchioles, cell replication, and tumors.

B.1. Delivery of Chemical to the Respiratory System

The respiratory system (nasal epithelium to alveoli) is the major interface between mammals and airborne chemicals in their environment. Inhalation of these chemicals delivers them directly to the cells lining the airways. Depending on the physico-chemical properties of the substance, at very low concentrations as much as 50% of the inhaled chemical in the airstream can be absorbed in the nose (Morris, 2000). In the presence of CYP metabolism inhibitors, about 10% of styrene is absorbed in the nasal region (Morris, 2000), but with metabolic activity up to 50% of the styrene is removed from the airstream in the upper respiratory tract of mice. These chemicals can also be absorbed directly into the cells of the terminal bronchioles (Clara cells) as well as the alveolar cells. When absorbed into alveolar cells, they pass into the blood capillaries and are distributed systemically in rats and mice, resulting in detectable blood concentrations of the parent compounds (Cruzan *et al.*, 1998, 2001).

For coumarin, naphthalene, styrene, and ethylbenzene, there is good evidence of distribution of the chemical from all routes of exposure to all tissues, including respiratory epithelium. When exposure is by the oral route, first pass metabolism in the liver dramatically reduces the amount of chemical that is distributed to tissues through the blood stream (Sarangapani *et al.*, 2002). However, metabolism and cytotoxicity in lung from oral exposure has been demonstrated for coumarin (NTP, 1993a), naphthalene (Buckpitt *et al.*, 2002), styrene (Green *et al.*, 2001a), and ethylbenzene (Stott *et al.*, 2003), indicating that these chemicals can be absorbed systemically and penetrate all organs after oral administration, and that the lung has a preferential capacity to metabolize systemically available concentrations of these compounds.

Qualitatively, delivery of these chemicals to lung cells does not appear to be species specific. This process is driven by the solubility of the chemicals in the various tissues, which should be approximately the same across species, including man, and by blood flow and minute volume, which may affect the quantity of these chemicals delivered to the lungs.

B.2. Metabolism in Lung

Many compounds are metabolized to cytotoxic metabolites by CYP2F2 in the Clara cells of the terminal bronchioles of mouse lung. The metabolite(s) responsible for cytotoxicity from most of these compounds in the terminal bronchioles have not been determined.

Coumarin: The major metabolite of coumarin in rats, mice, and humans is 7-hydroxycoumarin. However, coumarin is metabolized by CYP2F2 to coumarin-3,4-epoxide in mouse lung, which rearranges to 2-hydroxyphenylacetaldehyde (Born *et al.*, 2002) and causes mouse lung cytotoxicity and lung tumors. Inhibition of CYP2F2 by 5-phenyl-1-pentyne (5P1P) eliminated the bronchiolar cytotoxicity from coumarin (Born *et al.*, 2002). This metabolism occurs to a much lower extent in rats, which do not develop lung cytotoxicity or lung tumors (Felter *et al.*, 2006). Dihydrocoumarin is not capable of forming 3,4-epoxide and did not induce lung tumors in mice (NTP, 1993b).

Naphthalene: Pulmonary microsomes from mice metabolized naphthalene at approximately 8 times the rate of rat microsomes and produced mostly 1R,2S-naphthalene oxide, whereas rat microsomes produced mostly 1S, 2R- naphthalene oxide (Buckpitt *et al.*, 2002). Inhibition of CYP2F2 by 5P1P eliminated the bronchiolar cytotoxicity from naphthalene (Buckpitt *et al.*, 1995). Genter *et al.* (2006) demonstrated that CYP1A1 and CYP1A2 genes which are inducible by AHR in the mouse respiratory tract do not function to influence naphthalene toxicity, and confirm the results of Phimister *et al.* (2004) that CYP2F2 bioactivates naphthalene in lung and nasal tissues.

Styrene: For styrene, the first step in the major metabolic pathway is oxidation to S-styrene-7,8-oxide; this accounts for at least 80% of the metabolism of styrene in rats and mice (Sumner and Fennell, 1994; Cruzan *et al.*, 2002). It should be noted that oral administration of styrene-7,8-oxide to mice at 275 mg/kg/day did not result in increased lung tumors, even though PBPK models indicate this dose of SO would result in a higher lung level of SO than from metabolism of styrene at 40 ppm by inhalation (Sarangapani *et al.*, 2002). Further, Hofmann *et al.* (2006) demonstrated that *ex vivo* exposure to styrene in rat lungs at 1000 ppm (non-tumorigenic) produced 2.5 nmol styrene oxide/g lung vs. 0.25 from mouse lungs at styrene concentration of 40 ppm (tumorigenic). This led the authors to conclude that styrene oxide is not the agent responsible for mouse lung cytotoxicity from styrene exposure. In mouse lung, two alternate metabolic paths are prevalent; one involves formation of R-styrene-7,8-oxide and the other involves oxidation of the benzene ring (Cruzan *et al.*, 2002; Bartels *et al.*, 2005). Using selective inhibitors, Carlson determined that CYP1A, 2B, and 2E1 had little, if any, impact on Clara cell cytotoxicity of styrene, implying they are not involved in metabolic activation of styrene in the lung (Carlson 1997, Carlson *et al.*, 1998). Inhibition of

2E1, or the use of 2E1 knockout mice demonstrated that 2E1 plays some role in the acute liver cytotoxicity of styrene, but has no impact on the lung toxicity (Carlson, 2004; Vogie *et al.*, 2004). In studies of styrene, the inhibition of CYP2F2 by 5-phenyl-1-pentyne (5P1P) inhibited both the lung cytotoxicity and nasal cytotoxicity in CD-1 mice (Green *et al.*, 2001a, b). 4-Vinylphenol (4VP, 4-hydroxystyrene) is a minor urinary metabolite of styrene and has been used as a substrate for further ring-oxidized metabolites of styrene. 4-VP is 10 times as toxic to mouse lung as styrene and 5 times as toxic as styrene-7,8-oxide (Carlson *et al.*, 2002). Inhibition of CYP2F2 by 5P1P also inhibits the cytotoxicity of 4VP (Carlson, 2002), indicating that there is a subsequent metabolite of 4VP that is responsible for cytotoxicity. The metabolite(s) responsible for cytotoxicity from these compounds in the olfactory epithelium or terminal bronchioles have not been identified.

Ethylbenzene: *In vitro* studies examining comparative mouse, rat and human lung and liver microsomal metabolism of ethylbenzene have confirmed extensive metabolism in all three species to alkyl-oxidized metabolites, *e.g.*, 1-phenylethanol (mouse > rat ~ human; Saghir *et.al.*, 2006; 2007). 1-Phenylethanol was not pneumotoxic or tumorigenic when tested in high-dose oral subchronic and chronic rat and mouse bioassays (NTP, 1990a). No detectable lung toxicity was found from exposure to 1-phenylethanol, 2-phenylethanol, or phenylacetaldehyde in mice (Carlson *et al.*, 2002). Use of GSH-trapping to detect putative cytotoxic catechol and hydroquinone metabolites confirmed the *in vitro* formation of these metabolites in mouse, rat and human liver microsomes, and in mouse and rat, but not human, lung microsomes. Similar to the generation of alkyl-oxidized metabolites, mouse lung microsomes exhibited substantially higher metabolic activity (mouse lung GSH-derived metabolites approximately 10X > rat lung; human lung not detectable; mouse lung GSH metabolites approximately 2X > mouse liver; mouse liver approximately 10X > rat and human liver). Although ring-oxidized metabolites accounted for a relatively small fraction of overall ethylbenzene metabolism, their selective elevation in mouse lung microsomes is nonetheless consistent with the hypothesized mode of action attributing preferential formation of lung-derived cytotoxic, ring-oxidized metabolites as driving the mouse lung specific toxicity of ethylbenzene. Interestingly, both mouse and rat lung microsomes exhibited decreasing amounts of ring-oxidized metabolite formation with increasing concentrations of ethylbenzene, suggesting the possibility of cytochrome P450 suicide inhibition by reactive ring-oxidized metabolite(s). This observation would also be consistent with the hypothesis of the formation of reactive cytotoxic metabolites in mouse lung. 5P1P inhibition studies are currently in progress. 4- Hydroxyethyl-benzene is the only metabolite of ethylbenzene that has been demonstrated to cause mouse lung cytotoxicity in 3-day studies (Kaufmann *et al.*, 2005).

Cumene: In mice exposed to 14C-cumene, urinary metabolites included 4-(2-hydroxy-2-propyl) phenylsulfate, indicating ring oxidation (Ferguson *et al.*, 2008).

Data on the metabolism of these compounds in human lung tissue are limited because of the difficulty obtaining adequate specimens for testing. However, limited data indicate that these metabolites are either not produced in human lung or are produced to a much lower degree (Vassallo *et al.*, 2004; Buckpitt *et al.*, 1986; Cruzan *et al.*, 2002; Felter *et al.*, 2006). Baldwin *et al.* (2004) found no detectable CYP2F in any lung subcompartments in rhesus macaque. Thus human lung and nasal cells would not be expected to develop cytotoxicity from these compounds.

B.3. Cytotoxicity

Short term exposure to coumarin (Born *et al.*, 1998), naphthalene (West *et al.*, 2001), styrene (Cruzan *et al.*, 2002), and ethylbenzene (Stott *et al.*, 2003) all cause cytotoxicity in the terminal bronchioles of mouse lung, but not rat lung (Table 2). The target cells are the Clara cells lining the terminal bronchioles. Toxicity to alveolar cells does not occur. Single gavage doses of 150 and 200 mg/kg coumarin resulted in swelling and necrosis of Clara cells in the terminal bronchioles of male and female B6C3F1 mice (Born *et al.*, 1998). Doses below 150 mg/kg did not cause toxicity. While coumarin caused mouse lung cytotoxicity and lung tumors (NTP, 1993a), dihydrocoumarin did not (NTP, 1993b). Coumarin (NTP, 1993a) causes cytotoxicity in the terminal bronchioles, but since it was administered orally the olfactory epithelium was not examined.

The cytotoxicity from naphthalene is summarized by Buckpitt *et al.* (2002). Briefly, parenteral administration of 50 mg/kg naphthalene results in swelling of the Clara cells (O'Brien *et al.*, 1985); larger doses result in more severe effects, including a loss of apical blebs and decreased endoplasmic reticulum in Clara cells and denuding of Clara cells from the terminal bronchioles. For naphthalene, female mice are more susceptible than males. CYP2F2 bioactivates naphthalene in mouse lung terminal bronchiolar tissue to one or more reactive metabolites that induce cytotoxicity after depleting glutathione (Phimister *et al.*, 2004; Genter *et al.*, 2006). In rats, even at an ip dose of 1600 mg/kg, the Clara cells were apparently normal.

The cytotoxicity of styrene has been summarized by Cruzan *et al.* (2002, 2005). For styrene, cytotoxicity has been measured by increased cell replication following 3 inhalation (40 and 160 ppm) or ip (100 mg/kg) exposures (Green *et al.*, 2001a; Kaufmann *et al.*, 2005). Similarly, following 3 exposures, styrene metabolites styrene-7,8-oxide (100 mg/kg) and 4-hydroxystyrene (35 mg/kg), produced a greater increase in cell replication than the parent compound styrene (Kaufman *et al.*, 2005). In the chronic mouse study (Cruzan *et al.*, 2001), decreased staining of the Clara cells (an indicator of cytotoxicity) was reported in 50-70% of the mice

exposed to 20 ppm for 12, 18 or 24 months and in more than 80% of those exposed to 40, 80, or 160 ppm. Increased cell proliferation has been reported at concentrations of 40 ppm or greater (20 ppm has not been examined). Bronchiolar hyperplasia was seen in a few mice exposed to 40 ppm for 12 months and in most mice exposed to 80 or 160 ppm; by 24 months bronchiolar hyperplasia was seen in up to 40% of the mice exposed to 20 ppm and in more than 75% of those exposed to 40, 80 or 160 ppm (Cruzan *et al.*, 2001). Green and coworkers demonstrated that metabolism of styrene by CYP2F2 is necessary to cause the cytotoxicity (Green *et al.*, 2001a).

Exposure of B6C3F1 mice to tumorigenic 750 ppm ethylbenzene exposures resulted in significantly increased S-phase DNA synthesis in the small airways after 1 week treatment (measured by BrdU incorporation); S-phase synthesis remained elevated after 4 weeks of exposures (non-significant approximate 2X increase; Stott, 2003). In addition, a re-evaluation of the mouse lung tissues from the ethylbenzene bioassay identified the presence of multifocal bronchiolar/parabronchiolar hyperplasia at the 750 ppm tumorigenic exposure level (Brown, 2000).

Administration of these chemicals results in GSH depletion. Phimister *et al.* (2004) demonstrated that administration of naphthalene resulted in GSH depletion. They further reported that lung GSH depletion precedes cellular injury, that lung GSH is depleted by levels of naphthalene that do not deplete liver GSH, and that liver GSH is not able to maintain lung GSH at normal levels following naphthalene administration. Turner and coworkers have demonstrated glutathione (GSH) depletion in lung of mice administered 200 mg/kg styrene ip, which lasted through 6 hours, but returned to normal levels by 12 hours (Turner *et al.*, 2005).

B.4. Cell replication

The mouse terminal bronchioles respond to the cytotoxic injury by generating replacement Clara cells. Increased cell labeling after short-term exposure has been demonstrated for styrene, naphthalene, ethylbenzene, and coumarin. Long-term exposure results in continued bouts of cytotoxicity and cell replication. Continually elevated cell replication leads to overproduction of Clara cells, leading to cellular crowding, followed by hyperplasia which can eventually extend into alveolar ducts (Cruzan *et al.*, 2001). No increase in cell replication rates have been found in alveolar cells of mouse lungs from any of these compounds. No increase in cell replication rates was found in the lungs of rats exposed to styrene or ethylbenzene.

B. 5. Tumors

For coumarin (NTP, 1993a), naphthalene (NTP, 1992), styrene (Cruzan *et al.*, 2001), ethylbenzene (NTP, 1999), cumene (isopropylebenzene) (NTP, 2007a), alpha-methylstyrene (isopropenylbenzene) (NTP, 2007b), divinylbenzene (NTP, 2007c) and benzofuran (NTP, 1989), lung tumors were increased in mice, but not in rats. Tumors were found in the outer layer of the lung where the terminal bronchioles and alveoli intersect. Tumors generally encompass areas of alveoli and bronchioles and are termed "bronchioloalveolar adenomas" or "alveolarbronchiolar adenomas," depending on the pathologist. For all the chemicals in this class, tumors occurred late in life and were not life-shortening; *i.e.*, increased tumors were found only at study termination. In general, the increases were in benign tumors. In the case of styrene, increased lung tumors were found only at the end of the 24-month study, but not at the 12 and 18 month interim sacrifices (Cruzan *et al.*, 2001).

The incidence of lung tumors was not increased in mice exposed to dihydrocoumarin (not able to form 3,4-epoxide), 4-methylstyrene (not able to form 4-hydroxystyrene), mixture of 3- and 4-methylstyrene (vinyltoluene, not able to form 3- or 4-hydroxystyrene), styrene-7,8-oxide, or 1 phenylethanol (side-chain oxidation product of ethylbenzene).

C. Adequacy of Evidence of MOA in animals

C.1. Strength of Association

Chronic inhalation exposure of ethylbenzene, styrene, naphthalene, cumene, alpha-methylstyrene, divinylbenzene, and coumarin have all been shown to increase the incidence of lung tumors among mice, but not rats. Cytotoxicity and increased cell replication have been studied in coumarin, naphthalene, styrene, and ethylbenzene; in mice, all four cause terminal bronchiolar cytotoxicity and increased cell replication at exposure levels comparable to the tumorigenic levels (Table 2). For coumarin, naphthalene, and styrene, it has been demonstrated that inhibition of CYP2F2 inhibits the cytotoxicity and cell replication. Structurally similar chemicals (dihydrocoumarin, 2-, 3-, or 4-methylstyrene) that cannot be oxidized by CYP2F2 to active intermediates did not cause cytotoxicity or mouse lung tumors. Other chemicals have not been tested.

C.2. Consistency of Association

Cytotoxicity from these chemicals occurs in organs with high levels of CYP2F family. CYP2F2 (mouse) is expressed largely in Clara cells in the lung airways (most notably in the terminal bronchioles) and in the nasal olfactory epithelium, with little or none present in the liver. Extensive research has shown that there is a

strong association between CYP2F expression levels and tissue susceptibility to naphthalene cytotoxicity (Buckpitt *et. al.*, 2002). Styrene (Cruzan *et al.*, 1997, 2001), naphthalene (NTP, 1992), cumene (NTP, 2007a), and alpha-methylstyrene (NTP, 2007b) cause cytotoxicity in the terminal bronchioles and nasal olfactory epithelium in mice. Ethylbenzene causes cytotoxicity in the terminal bronchioles, but not in the nasal olfactory epithelium at the concentrations tested (NTP, 1999). In rats, CYP2F4 is expressed mainly in the nasal olfactory epithelium, with lesser amounts in the lung. Styrene (Cruzan *et al.*, 1997, 1998), naphthalene (NTP, 2000), cumene (NTP, 2007a), and alpha-methylstyrene (NTP, 2007b) cause cytotoxicity in the nasal olfactory epithelium of rats, but not in the lung terminal bronchioles. Ethylbenzene does not cause cytotoxicity in either lung or olfactory epithelium in rats (NTP, 1999). Coumarin does not cause cytotoxicity in rat lung or nasal olfactory epithelium. In humans, CYP2F1 is expressed at very low levels in the lung, much lower than CYP2F4 in the rat. Therefore, it is not surprising that these chemicals have not been reported to cause cytotoxicity in human lung cells.

C.3. Specificity of Association

Mice have a much greater number of Clara cells than do rats, which have a much greater number than humans. In addition, mouse Clara cells have much more CYP2F2 than the amount of CYP2F4 found in rat Clara cells. Human lung Clara cells have barely detectable levels of CYP2F1. Thus mice have the greatest number of target cells for toxicity, and those target cells have the greatest capacity to produce toxic metabolites.

Toxicity in mice occurs in 2 organs which contain high levels of CYP2F2: nasal olfactory mucosa (chronic cytotoxicity, limited cellular replacement, cells replaced with respiratory-like cells), and lung (chronic cytotoxicity, rapid cellular replacement in kind, hyperplasia). Toxicity in both olfactory mucosa and Clara cells is prevented if CYP2F2 is inhibited by 5P1P. In rat lung and liver, with very little CYP2F4, these chemicals are metabolized primarily via CYP2E1. Rat nasal olfactory tissue contains a large amount of CYP2F4, in addition to CYP2E1 (Green, 2001b). In rat nasal olfactory tissue, large amounts of the toxic metabolites from these compounds are formed and cytotoxicity is seen from many of them.

D. Qualitative Relevance of the Animal MOA for Humans

The key events for this mouse lung tumor MOA are presented in Table 2 (see next page).

Lung tumors are quite prevalent in humans, mostly related to cigarette smoking. These are thought to arise from bronchiolar cells and may involve cytotoxicity, as well as genotoxicity. This suggests that cytotoxicity in bronchioles of humans from chemicals could contribute to the formation of lung tumors.

The MOA proposes that the toxic effects in mice are due to metabolism by CYP2F2. Rats have lower levels of CYP2F4 in terminal bronchioles and do not produce sufficient metabolites to cause cytotoxicity or lung tumors. Humans have much lower amounts of CYP2F1 and one would expect they would produce much lower levels of cytotoxic metabolites than in mice or even rats. If human CYP2F1 could produce sufficient metabolites from a chemical to produce bronchiolar cell cytotoxicity, it could conceivably lead to lung tumors.

Table 2. Dose and Temporal Relationships of Key Events in Mice

Chemical	Metabolism by CYP2F2	Acute Cytotoxicity	Sustained Cytotoxicity	Hyperplasia	Tumors
Styrene	yes	40 ppm*	20 ppm	160 ppm 3 months to 20 ppm after 2 years	Only at 2 years: 40 ppm - males and 20 ppm -females
Ethylbenzene	yes	750 ppm	750 ppm	750 ppm	750 ppm males only
Naphthalene	yes	8 ppm	30 ppm	30 ppm	30 ppm females only
Cumene	Not tested	Not tested	250 ppm males 125 ppm females	250 ppm males 125 ppm females	250 ppm males 125 ppm females
Alpha-Methylstyrene	Not tested	Not tested	300 ppm females	300 ppm females only	100 ppm females only (not significant)
Divinylbenzene	Not tested	Not tested	10 ppm males and females	10 ppm males and females	10 or 100, not 30 females only
Coumarin	yes	150 mg/kg by gavage	None reported	None reported	200 mg/kg/day gavage males and females 275 mg/kg/day in diet – no increase
Benzofuran	Not tested	Not tested	120 mg/kg by gavage	120 mg/kg	120 mg/kg males and females

*lowest concentration tested

E. Quantitative Relevance of the Animal MOA for Humans

Given that the qualitative impacts of the proposed MOA on tumor outcomes are not fully defined, quantitative differences between mice and humans must also be considered and include:

(1) Rodent exposures in the bioassays are orders of magnitude higher than expected human exposure.
(2) Mouse lung has a larger fraction than the human lung with respect to Clara cells (Plopper *et al.*, 1980a, b).
(3) Rates of metabolism for these chemicals in lung microsomes exhibit clear species differences, with rates in mice being greater than the corresponding rates in humans (Green *et al.*, 2001; Vassallo *et al.*, 2004; Saghir *et al.*, 2006).
(4) Background rates for lung tumors are higher in male mice (~14%) than in humans (~7%, SEER, 2006).

Given these species differences, the MOA is assumed to be plausible in humans,

but humans are expected to be much less sensitive than mice to the pulmonary effects of these chemicals. Because rat lungs contain more CYP2F4 than human lungs contain CYP2F1 and rats do not develop cytotoxicity or lung tumors from these chemicals, it is *very unlikely* that any chemical that causes mouse lung tumors by this MOA and does not cause rat lung tumors will cause human lung tumors.

Conclusion on Mode of Action: The mouse lung tumors are generated following CYP2F2 metabolism in terminal bronchioles; the resulting unique metabolites cause cytotoxicity, leading to regenerative hyperplasia and eventually tumors.

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