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October 26, 2009

John Burklow
Associate Director for Communications
Office of the Director
National Institutes of Health
Building 1, Room 344
9000 Rockville Pike
Bethesda, MD 20892

Re: Request for Correction of Information under the Information Quality Act

Dear Mr. Burklow:

This Request for Correction (RFC) of information is submitted by the Styrene Information and Research Center (SIRC) under the Information Quality Act (IQA)[1] and implementing guidelines issued by the Office of Management and Budget (OMB), [2] the U.S. Department of Health and Human Services (HHS)[3] and the National Institutes of Health (NIH).[4]

SIRC seeks corrections to the "Final Report on Carcinogens Background Document for Styrene" issued by the National Toxicology Program (NTP) on Sept. 29, 2008 (Background Document).[5] The Background Document has been generated and disseminated by NTP in connection with its consideration of styrene for potential listing in the upcoming 12th Report on Carcinogens (RoC). The Background Document is a final document on which NTP has already relied (e.g., in

[1] Pub. L. No. 106-554, § 515, 114 Stat. 2763A-153 to 2763A-154, 44 U.S.C. § 3516 note (2000).

[2] *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies*, 67 Fed. Reg. 8452 (Feb. 22, 2002).

[3] HHS, *Guidelines for Ensuring the Quality of Information Disseminated to the Public*, available at http://www.hhs.gov/infoquality/part1.html.

[4] NIH, *Guidelines for Ensuring the Quality of Information Disseminated to the Public*, available at http://aspe.hhs.gov/infoquality/Guidelines/NIHinfo2.shtml.

[5] http://ntp.niehs.nih.gov/files/Styrene_Background_Document_%289-29-08%29F%5B1%5D.pdf

preparing the draft substance profile for styrene). Thus, it is properly subject to correction now under the IQA. Indeed, if the Background Document is not corrected, it follows that any discussion of styrene in the 12th RoC will be similarly flawed under the IQA, and any listing of styrene in that report will be arbitrary and capricious under the Administrative Procedure Act (APA).

The balance of this document contains: (*i*) detailed descriptions of the portions of the Background Document that require correction, (*ii*) the specific reasons why those portions do not comply with IQA requirements, and (*iii*) suggested recommendations for revising the Background Document. The following discussion also explains how the Background Document currently affects, and will in the future affect, SIRC and its members. Complete contact information for SIRC's Executive Director is presented on the first and last pages of this request.

Unless otherwise indicated, all studies referenced are listed in the Background Document (pages 385-454). Similarly, abbreviations and terminology follow the usage in the Background Document (pages xxiii-xxx and 455-461). Copies of those sections of the Background Document are enclosed for the reader's convenience.

SIRC's request is organized as follows:

I. EXECUTIVE SUMMARY

The Background Document is "influential scientific information" that "present[s] information on health effects," and, therefore, is subject to the most demanding requirements of OMB's, HHS's and NTP's IQA Guidelines. Its failure to meet the objectivity and utility requirements of the Guidelines is extensive and fundamental, and invalidates its analysis and presentation of human, animal and mechanistic data.

Human Data:

- The draft Background Document correctly concluded that workers in the reinforced plastic (RPC) industry are the most relevant study population, because they – particularly laminators – have the highest cumulative exposure to styrene and are sufficiently numerous.
- The best available science regarding RPC does not indicate increased cancer incidence. Kolstad et al. (1995, 1994) is methodologically unsound because it did not actually distinguish between high and low exposure populations, and did not identify if any person with cancer was actually exposed to styrene. The final Background Document's characterization of Kogevinas et al. (1994a, 1993) as showing an association between styrene exposure and cancer is inaccurate and biased; the studies' authors themselves stated that their work did not show such an association, but at best did not exclude it. Other reviews of Kogevinas et al. have endorsed that conclusion.
- The final Background Document's shift to styrene-butadiene rubber (SBR) workers as the relevant population is unexplained and thus incomplete and non-transparent. It was clearly based on the Expert Panel's recommendation, but that recommendation does not represent sound and objective scientific practice, since RPC workers actually have higher cumulative exposures. The Expert Panel also misinterpreted Delzell et al. (2006) as showing evidence of cancer among SBR workers; in comments on that report filed with NTP, Delzell herself rejected this interpretation. By adopting the Expert Panel's view, the final Background Document (finalized prematurely before the close of the comment period on the Expert Panel's report) is thus inaccurate and biased.
- The Background Document's claim that all forms of leukemia arise from the same stem cell is based on unsound method and is unreliable.
- The Background Document's discussions of the association of styrene with breast cancer, lymphohematopoietic cancer, and pancreatic cancer all conflict with the weight of

available scientific studies, which the Background Document ignores, in violation of sound scientific methods and NIH's obligation (and undertaking) to be "comprehensive."

- Findings that are not statistically significant may be useful for guiding further research, but NTP does not follow "sound statistical . . . method []" or "sound . . . scientific practice []" when it bases conclusions regarding "reasonably anticipated" carcinogenicity on non-statistically significant findings.

Animal Data:

- The Background Document found "increased" incidence in mouse tumors in two National Cancer Institute (NCI) studies that NCI itself said provide, respectively, "no more than suggestive evidence" and "no evidence" of tumors. It did so by substituting historical control data from other laboratories for the historical control data contained in the NCI (1979a) study, in violation of sound and objective scientific practices, and by introducing interpretive bias into its characterization of the NCI (1979a) study.
- The Background Document combined data on fibroadenomas and adenocarcinomas, even though doing so does not represent sound and objective scientific practice and is misleading.
- The Background Document deleted references in the draft to decreased incidence of tumors on the basis of unsupported statements about historical experience, in violation of NTP's own policy on use of historical controls.

Mechanistic Data:

- The critical question of styrene metabolism is treated in a very biased and incomplete fashion in the Background Document. First, it presents two competing theories on how styrene metabolites cause mouse lung tumors as if both were still plausible, when in fact the theory that genotoxic events from styrene-7,8-oxide (SO) lead to lung tumors has been discredited in favor of the theory that cytotoxic styrene metabolites generated by cytochrome P450 2F2 (CYP2F2) cause cell proliferation that leads to tumors. Second, it continues the draft's failure even to cite the finding of Hofmann et al. (2005) that levels of SO in rat lungs at eight times the tumorigenic level in mice do not produce tumors in rats, and other data that indicate mouse lung tumors are unrelated to SO. Third, it does not describe the crucial role played by CYP2F2 in catalyzing the ring oxidation of styrene to 4-vinylphenol in the mouse lung. Finally, it fails to note the concordance of studies on the much greater proliferative effect of 4-vinylphenyl compared to SO.
- The Background Document's genotoxity discussion is neither objective nor useful. On the topic of chromosomal aberrations and sister chromatid exchanges, it omits criticisms of the cited human studies, fails even to note the many contrary studies, and does not

address the lack of increases in chromosomal aberration or micronuclei in controlled experiments in laboratory animals. The discussion of DNA adducts does not explain that adducts are biomarkers of exposure, not indicators of adverse health effects. The discussion of DNA strand breaks does not describe the severe limitations of the COMET assays employed.

The Background Document also fails to address the lack of concordance between these three categories of data, thus downplaying the inconsistency inherent in the three presentations.

The Expert Panel's peer review of the draft Background Document does not immunize the final Background Document from this RFC. First, the presumption of objectivity that normally attaches to documents that have been properly peer reviewed does not apply in this case, for two reasons:

- The actions and statements of the Expert Panel, virtually all of which were adopted in the final document, consistently bias the final document and render it less complete and less scientifically sound than the draft.
- NTP did not wait for the final Expert Panel report before NTP finalized the Background Document. In addition, the Background Document was finalized several weeks before the close of the public comment period on the Expert Panel's report. Comments subsequently (but timely) filed by SIRC and others contained fundamental criticisms of the Expert Panel's *draft* report, but NTP's premature action prevented it from even considering whether, in light of those criticisms, it ought not adopt the Expert Panel's draft views in the final Background Document.

Second, peer review does not create a presumption of utility. The Background Document violates the utility requirement of the IQA because it is so fundamentally unreliable and misleading that it is not useful to policymakers at NTP or other federal agencies, or the public.

A complete and objective analysis and presentation of the data regarding styrene leads to the conclusion that styrene is not a human carcinogen, and produces tumors in only in the lungs of some mouse strains because of cell proliferation caused by styrene metabolites (principally 4-vinylphenyl) created by a metabolic process involving CYP2F2 that is irrelevant to humans (or rats, for that matter). Unfortunately, and due in part to the recommendations of the Expert Panel, the final Background Document is consistently skewed to lead to the inaccurate conclusion that

styrene is a human carcinogen based on a genotoxic mechanism. The Background Document's flaws are so significant that, not only does it violate IQA requirements, but a final listing decision based on it would be arbitrary and capricious under the Administrative Procedure Act. While listing decisions are policy choices, to be sustainable, those policy decisions must be based on a minimum quantum of quality information. NIH does not have policy discretion to act on the basis of defective and misleading information like that contained in the final Background Document.

II. IMPACT OF THE BACKGROUND DOCUMENT ON SIRC AND ITS MEMBERS

Listings and classifications in the Report on Carcinogens are widely recognized as highly influential and significant. The dissemination of incorrect or misleading information on styrene in connection with development of the 12th RoC is currently having an adverse impact on SIRC and its member companies. If information that does not meet IQA requirements is then used as a basis for listing styrene as a carcinogen in the 12th RoC, that will lead to even greater direct and substantial economic and reputational damage to the industry that SIRC represents, as explained at length below. It is thus of the utmost importance that the Background Document and other pronouncements by NTP be accurate, reliable, unbiased, clear, complete and transparent in substance and presentation.

A. Adverse Effects of the Background Document and a Potential Carcinogen Listing

The Styrene Information and Research Center, Inc. is a nonprofit trade association whose members include major producers and users of styrene monomer and downstream products. SIRC's mission is to evaluate existing data on potential health effects of styrene, and to develop additional data where it is needed. Since its founding in 1987, SIRC has expended over $14 million on research assessing the possible health and environmental effects of styrene. More information on SIRC can be found at http://www.styrene.org.

We expand on potential impact by considering two of the many markets in which styrene-derived products play a significant role: food contact applications and reinforced

plastics/composites. We then summarize the overall effects on the national economy of listing styrene in an RoC.

Styrene is used to make polymerized styrene, plastics and other products. The key features of many styrenic products are that they are lightweight, strong, and easy to clean. Businesses use styrene to make products that promote health, safety, and a higher standard of living, including:

- Bike helmets
- Transport equipment for vaccines, transplant organs, and blood supplies
- Blood-analysis and dialysis machines
- Refrigerators, microwave ovens, and small kitchen appliances
- Countertops and shower surrounds
- Insulating food-service containers
- Shipping and storage containers for produce, dairy, eggs, and other agricultural products
- Computers, televisions, and video-game consoles
- Automotive tires
- Trucks, cars and boats
- Carpets and furniture
- Traffic-safety equipment

For many products styrene is the primary chemical of choice for the specific application. For example, no reasonably available material is strong and pliant enough to replace styrenic plastic in bike helmets. For other uses, styrene is only one option among several. For example, styrenic plastics used to store and ship milk, eggs, fruit, and other produce are lighter and stronger than their alternatives, but they could be replaced with paper or glass. Food-service containers for quick-serve restaurants that are made from styrene provide outstanding insulation and cleanliness, but they could be replaced with other plastic containers or with paper. Styrenic plastics for shower surrounds and countertops are affordable and inexpensive to transport, but they could be replaced with granite or tile. Such substitutions would inherently come at some cost, however – whether in dollars or performance – since customers would use those other substitutes now if they were cheaper or performed better. They would also have environmental and health costs, as explained below.

1. *Food Packaging/Food Service*

Food packaging and foodservice are highly competitive industries. Since these industries offer products that either come into contact with food that is ingested or are visibly present in the home, they are extremely vulnerable to consumer perceptions of toxicity. Industry associations have received many inquiries from consumers, schools, governing agencies, and purchasing bodies about the safety and health of polystyrene for food-contact uses, including questions about the possible migration of styrene from packaging to food. SIRC has no doubt that the course of NTP's current consideration of styrene has prompted such inquiries. If styrene is listed as a carcinogen in the 12th RoC, businesses in food packaging and foodservice would be swamped with calls from customers concerned about toxicity. They would immediately begin to assess their options for packaging, and many would deselect styrenic products.

If styrene is listed in the 12th RoC, that fact will be widely and promptly known and disseminated throughout the trade press and other media. It will precipitate abrupt deselection of styrenics from the portion of the market that will not wait for SIRC or others to explain whether levels of current exposure actually result in any significant risks. This is what happened during a similar listing issue in Japan. In our experience, deselections like these are nearly impossible to reverse, because they involve redesigning large systems of packaging, printing, loading and marketing.

Deselection of products derived from styrene would harm not only those who produce and use styrenic packaging, but also the environment, consumers, and the public health. Styrenic containers reduce consumption of fossil fuels and greenhouse gas emissions because they can be transported more efficiently than heavier materials such as wooden crates or glass milk bottles. Styrene containers can be rinsed and recycled, unlike porous materials such as cardboard cartons or paper berry baskets, which can seldom be separated and effectively cleaned of food waste.

Styrenic containers promote nutrition and health by making fruits, vegetables, and dairy products more affordable. In addition to being less expensive to transport, styrene containers reduce waste and spoilage. Styrenic containers help prevent food-borne illness because they reduce contamination from bruising and breakage.

An inappropriate listing of styrene as a carcinogen would create a long-lasting misperception about the potential human health impacts of styrenic containers. It would also lead to economic disruption without countervailing benefits, beginning with inefficiencies in foodservice and food container markets and then exponentially expanding its unintended effects.

2. *Cast Polymer Styrenics*

Styrenic products include materials and products for use in the home, such as counter tops, lavatories, vanities, shower receptors, and bathtubs. These "cast polymer" styrenic products are man-made, chemically-bonded, mineral-filled, polymeric materials that are molded and hardened to a solid material in a variety of shapes that meet diverse design needs. The manufacturing process permits a range of uses almost impossible to achieve with quarried stone.
Cast polymer is stronger and less brittle than granite. It is more durable than porcelain. Cast polymer is affordable and inexpensive to transport. It could be replaced with granite or tile, but again, only at some cost.

The home building and home improvement industries are highly competitive, especially for kitchen and bath surface products. Since these products are visibly present in the home, they are also highly vulnerable to consumer perceptions of toxicity. If styrene is inappropriately characterized or listed as a carcinogen, purchasers of kitchen and bath surface products would deselect styrenic products. Competitors would quickly and widely publicize the listing decisions, raising unfounded concerns about product toxicity and creating a false impression that cast polymer products are dangerous. Small cast polymer businesses would have a much harder time hiring and retaining good employees because of the false impression that workplaces with styrene are unsafe.

Listing styrene would also create a false impression that cast polymer businesses are not safe neighbors. Neighbors of cast-polymer businesses will use the information in their attempts to

drive these small businesses out of their neighborhoods through zoning or nuisance actions, by publicizing the listing decision to local government agencies.

If styrene is inappropriately listed as a carcinogen by NTP, many small, family-owned cast-polymer businesses will be forced to close because of lost market share and changes in local land-use regulation. These closures would further disrupt the home-building and home-improvement industries generally. These economic disruptions would begin with small family-owned cast-polymer businesses and then spread to the home improvement and home building industries. Wrongly listing styrene as a carcinogen would create a long-lasting misperception that styrene and its products are dangerous to human health – when, in fact, styrenic plastics promote good health and sound business.

3. *Overall Impacts of a Carcinogen Listing*

Stepping back from these two examples, the overall U.S. styrene industry is a diversified industry of approximately $28 billion comprising hundreds of companies with thousands of facilities that provide directly some 128,000 jobs throughout the country in more than 5,000 plants with an annual payroll that exceeds $4 billion. The industry provides essential raw materials and products for nearly all major American industries, from automobiles and construction to electronics and packaging. Manufacturers produce a wide variety of everyday goods from styrene-based resins ranging from cups and utensils to furniture, bathroom and kitchen appliances, hospital and school supplies, sports and recreational equipment, consumer electronics, automobile parts, and durable lightweight packaging of all kinds.

Styrene is the foundation for corrosion-resistant resins that serve an important role in environmental protection and renewable energy applications. Stack liners for challenging industrial applications and jet bubble reactors for coal desulfurization are manufactured from styrene-based resins that are fiber-reinforced. Styrene-based polymers are also used to manufacture blades for wind-power generators, solar photovoltaic collectors, and underground fuel storage tanks, to name a just few examples. These end-products are typically certified under

applicable standards to ensure safe and effective performance from a number of perspectives, such as the nationally recognized NSF International health effects standard for all materials that contact drinking water.[6]

Major styrene manufacturing states include California, Florida, Georgia, Illinois, Louisiana, New Jersey, Ohio, Pennsylvania, Texas, and West Virginia. Styrene monomer and derivative polymer plants are worth nearly $9 billion. The industry contributes tax revenues of $7 billion annually and almost $540 million to the U.S. trade balance.

The concerns expressed above were endorsed recently by a judge on the California Superior Court, who preliminarily enjoined the state's Office of Environmental Health Hazard Assessment from listing styrene on the Proposition 65 list of chemicals "known to the State to cause cancer."[7] In support of its conclusion that such listing would cause "immediate and irreparable harm" to SIRC and its members, the court stated:

> The court agrees with plaintiff that the designation of a product as a carcinogen, particularly one associated with food, could have a devastating effect on that product's use. Such a designation would likely have the intended "stigmatizing" effect. . . . The fact that plaintiff need not print a warning on its products immediately does not mean that the designation as a carcinogen would not receive immediate and wide-spread publicity. Further, once identified as a carcinogen, it would be difficult to undo such a designation in the event that plaintiff were to prevail in this litigation.[8]

B. SIRC and Its Members Are Affected Persons Entitled to Obtain Correction of the Background Document

As explained above, because the Background Document is not accurate, reliable, unbiased, clear, and complete, it is having adverse economic, health and safety impacts on SIRC, its members, various downstream industries, and the general public. The Background Document's faulty information also currently causes reputational damage to SIRC and its members, as they are

[6] NSF/ANSI Standard 61: Drinking Water System Components -- Health Effects (2008).

[7] SIRC v. OEHHA, No. 34-2009-00053089-CU-JR-GDS (Cal. App. Dep't Super. Ct. Aug. 12, 2009).

[8] *Id*., slip op. at 4 (citation omitted).

viewed critically by many for making a product that appears to be more hazardous than it in fact is. Because the Background Document is a final NTP document, currently being disseminated by NTP, that adversely affects SIRC and its members, NTP is obligated to correct the Background Document's IQA shortcomings.

Those shortcomings are also adversely affecting SIRC and its members through their impact on NTP's ongoing consideration of listing styrene. SIRC recognizes that Background Documents officially "do not contain any opinion regarding the listing status for the candidate substance."[9] But Background Documents do effectively determine the content of the NTP's draft Substance Profiles, which do embody "the NTP's policy decision regarding listing status."[10] The draft Substance Profile for styrene, which was released earlier this year, is essentially a condensed version of the Background Document, supplemented by interpretive conclusions about how information in the Background Document supports styrene's classification as "reasonably anticipated to be a human carcinogen."[11]

And the draft Substance Profile does indeed appear to have inappropriately skewed the views of the members of NTP's Board of Scientific Counselors (BSC). In their discussion this February, numerous BSC members criticized the omissions that they perceived in the document, based on what they already knew, even as they concluded that it supported a "reasonably anticipated" characterization.[12] One can only conclude that, if the draft Substance Profile had actually presented the data in a complete and objective fashion and the BSC members had appreciated the limited and confounded nature of that data, they might well have reached a different conclusion.

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[9] NTP Report on Carcinogen Process, "Draft Background Documents," available at http://ntp.niehs.nih.gov/index.cfm?objectid=FA93DE50-F1F6-975E-7DA225D6FBE45515.

[10] *Id.*, "Peer Review of Draft Substance Profiles."

[11] http://ntp.niehs.nih.gov/files/StyreneProfile1_5_ref_change_v3.pdf.

[12] *See* NTP BSC, Summary Minutes of February 24, 2009 Meeting, available at http://ntp.niehs.nih.gov/ntp/About_NTP/BSC/2009/February/minutes022409.pdf, at 25 (Dr. Eastman: "[The draft] did not reflect the range of findings or the controversy around styrene" and "does not reflect the range of the epidemiological results, the variability of the studies, or the controversial nature of the conclusions."), 27 (Dr. Friedman-Jimenez: "The profile could benefit from more inclusive and critical discussion of negative and statistically insignificant findings."); *see also id.* at 24 (Dr. Pino, noting omission of Wong study), 28-29 (Dr. Hines, criticizing mechanistic discussion), 30-31 (Dr. Loomis, noting "common problems" with styrene epidemiology studies).

While the draft Substance Profile contains a disclaimer that it "should not be construed to represent final NTP determination or policy," it is difficult to imagine NTP coming to a different conclusion in the final version of the Substance Profile – *i.e.*, the 12th RoC – if it continues to rely upon the inaccurate, unreliable and biased information contained in the Background Document and the document's unclear and incomplete presentation of information.

The draft Substance Profile for styrene is scheduled to be finalized as the relevant portion of the 12th RoC, and once approved by the NTP Director and the Secretary of Health and Human Services, it will represent the policy of the U.S. Government. If the 12th RoC finalizes the proposed characterization of styrene as a "reasonably anticipated" carcinogen, the market and reputational injuries recounted above will be inevitable and substantially irreversible. A 'de-listing' of styrene would take years, as likely would a correction of the 12th RoC under the IQA. A properly revised Background Document, however, could well lead to a different result, thereby averting this harm. Thus, it is essential to SIRC and its members that the Background Document be corrected now.

Beyond its market impact, a listing of styrene in the 12th RoC would also have direct legal consequences. Under California's Proposition 65, an RoC listing results in a determination by the state's Office of Environmental Health Hazard Assessment that the substance has been determined by an "authoritative body" to cause cancer.[13] Several other state statutes also mandate that regulatory actions flow from an RoC listing.[14] These legal consequences are the reasons that two federal courts have held RoC listings to be judicially reviewable.[15] They are also reasons why NIH should correct the Background Document now, rather than allowing its defects to impair the 12th RoC.

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[13] *See* Cal. Code Regs. tit. 27, § 25306 (2009).

[14] *See* 820 Ill. Comp. Stat. 255/3-m (2009); Mass. Gen. Laws ch. 111F, § 4(b) (1985); 35 Pa. Stat. Ann. § 7301 *et seq.* (West 1988).

[15] *See Tozzi v. DHHS*, 271 F.3d 301, 310-11 (D.C. Cir. 2001); *Synthetic Organic Chemical Mfrs. Ass'n v. DHHS*, 720 F. Supp. 1244, 1248 (W.D. La. 1989).

III. IQA BACKGROUND AND APPLICABILITY TO THE BACKGROUND DOCUMENT

A. Overview

Congress enacted the IQA to "ensur[e,] and maximiz[e,] the quality, objectivity, utility and integrity of information . . . disseminated by Federal agencies" like NIH.[16] To do so, it required the Office of Management & Budget (OMB) to issue government-wide implementing guidance.[17] It also instructed each agency to issue its own guidelines, which have two functions: (i) to apply the OMB Guidelines to the agency's particular circumstances, and (ii) to "establish administrative mechanisms allowing affected persons to seek *and obtain* correction of information . . . disseminated by the agency that does not comply with the [OMB] guidelines. . . ."[18] OMB issued its guidelines in final form in February 2002. HHS has issued department-wide guidelines,[19] and NIH has issued its own agency-specific guidelines.[20] The Reports on Carcinogens must meet all three sets of guidelines.

OMB's Guidelines require all disseminations to meet "a basic standard of quality . . . appropriate to the nature and timeliness of the information . . . ."[21] They define "quality" in terms of objectivity, utility and integrity.[22] "Objectivity" is centrally relevant in cases of scientific health assessments such as the RoC. Objectivity has significant consequences both for the substance of such information and the way it is presented, as discussed below. "Utility" is also important in this case, as it refers to the usefulness of the information to its intended users, including the public.[23]

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[16] Pub. L. No. 106-554, *supra* note 1, at § 515(a).

[17] *See* 67 Fed. Reg. 8452, *supra* note 2.

[18] Pub. L. No. 106-554, *supra* note 1, at § 515(b)(2)(B) (emphasis added).

[19] *See* HHS Guidelines, *supra* note 3.

[20] *See* NIH Guidelines, *supra* note 4.

[21] 67 Fed. Reg. at 8458.

[22] *Id*. at 8459; *cf*. 44 U.S.C. § 3504(e)(1)(B) (2006).

[23] *Id*.

B. Objectivity -- Substance

From a substantive perspective, "objectivity" means that information must be *accurate, reliable* and *unbiased*.[24] Scientific information must be generated using sound statistical and research methods.[25] "Influential" scientific information must be sufficiently transparent to be reproduced, subject to several caveats.[26] This means, with respect to analytical results, that agencies must provide "sufficient transparency about data and methods that an independent reanalysis could be undertaken by a qualified member of the public."[27] Part III.D below demonstrates that the Background Document is "influential."

Influential information regarding risks to health, safety or the environment must be based on requirements, drawn from the Safe Drinking Water Act (SDWA), to use "the best available, peer-reviewed science and supporting studies conducted in accordance with sound and objective scientific practices; and . . . data collected by accepted methods or best available methods. . . ."[28] Part III.E below explains why these requirements apply to the Background Document.

C. Objectivity -- Presentation

From the perspective of presentation, "objectivity" means that information must be presented in an *accurate, clear, complete* and *unbiased* manner, which includes presentation in the proper context.[29] The sources of the information must be disclosed subject to confidentiality and privacy limits, and where appropriate, data should have full, accurate and transparent documentation, with sources of error identified.[30] Scientific, financial and statistical information must be accompanied by supporting data and models.[31] Influential information regarding risks to health, safety or the environment must additionally meet requirement drawn from the SDWA;

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[24] 67 Fed. Reg. 8549 (emphasis added).

[25] *Id*.

[26] *Id*. at 8460.

[27] *Id.*

[28] *Id*.; *see also* 42 U.S.C. § 300g-1(b)(3)(A) (2006).

[29] 67 Fed. Reg. at 8459 (emphasis added).

[30] *Id*.

[31] *Id*. at 8460.

i.e., it must be comprehensive, informative and understandable, and must specify, among other things: (i) each significant uncertainty and studies that would assist in resolving the same; and (ii) peer-reviewed studies that support, are directly relevant to or fail to support estimates and methodologies used to reconcile inconsistencies in data.[32]

D. The Background Document Is "Influential"

Under the objectivity requirement, higher standards apply to scientific, financial and statistical information that is "influential"; *i.e.*, has a clear and substantial impact on important public policies or private sector decisions.[33] It is clear from the NIH Guidelines and NIH's responses to correction requests involving Reports on Carcinogens that NIH regards them as influential scientific information.[34] Without doubt, the Background Document also constitutes influential scientific information; it will be outcome determinative with regard to classification of styrene under the Report on Carcinogens process:

- The Background Document is the key document reviewed by the Expert Panel and the basis for the Expert Panel's recommendation on classification in the Report on Carcinogens.[35]
- The Background Document is the basis for the draft Substance Profile and will be the basis for the final Substance Profile.
- The Background Document is cited 14 times in the 11-page narrative of the draft Substance Profile for styrene.[36] These references include human, animal and other relevant data; that is, the very heart of the Substance Profile and the basis for classification.
- Other than its cancer classification statements, the draft Substance Profile is a really nothing but a short summary of the Background Document.

While the Background Document does not include an express classification, the characterization of the scientific literature on styrene in the Background Document certainly points towards and is intended to be consistent with the classification of styrene in the Substance Profile.

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[32] *Id.*; *see also* 42 U.S.C. § 300g-1(b)(3)(B).

[33] 67 Fed. Reg. at 8460.

[34] *See* NIH Guidelines, *supra* note 4, at § V.2.d. ("One of our most visible publications is the Report on Carcinogens . . . .").

[35] *See* http://ntp.niehs.nih.gov/?objectid=DFAF3D96-F1F6-975E-70E9156852E58764&#20080722.

[36] *See* http://ntp.niehs.nih.gov/files/StyreneProfile1_5_ref_change_v3.pdf.

E. Relevant SDWA Requirements Apply to the Background Document

As explained Parts III.C & D above, the substance and presentation of influential information regarding risks to health, safety or the environment must meet requirements drawn from the Safe Drinking Water Act (SDWA). To recap, it must:

- Use "the best available, peer-reviewed science and supporting studies conducted in accordance with sound and objective scientific practices; and . . . data collected by accepted methods or best available methods"; and
- "[B]e comprehensive, informative and understandable" and "specify, to the extent practicable . . . (iv) each significant uncertainty and studies that would assist in resolving the same; and (v) peer-reviewed studies that support, are directly relevant to or fail to support estimates and methodologies used to reconcile inconsistencies in data."

NIH's IQA Guidelines appropriately and expressly apply most of these requirements to the RoC process:

> NIEHS and NTP procedures conform to accepted NIH scientific practices where quantitative and qualitative scientific conclusions are based on: (1) The best available science and supporting studies, particularly peer-reviewed studies, conducted in accordance with sound and objective scientific practices; and (2) data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justifies use of the data). The NIEHS and NTP make every effort to ensure that the presentation and dissemination of information about environmental health is comprehensive, informative, and understandable.[37]

SIRC is aware of NTP's contention that the RoC "does not deal with analysis of risks to human health, safety and the environment and therefore is not subject to the SDWA [requirements]."[38] There are three responses to this claim.

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[37] *See* NIH Guidelines, *supra* note 4, at §§ V.2.d, VII.

[38] Letter to Neil J. King from Samuel H. Wilson, M.D. (Oct. 27, 2004).

First, it is clear that the NIH Guidelines do in fact adopt the SDWA requirements, at least to the extent just quoted.

Second, under the SDWA itself, the statutory requirements that OMB incorporated into its objectivity standard relate to EPA actions associated with issuance of national primary drinking water standards, with the "substantive" requirements applicable "to the degree that [such] action is based on science" [39] and the "presentational" requirements applicable to EPA "presentation of information on health effects." [40] Thus, under the SDWA, these requirements are applicable, depending on their relevance, to all stages of the risk assessment or analysis process, including the initial step of *hazard identification* – the role that the RoC plays – and not just EPA's final risk characterization. And while some of the "presentational" requirements speak of "estimate[s] of risk," two of them do not:

> (iv) each significant uncertainty and studies that would assist in resolving the same; and
>
> (v) peer-reviewed studies that support, are directly relevant to or fail to support estimates and methodologies used to reconcile inconsistencies in the scientific data.[41]

NTP should thus apply these two requirements to its RoC process.

Third, NIH's position reflects a crabbed reading of OMB's IQA Guidelines, which do not limit application of the SDWA requirements to risk *characterization*, the last stage of the risk assessment or analysis process. Rather, those requirements apply to all stages of the risk assessment or analysis process, including hazard identification. Federal agency risk assessments routinely incorporate elements performed by other agencies (such as NIH), and it only makes sense for those elements to be subject to the SDWA requirements at the time they are originally disseminated by those other agencies. It would be difficult and highly impractical, for example, for EPA to apply the SDWA requirements to the RoC at the time it was generating a risk assessment for a chemical listed therein. In sum, while NTP is authorized to "adapt" the OMB

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[39] 42 U.S.C. § 300g-1(b)(A). The preamble to OMB's IQA Guidelines specifically recognizes that these SDWA requirements "adopt[] a basic standard of quality for the use of science in agency decisionmaking." 67 Fed. Reg. at 8457.

[40] 42 U.S.C. § 300g-1(b)(3)(B).

[41] *Id.* at §§ 300g-1(b)(3)(B)(iv).

IQA Guidelines to its particular circumstances, it does not have discretion to do so in a way that ignores the clear intent of those guidelines.

F. The Background Document Must Have Utility

As noted above, the OMB IQA guidelines define "utility" to "refer[] to the usefulness of the information to its intended users, including the public. In assessing the usefulness of information that the agency disseminates to the public, the agency needs to consider the uses of the information not only from the perspective of the agency but also from the perspective of the public."[42] This often underestimated requirement is important because it goes to the heart of why an agency is disseminating information in the first place. To the extent a Background Document misstates or overstates the carcinogenicity of a substance, for example, it (and documents based on it, like an RoC) is not useful to other federal or state agencies, whose regulatory or policy resources will now be misdirected. It is also not useful to the public, which will now be misled as to the prevalence of carcinogens in their environment and into taking unnecessary actions to protect themselves from substances that are not, in fact, so hazardous.

G. Statements Contained in the Expert Panel Report Are Subject to Correction to the Extent the Background Document Adopts It

The Expert Panel's report and deliberations are discussed at several points in this request. We are mindful that NTP has previously taken the position that, because the Expert Panel is an independent advisory committee operating under the Federal Advisory Committee Act (FACA),[43] its reports are not agency disseminations subject to the IQA.[44] Whether or not this position is correct as a general matter, it is clear that whenever NTP has incorporated the recommendations of the Expert Panel into the Background Document in a way that at least "reasonably suggests that the agency agrees with the information, this appearance of having the information represent agency views makes agency dissemination of the information subject to

[42] *Id*.

[43] 5 U.S.C. app. § 2 (2006).

[44] *See* http://ntp.niehs.nih.gov/go/29353 (discussing the RoC Subcommittee of the Board of Scientific Counselors).

th[e IQA] guidelines."[45] Indeed, unless NTP has provided some other scientific rationale in the Background Document for a particular statement or action attributed to the Expert Panel, one can only assume that NTP is relying on the rationale expressed in the Expert Panel report. In this request, SIRC has limited its critique of the statements of the Expert Panel to instances where the Background Document has incorporated them and they are directly relevant to its compliance with the IQA.

H. The Identified IQA Deficiencies Will Invalidate Subsequent Documents in the RoC Process Regarding Styrene

A fundamental admonition of the OMB Guidelines is that "[a]gencies shall treat information quality as integral to every step of an agency's development of information, including creation, collection, maintenance, and dissemination."[46] The NIH Guidelines adopt this concept:

> The quality assurance process begins at the inception of the information development process. NIH is committed to integrating the principle of information quality into every phase of information development, including creation, collection, maintenance, and dissemination.[47]

The point of this requirement is not merely that agencies get it right the first time, so they do not have to face the delays and costs of a correction request after the fact; rather, OMB's instruction recognizes the persistent or cumulative effect of an initial failure of information quality: if an agency begins with information that is inaccurate, unreliable, biased or incomplete, those failings will infect subsequent "information products" that the agency develops based on the original information. Applied to the present case, this means if a Background Document is seriously flawed, the substance profile and the final RoC, which are based upon the Background Document, will be similarly flawed. Part IV.C below provides an example of how the flawed analysis and presentation of animal data in the Background Document leads the draft substance profile to come to the erroneous conclusion that there is "sufficient" animal evidence of styrene carcinogenicity to support a finding of "reasonably anticipated to be a human carcinogen." An objective analysis and presentation of the data would conclude that: (i) tumors among animals

[45] 67 Fed. Reg. at 8454.

[46] *Id.* at 8459.

[47] *See* NIH Guidelines, *supra* note 4, at introduction.

are seen only in the mouse lung; and (ii) those tumors are caused by a mechanism of action that is not relevant to humans.

This outcome is not inevitable – if the defective Background Document is corrected, the later documents based on it may be sustainable. But correction of the Background Document is necessary.

In this case, moreover, the flaws of the Background Document are of such magnitude that, not only does it violate IQA requirements, but a final listing decision based on it will be arbitrary and capricious under the APA.[48] SIRC appreciates NIH's view that the choice to list a chemical as carcinogenic, and the particular characterization attached to it (i.e., known or reasonably anticipated), are policy decisions. But to be sustainable, those policy decisions must be based on a minimum quantum of quality information. NIH does not have policy discretion to act on the basis of defective and misleading information.

IV. DETAILED DISCUSSION OF IQA DEFICIENCIES AND REQUIRED CORRECTIONS

A. Overview

This portion of SIRC's request explains in great detail exactly how the Background Document falls short of the applicable IQA standards summarized above. To help relate these defects to the questions that the Background Document is intended to address, these criticisms are organized along the three prongs of the RoC listing criteria for "reasonably anticipated" carcinogens: human studies, animal studies, and mechanistic considerations. Finally, this portion demonstrates the Background Document's failure to discuss the inconsistencies and lack of concordance among these three types of data.

For each issue identified under these four headings, the request first explains why the relevant portion(s) of the Background Document do(es) not meet IQA requirements. It then describes what changes to the Background Document would be required to satisfy those requirements.

[48] 5 U.S.C. § 706(2)(A) (2006).

Because so many of these problems are carried into the draft Substance Profile, the final version of that document will need to be corrected consistently in conformity to the revised Background Document.

B. Human Data

This part of SIRC's request demonstrates why the Background Document's discussion of human cancer studies fails to meet IQA requirements. As it explains in great detail:

- The draft Background Document correctly concluded that workers in the reinforced plastic (RPC) industry are the most relevant study population, because they – particularly laminators – have the highest cumulative exposure to styrene and are sufficiently numerous.
- The best available science regarding RPC does not indicate increased cancer incidence. Kolstad et al. (1995, 1994) is methodologically unsound because it did not actually distinguish between high and low exposure populations and did not identify if any person with cancer was actually exposed to styrene. The final Background Document's characterization of Kogevinas et al. (1994a, 1993) as showing an association between styrene exposure and cancer is inaccurate and biased; the studies' authors themselves stated that their work did not show such an association, but at best did not exclude it. Other reviews of Kogevinas et al. have endorsed that conclusion.
- The final Background Document's shift to styrene-butadiene rubber (SBR) workers as the relevant population is unexplained and thus incomplete and non-transparent. It was clearly based on the Expert Panel's recommendation, but that recommendation does not represent sound and objective scientific practice, since RPC workers actually have higher cumulative exposures. The Expert Panel also misinterpreted Delzell et al. (2006) as showing evidence of cancer among SBR workers; in comments on that report filed with NTP, Delzell herself rejected this interpretation. By adopting the Expert Panel's view, the final Background Document (finalized prematurely before the close of the comment period on the Expert Panel's report) is thus inaccurate and biased.
- The Background Document's claim that all forms of leukemia arise from the same stem cell is based on unsound method and is unreliable.
- The Background document's discussions of the association of styrene with breast cancer, lymphohematopoietic cancer, and pancreatic cancer all conflict with the weight of available scientific studies, which the Background Document ignores, in violation of sound scientific methods and NIH's obligation (and undertaking) to be "comprehensive."
- Findings that are not statistically significant may be useful for guiding further research, but NTP does not follow "sound statistical . . . method[]" or "sound . . . scientific

practice[]" when it bases conclusions regarding "reasonably anticipated" carcinogenicity on non-statistically significant findings.

1. *Shift from the Reinforced Plastics Industry to the SBR Industry*

The Draft Background document identified workers in the reinforced plastics (RPC) industry as the most relevant population to study. In contrast, the final version shifted its focus to styrene-butadiene rubber (SBR) workers. In doing so, however, the Background Document failed to meet the reliability requirement of the IQA, because lower exposure studies with confounding exposures cannot be more significant then higher exposure studies with few confounding exposures. It also failed the clarity and completeness requirements, as it provided no explanation of this profound shift.

The draft Background Document stated (page 133, emphasis supplied):

> Workers in the reinforced plastics industry have experienced styrene exposure levels and cumulative styrene exposure considerably higher than workers in the styrene-butadiene rubber and styrene monomer and polymer industries (Delzell *et al*. 2001, Jensen *et al.* 1990, Kogevinas *et al*. 1994b, Macaluso *et al*. 1996, Macaluso *et al*. 2004, Thiess and Friedheim 1978). Furthermore, the reinforced plastics industry, unlike the two other industries, is characterized by exposure to few other suspected carcinogens (Jensen et al. 1990). **Thus, workers from the reinforced plastics industry are the most relevant study population.** Results for workers biomonitored for styrene also are highly informative, because their styrene exposure was well characterized and represented levels experienced in the reinforced plastics industry (Anttila *et al*. 1998).

In the final Background Document (page 156, lines 1-17), the highlighted sentence from the draft document was deleted without explanation. This change clearly was based, however, on a recommendation from the Expert Panel. Moreover, because NTP adopted the Expert Panel's position without further explanation, the approach of the Expert Panel is properly subject to this correction request.

The Expert Panel's Report stated (page 9):

> The panel does not agree that the reinforced plastics industry or the biomonitored populations are the best for evaluating carcinogenicity of styrene. As noted previously, the reinforced plastics industry has many short-term workers who have not had an adequate duration of exposure to carcinogenic agents, so the at-risk population is too small to adequately assess risks. There is only one study of biomonitored workers starting

> in the late 1970's [Antilla *et al.* 1998], but there is no information about duration or levels of exposure and a comparison of risks by exposure. The population is also quite small. In comparison, the SBR industry studies have several advantages for evaluating carcinogenicity due to long-term exposure, long follow-up and extensive analysis.

The Expert Panel asserted that the RPC studies should be discounted because there were a large number of short-term workers. However, there is no appreciable difference between the two cohorts in the number of workers exposed for more than 10 years. In the Kogevinas and Wong RPC cohorts combined, there were ~6,700 persons exposed to styrene for greater than 10 years, while in the Delzell SBR cohort that the Expert Panel cited (with a median duration of exposure of 11 years), there were ~8,960 persons exposed for greater than 11 years. The Panel did not offer any explanation as to how they determined the SBR cohort was adequate but the RPC cohort was not.

Nor could the Expert Panel have done so. If styrene is acting as a non-threshold (genotoxic) carcinogen, as the Panel suggests, then risk should be equally increased in short and long-term workers as a function of their cumulative exposure. The theory of a genotoxic mode of action (MOA) is that the carcinogen causes DNA damage, with the risk of developing cancer being proportional to the number of "hits" of the carcinogen on the DNA. This MOA clearly entails that cumulative exposure is more important than duration of exposure as a risk factor for genotoxic carcinogens. The median cumulative exposure in the Delzell SBR cohort was 13 ppm-years (~8,962 persons exposed to greater than 13 ppm-years). In the Kogevinas RPC cohort, ~30,516 persons were exposed to greater than 75 ppm-years styrene. In the Wong RPC study, ~11,870 were exposed to greater than 10 ppm-years and ~7,910 of those were exposed to greater than 30 ppm-years. In each of the Wong and Kogevinas RPC studies, therefore, there were more workers with equal or greater cumulative styrene exposure than in the Delzell SBR cohort, yet neither the Wong nor Kogevinas study showed the effects purported to be shown in the SBR cohort.

It is not the duration of exposure to the carcinogenic agent that matters most, as is suggested by the Panel, but rather that there be an adequate follow-up time so that any induced cancer incidence or mortality will be observable. Follow-up time in the RPC workers is clearly

comparable to that of SBR workers. In addition, RPC workers had much higher exposure levels than SBR workers, so, if styrene is a human carcinogen, risks should be higher in the RPC industry. Based on duration of exposure, the RPC studies provide numbers of persons exposed for long periods of time (greater than 10 years) that are comparable to the number in the SBR study.

The results of the RPC studies cannot be dismissed on the basis that too few persons were exposed long-term. Based on cumulative exposure, the RPC studies provide more persons with greater exposure than the SBR cohort. As noted in Boffetta et al. (2009)[49]:

> Studies of workers employed in the manufacture of glass fibre-reinforced plastics such as boat and automobile parts, tanks, and bath units are particularly informative with respect to the potential carcinogenicity of styrene because exposure levels are typically higher than in other industries.

Thus, based on either duration of exposure, as suggested to be most important by the Expert Panel, or cumulative exposure, as suggested to be most important by Drs. Delzell, Teta, Goodman, and Rhomberg in their comments submitted to the NTP, the RPC studies provide (i) an equivalent number of workers exposed for more than 10 years and (ii) more than 30,000 workers with greater cumulative exposures than the SBR studies, and thus should provide the best assessment of styrene's cancer risk in humans. Yet cancer risks are not higher in the RPC industry, and these RPC studies are not supportive of a causal association.

In conclusion, then, the final Background Report shifted its focus from the most relevant population – one with greater cumulative exposure, but in which elevated cancer risks are not seen – to a less relevant population – one that has less cumulative exposure and is more ambiguous regarding carcinogenicity. Because this shift is methodologically unsupportable, the Background Document does not employ "sound and objective scientific practice" and is unsound and unreliable. The Background Document offered no explanation for its shift, but clearly was responding to the direction of the Expert Panel – which similarly did not explain its rationale.

[49] A manuscript was previously provided to NTP and the paper is scheduled to be published in the November issue of the Journal of Occupational and Environmental Medicine.

Because the unexplained shift was apparently result-oriented, the Background Document is incomplete and biased.

Corrective Action: The language from the Draft Background Document should be reinstated, as the data do not support the Expert Panel's comments. The final Background Document (page 156, lines 1-17) should be changed to read:

> Workers in the reinforced plastics industry have experienced styrene exposure levels and cumulative styrene exposure considerably higher than workers in the styrene-butadiene rubber and styrene monomer and polymer industries (Delzell *et al*. 2001, Jensen *et al.* 1990, Kogevinas *et al*. 1994b, Macaluso *et al*. 1996, Macaluso *et al*. 2004, Thiess and Friedheim 1978). Furthermore, the reinforced plastics industry, unlike the two other industries, is characterized by exposure to few other suspected carcinogens (Jensen et al. 1990). **Thus, workers from the reinforced plastics industry are the most relevant study population.** Results for workers biomonitored for styrene also are highly informative, because their styrene exposure was well characterized and represented levels experienced in the reinforced plastics industry (Anttila *et al*. 1998).

2. *Mischaracterization of Kolstad et al. (1995, 1994)*

The principal RPC study on which the draft Background Document relied was Kolstad et al. (1995, 1994). And yet, despite focusing on the most relevant study population, that work does not actually support any association of styrene with cancer among exposed workers, as SIRC explained in its comments on the draft. While NTP attempted to address this shortcoming in the final Background Document, primarily by shifting focus to the SBR industry, it left uncorrected its flawed discussion of the Kolstad work. Thus, pages 93-96 and 103 of the Background Document continue to make assertions based upon that work that are not supported by it and are inconsistent with the objectivity requirement of the IQA.

The Kolstad cohort was treated in the draft Background Document as evaluating high and low exposure, but there is no individual exposure assessment in the study. As explained below, the Background Document inaccurately describes the methodology employed by the study and the resulting data. More problematic, the methodology and data are so unreliable and unsound that one cannot reasonably conclude that the study provides evidence of increased cancer from styrene exposure. It therefore lacks utility as well as objectivity.

Kolstad et al. reviewed the Danish industry registry for companies that might be involved in reinforced plastics. They then asked the company owners if they were involved in RPC. They also asked two suppliers of styrene-based resin material to identify if each company listed was involved in RPC. The resin suppliers identified 386 companies with 36,525 employees as involved to some extent in RPC. The company owners identified 277 companies with 28,518 employees as ever involved in RPC. The suppliers and owners agreed on 233 companies with 26,784 employees. Kolstad et al. performed the rest of the analyses using the responses from the resin suppliers. (They did not explain this choice, but presumably it was because they found a significant increase using the suppliers' assessment and did not find one using owners' assessment in a subset of the cohort.) In reviewing that choice, the European Chemicals Agency concluded: "Therefore, the accuracy of the number of exposed workers used in the calculations of standardised incidence ratios (SIRs) was highly questionable."[50]

Kolstad et al. further asked the suppliers if more or less than 50% of each company's workforce was involved in RPC. The authors' evaluation of "high" versus "low" exposures was thus based solely on whether more or less than 50% of the workers may have been involved in RPC. In fact, however, *RPC workers* in the category where less than 50% of employees were involved in RPC had the same exposure as RPC workers in companies where more than 50% of employees were involved in RPC – there were just RPC workers per company in the "high" category. But RPC workers in the "low" category – as a class – cannot be deemed to have had low exposure.

What's more, no attempt was made to determine how many workers were laminators, the group with by far the highest styrene exposures of any workers in the RPC industry. The authors estimated that 43% of the cohort was involved in RPC, but not all of those were laminators.[51] In

[50] European Chemicals Agency, "Styrene CAS No: 100-42-5 EINECS No: 202-851-5 ANNEX XV TRANSITIONAL REPORT - Documentation of the work done under the Existing Substance Regulation (EEC) No 793/93 and submitted to the European Chemicals Agency according to Article 136(3) of Regulation (EC) No 1907/2006" at 257 (2009)(hereinafter cited as "EU Risk Assessment Report") available at http://echa.europa.eu/chem_data/transit_measures/annex_xv_trans_reports_en.asp.

[51] Table 3-1 of the Background Document describes Kolstad's "high exposed" workers by saying "[a]n estimated 43% of the study population were laminators." That is not true; this group comprises companies where 50 to 100% of employees were thought to be involved in some phase of RPC. The table and text

a typical RPC facility, only 10 to 20% of the workforce are laminators. Thus, one can estimate that between 4 and 9% of the cohort were laminators. There were 32 cases of leukemia among those who worked less than one year and were more than 10 years from first employment; this was reported as a significant increase. No attempt was made to determine if any of the 32 cases was actually exposed to styrene. After discussing this work, the European Chemicals Agency concluded: "Overall, there is no evidence from this study that styrene exposure induces an increased cancer risk."[52]

Corrective Action: The discussion of Kolstad et al. (1995, 1994) (Background Document pages 93-96 and 103) must be modified to accurately describe the study methodology and contextualize the study within the framework of the study authors' approach to exposure characterization. While a statement on page 94 of the Background Document references the "methodological limitation" of the study,[53] the Background Document proceeds to ignore this limitation when characterizing and drawing conclusions from Kolstad et al. (1995, 1994).[54] An accurate characterization of the study would be that it does not provide evidence of increased cancer from styrene exposure. A corrected summary would read:

> In Kolstad et al. (1995, 1994), the authors reviewed the Danish industry registry for companies that might be involved in reinforced plastics (RPC). They then asked the company owners if they were involved in RPC. They also asked two suppliers of styrene-based resin material to identify if each company listed was involved in RPC. The resin suppliers identified 386 companies with 36,525 employees as involved to some extent in RPC. The company owners identified 277 companies with 28,518 employees as ever involved in RPC. The suppliers and owners agreed on 233 companies with 26,784 employees. Kolstad et al. performed the rest of the analyses using only the responses from the resin suppliers, without providing any justification for that choice.

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regarding Kolstad must be modified to correctly state the make-up and exposure of the cohort and correctly assess the conclusions that can scientifically be drawn from this study.

[52] EU Risk Assessment Report, *supra* note 50.

[53] The Background Document states: "Nevertheless, the post hoc decision to rely solely on the dealers' estimates of exposure, together with a lack of exposure measurements (except in a small sample of the companies included in the study in a separate survey) represents a methodological limitation of this study." This is a remarkable understatement.

[54] This error is repeated in the draft substance profile and that document also must be revised based on the same error.

> Kolstad et al.'s evaluation of high versus low exposures was based on whether more or less than 50% of the workers may have been involved in RPC, based on questions directed to the suppliers. In fact, the RPC workers in the category where fewer than 50% of employees were involved in RPC had the same exposure as RPC workers in companies where more than 50% of employees were involved in RPC, and were thus incorrectly deemed to have low exposure.
>
> The authors estimated that 43% of the cohort was involved in RPC, but not all of those were exposed to styrene. No attempt was made to determine how many of these workers were laminators, the group with the highest styrene exposures. In a typical RPC facility only 10 to 20% of the workforce were laminators. Thus, one can estimate that between 4 and 9% of the cohort were laminators. There were 32 leukemias among those who worked less than one year and were more than 10 years from first employment; this was reported as a significant increase. No attempt was made to determine if any of the 32 cases was actually exposed to styrene. Based on this methodology and data, it is not reasonable to conclude that this study provides evidence of increased cancer from styrene exposure.

3. *Kogevinas (1994a, 1993) Mischaracterized*

The second study involving RPC workers on which the Background Document principally relies is Kogevinas et al. (1994a, 1993). Again, although the final Background Document shifted its focus to SBR workers as the most relevant population, it retained erroneous statements about that study at pages 96-98 and 105-106. In particular, it reinterprets the Kogevinas study to assert an association between neoplasms of the lymphatic and hematopoietic tissues among workers exposed to styrene, even though the authors themselves did not conclude from their study that styrene causes cancer. Independent reviews of these studies have agreed with the authors, not with the NTP (Boffetta et al. 2009). Indeed, in this study there were more cases of significant *deficits* of cancer than *increases* of cancer. No consistent pattern of increases was found among studies. The Background Document's interpretation of these studies is thus inaccurate at best and biased at worst. An objective reading of this study is that the epidemiologic evidence the authors evaluated does not support a causal relationship between styrene exposure and any type of human cancer.

The Kogevinas study comprised eight subcohorts that had different criteria for inclusion, different exposure assessments, and different years of follow-up (with an average of 13 years). One of the subcohorts was the group of workers from the Kolstad study where more than 50% of the workers of a company were estimated to have been involved in RPC. These were included

under the job category of "unspecified tasks" because there was no way to determine which ones were laminators and which had other jobs in RPC. Kogevinas and coauthors found no increase in any cancer type by job classification across the eight subcohorts; most important, there was no increased cancer risk among the highest exposed workers, the laminators.

The authors also estimated a cumulative exposure and duration of exposure for each member of the cohort. They then estimated an average exposure by dividing the cumulative exposure by the duration. The authors reported a significant increased trend in total lymphohematopoietic cancers in relation to average exposure, but not in relation to cumulative exposure or to exposure duration. It is erroneous and unreliable, however, to regard a statistically significant difference in the dividend (average exposure) as a causal effect when neither the numerator (cumulative exposure) nor the denominator (duration of exposure) was significant.

Table 3 in Kogevinas *et al.* (1994) lists 74 standardized mortality ratios (SMRs) and their 95% confidence intervals (CIs), each representing an analysis of a particular subset or categorization of the subjects in a study. Of these, six were statistically significant:

- all neoplasms (SMR = 91, 95% CI = 83-99)
- all neoplasms, < 10 years since first exposure (SMR = 84, 95% CI = 72-97)
- lymphatic and hematopoietic cancers, < 2 years exposure, < 10 years since first exposure (SMR = 43, 95% CI = 16-93)
- non-Hodgkin's lymphoma (SMR = 0, 95% CI = 0-99); and
- lymphatic and hematopoietic cancers, < 2 years exposure, 10-19 years since first exposure (SMR = 183, 95% CI = 112-283)
- leukemia, < 2 years exposure, 10-19 years since first exposure (SMR = 215, 95% CI = 103-395).

The two statistically significantly increased SMRs do not represent evidence that styrene exposure caused increased cancer among members of this cohort. Based on a 5% probability of false positive results, out of 74 standardized mortality ratios (SMRs), one would expect four statistically significant – *but false* – outcomes in this study, either false positives or false negatives. Here, four of the six statistically significant results show significant decreases in cancer incidence. Unless one assumes all four of the false positive results were the four decreases, one or two of the significant increases is likely to represent a false positive. Thus,

Kogevinas et al. (1994) does not present data supporting a causal connection between styrene exposure and the cancer types being analyzed.

On page 176, the Background Document states "Kolstad *et al.* 1994 reported a non-significantly increased incidence for all lymphohematopoietic malignancies (SIR = 1.20; 95% CI = 0.98 to 1.44, 112 observed cases) among Danish workers (which overlaps with the international study reported by Kogevinas *et al.* (1994a, 1993). No increase in lymphohematopoietic cancer mortality was observed for the two U.S. studies (Ruder *et al*. 2004, and Wong *et al*. 1994)." The misleading implication of that statement is that "all lymphohematopoietic" malignancies were increased in the Kogevinas study. However, neither Table 2 nor Table 3 of Kogevinas reported increased risk from "all LH" malignancies.

The authors did not conclude that this study provided evidence of carcinogenicity from styrene, but only that it did not exclude that possibility: "These findings leave open the possibility of an excess risk of neoplasms of the lymphatic and hematopoietic tissues among workers exposed to styrene." NTP's reinterpretation of this study as providing an association is thus inaccurate at best and biased at worst.

Corrective Action: The Background Document (pages 96-98 and 105-106) must be revised to note that Kogevinas (1994a, 1993) does not demonstrate any statistical association of neoplasms of the lymphatic and hematopoietic tissues among workers exposed to styrene, although the study did not exclude such a possibility.[55] Suggested substitute language follows.

> The Kogevinas study comprised eight subcohorts that had different criteria for inclusion, different exposure assessments, and different years of follow-up (with an average of 13 years). The workers in the Kolstad study were from companies where more than 50% of the workers were estimated by the companies' suppliers to have been involved in reinforced plastics/composites (RPC).
>
> There was no increase in any cancer type by job classification. Kogevinas and coauthors estimated a cumulative exposure and duration of exposure for each member of the cohort. They then estimated an average exposure by dividing the cumulative exposure by the duration. The authors reported a significant increased trend in total lymphohematopoietic

[55] This error was carried over into the draft substance profile and that document must be revised based on the same error.

cancers in relation to average exposure (albeit only by excluding one of the subcohorts), even though there was no significant trend in either cumulative exposure or exposure duration. It is erroneous and unreliable, however, to regard a statistically significant difference in the dividend (average exposure) as a causal effect when neither the numerator (cumulative exposure) nor the denominator (duration of exposure) was significant.

Table 3 in Kogevinas *et al.* (1994) lists 74 SMRs and their 95% CIs, each representing an analysis of a particular subset or categorization of the subjects in a study. Of these, six are statistically significant:

- all neoplasms (SMR = 91, 95% CI = 83-99)
- all neoplasms, < 10 years since first exposure (SMR = 84, 95% CI = 72-97)
- lymphatic and hematopoietic cancers, < 2 years exposure, < 10 years since first exposure (SMR = 43, 95% CI = 16-93)
- non-Hodgkin's lymphoma (SMR = 0, 95% CI = 0-99); and
- lymphatic and hematopoietic cancers, < 2 years exposure, 10-19 years since first exposure (SMR = 183, 95% CI = 112-283)
- leukemia, < 2 years exposure, 10-19 years since first exposure (SMR = 215, 95% CI = 103-395).

Four of these six are significant decreases in cancer incidence. Based on a 5% probability of false positive results, one would expect 4 statistically significant outcomes in this study. Unless one assumes all four of the false positive results were the four decreases, one or two of the significant increases is likely to represent a false positive.

The authors did not conclude that this study provided evidence of carcinogenicity from styrene, stating: "These findings leave open the possibility of an excess risk of neoplasms of the lymphatic and hematopoietic tissues among workers exposed to styrene." NTP concurs with the authors that the study does not demonstrate a causal association but only does not exclude that possibility.

4. *Misinterpretation of Delzell Studies*

Because NTP switched the focus of its human cancer analysis from the reinforced plastics industry to the SBR industry, the final Background Document contains an extended and substantial discussion of studies of styrene-butadiene rubber (SBR) industry workers by Delzell et al. (2006).[56] While that discussion is consistent with the approach of the Expert Panel, Delzell herself disagreed with the Expert Panel's conclusions. By adopting those conclusions, the Background Document has employed unsound and unobjective methods and is thus unreliable.

[56] Delzell et al. is addressed in six pages of the draft Background Document (pages 97-102) versus 13 pages in the final Background Document (pages 113-126)

NTP re-interpreted Delzell et al. to assert an association when the authors themselves did not conclude that styrene caused cancer. Independent reviews of these studies agreed with the authors, not with the NTP (Boffetta et al. 2009). There were more cases of significant *deficits* of cancer than *increases* of cancer. No consistent pattern of increases was found among studies. As Dr. Delzell points out in her comments, her data do not provide convincing evidence. The data is at best weak, but better characterized as unsupportive. Therefore, the available epidemiologic evidence does not support a causal relationship between styrene exposure and any type of human cancer, in contrast to the Background Document's declaration. This conclusion is supported by the European Union's extensive analysis of Delzell et al., which states:

> Overall, this large cohort-mortality study shows a clear excess of leukaemia among workers in the SBR industry. . . . However, the evidence does not indicate any causal association with occupational exposure to styrene. There is no consistent trend in mortality rates with increasing styrene exposures, particularly when correction is made for the other occupational exposures in these plants.[57]

After discussing other SBR studies, the EU concluded:

> In the styrene-butadiene rubber industry, several studies have pointed to an increased risk of cancer of the lymphatic and haematopoietic systems. However, detailed analysis of these data, together with the general toxicological picture for styrene and butadiene (see butadiene EU RAR), suggests that where increases are due to occupational exposure, it is butadiene, not styrene, that is the causative agent. In conclusion, based on human studies, there is no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene.[58]

The Expert Panel asserted that there is increased risk of Non-Hodgkin lymphoma (NHL), and NHL combined with chronic lymphocytic leukemia (CLL), caused by styrene and not by butadiene in the SBR cohort. It said:

> In the Delzell study there was an exposure-response relationship for NHL and NHL plus chronic lymphocytic leukemia (CLL) that was not attenuated by control for butadiene and only mildly attenuated by control for dimethyldithio-carbamate (DMDTC) (which may not have been appropriate to control for).

[57] EU Risk Assessment Report, *supra* note 50, at 268.

[58] *Id*. at 271.

As noted in the comments by Dr. Delzell provided to NTP, neither her University of Alabama (UAB) report, nor her manuscripts, contained any statistical analysis for exposure-response trends for NHL or NHL-CLL and styrene.

Even more problematic, the Expert Panel said: "It is very unlikely that such a strong exposure-response trend could be due to chance, bias, or confounding." This is a controversial statement, and one for which the Panel provided no basis. In fact, the data do not reflect a strong exposure-response. None of the values for styrene after adjustment for butadiene were significantly different from unexposed workers or were different from values for butadiene adjusted for styrene. Dr. Delzell observed that exposure to butadiene is a complicating factor in interpreting effects of styrene in the SBR workers. While the UAB group found little evidence of an association between butadiene and NHL, butadiene was positively associated with CLL.

In addition, the elevated relative risk (RR) values for NHL and CLL-NHL among workers with nonzero exposure to styrene reflect, to some extent, unexplained and substantial deficits of deaths from NHL and CLL-NHL in the styrene-unexposed group. These deficits were seen when the workers unexposed to styrene were compared to the general population at large (Delzell et al., 2006, tables 21 and 22). The deficit observed in this "external" comparison was statistically significant for CLL-NHL, based on 2 observed compared to 8.1 expected deaths during the time period 1968-1998 (standardized mortality ratio=0.25, 95% confidence interval, 0.03-0.89). This deficit may have been due to chance, but another possible explanation is the presence of an unidentified confounder. If confounding explains this large deficit of CLL-NHL deaths among workers unexposed to styrene, it is not known if using internal comparison procedures (as done for analyses reported in table 3-3 of the Background document) removed such confounding. The Styrene Expert Panel's Subgroup Report for Section 3 (page 3, second paragraph) suggests that the fact that "workers who survive to go from low to high categories will contribute many person-years to low dose groups as they accumulate dose…could account for the very low SMRs seen in the "0" and lowest dose categories." The rationale for this explanation is neither clear nor plausible.

The Panel's justification document emphasizes the inevitable misclassification of exposure to styrene in the epidemiologic studies. However, it errs in repeatedly stating that such misclassification "would be expected to be nondifferential and to bias any measures of association towards no effect" (page 161). This statement is not necessarily true in analyses of exposure-response in which RRs are calculated for each exposure category, compared to a nonexposed or lowest exposure referent category. More important, a presumably underestimated exposure-response association is not tantamount to evidence supporting a true causal relationship. At most, it may be reasonable to argue that lack of an association in a study with an unusual amount of misclassification does not constitute strong evidence against the existence of a true causal relationship, when there are other studies that support such a relationship. In the case of styrene, the other studies do not support such a relationship. Therefore, the Panel's misclassification analysis is flawed and should not be carried over into the Background Document.

When discussing Delzell et al., the Expert Panel's Dr. Matanowski stated:

> If you recombine the groups as we've done in looking at the tables we've recombined these that say have five groups into three groups you find a nice monotonic linear exposure. So it has to do with grouping to some extent.

This statement by Dr. Matanowski demonstrates that the Expert Panel performed a new analysis by combining different groups. In addition to being apparently spontaneous or off-the-cuff, this analysis is undocumented: nothing showing the recombined groups appears in either the Background Document or the Expert Panel materials. Documents provided by NTP in response to a Freedom of Information Action (FOIA) request did not contain any materials documenting this reanalysis of the three recombined groups. This lack of transparency and reproducibility does not conform to IQA requirements and casts doubt on the reliability of the final Background Document. (It also undermines the presumption of objectivity that ordinarily would be created by the Expert Panel's peer review, as discussed in Part V below.)

Dr. Delzell reviewed the final Background Document and her comments were submitted to NTP. Because NTP's characterization of styrene relies heavily on studies by Dr. Delzell and her colleagues, we quote at length from Dr. Delzell's comments. They demonstrate the unsound and

biased nature of the Expert Panel's conclusions. Due to its 2.5-page length, we italicize the quotation of Dr. Delzell's comments.

One important conclusion of the Styrene Expert Panel was, "there was limited evidence for the carcinogenicity of styrene in humans…" (Recommendation for listing status, page 2). I agree that the epidemiologic data and other biologic data on styrene are not sufficient for concluding that exposure to this chemical causes cancer in humans.

Another central conclusion of the Styrene Expert Panel was:

> *"The strongest evidence for cancer in humans is the association between styrene exposure and non-Hodgkin lymphoma (NHL). This evidence comes from the Delzell et al. (2006) analysis in the styrene-butadiene industry and the Kogevinas (1994a) study in the reinforced plastics industry." (Recommendation for listing status, page 2)*

To the extent that the above statement implies that the epidemiologic results for NHL from the two studies constitute strong evidence of a causal relation with styrene, I do not agree. Results for styrene and NHL from both studies are unconvincing.

With regard to the UAB study, the Styrene Expert Panel noted:

> *"In the Delzell study there was an exposure-response relationship for NHL and NHL plus chronic lymphocytic leukemia (CLL) that was not attenuated by control for butadiene and only mildly attenuated by control for dimethyldithiocarbamate (DMDTC) (which may not have been appropriate to control for). It is very unlikely that such a strong exposure-response trend could be due to chance, bias, or confounding." (Recommendation for listing status, page 2).*

The assertion in the second sentence of the above statement is controversial. As the Background document points out frequently, the papers and report on the UAB study did not include any statistical tests of exposure-response trends for styrene and NHL or NHL/CLL. Such tests have been performed and reported for exposure-response data on butadiene and styrene and leukemia (Delzell et al., 2006, Table 16, page 34), but not for styrene and NHL or CLL-NHL. Among workers with nonzero exposure to styrene, the rate ratio (RR) for NHL rose with increasing levels of cumulative styrene exposure, with butadiene-adjusted RRs of 1.7, 1.8, 2.3 and 3.2 for styrene ppm-years categories of >0-<8.3, 8.3-<31.8, 31.8-<61.1 and 61.1+, respectively (Delzell et al., 2006, Table 18, page 37). However, these data were imprecise, and none of the butadiene-adjusted RRs was statistically significant. The corresponding butadiene-adjusted RRs for CLL-NHL were 2.2, 2.2, 2.7 and 3.1 (none was statistically significant) (Delzell et al., 2006, Table 19, page 37). [The Background document correctly cites these results on pages 125 and 126 (Table 3-3), page 125 incorrectly cites the relevant table for NHL and CLL-NHL as 3-2, a table that presents results for leukemia, not NHL or CLL-NHL.]

The latter results for CLL-NHL were driven by the data for NHL. After adjustment for butadiene, there was no evidence of exposure-response for styrene and CLL among those with higher exposure to styrene: in an unpublished "two-agent model" containing terms for age, years since hire, ppm-years of exposure to butadiene (<33.7, 33.7-<425.0, 425.0+) and ppm-years of exposure to styrene (<8.3, 8.3-<61.1, 61.1+), RRs for styrene and CLL were

1.4 (95% CI, 0.4-4.4) for 8.3-<61.1 ppm-years and 1.3 (95% CI, 0.3-5.4) for 61.1+ ppm-years. In this analysis, RRs for butadiene and CLL were 1.3 (95% CI, 0.4-4.1) for 33.7-<425.0 ppm-years and 3.2 (95% CI, 0.8-13.0) for 425.0+ ppm-years. [Pages 124 and 180 incorrectly refers to styrene exposure categories as "terciles"; rather, the three categories were (unexposed plus quartile 1), (quartiles 2 and 3 combined) and quartile 4.]

In addition, the elevated RRs for NHL and CLL-NHL among workers with nonzero exposure to styrene reflect, to some extent, unexplained and substantial deficits of deaths from NHL and CLL-NHL in the styrene-unexposed group. These deficits were seen when the workers unexposed to styrene were compared to the general population at large (Delzell et al., 2006, tables 21 and 22). The deficit observed in this "external" comparison was statistically significant for CLL-NHL, based on 2 observed compared to 8.1 expected deaths during the time period 1968-1998 (standardized mortality ratio=0.25, 95% confidence interval, 0.03-0.89). This deficit may have been due to chance, but another possible explanation is the presence of an unidentified confounder. If confounding explains this large deficit of CLL-NHL deaths among workers unexposed to styrene, it is not known if use internal comparison procedures (as done for analyses reported in table 3-3 of the Background document) removed such confounding. The Styrene Subgroup Report for Section 3 (page 3, second paragraph) suggests that the fact that "…workers who survive to go from low to high categories will contribute many person-years to low dose groups as they accumulate dose…could account for the very low SMRs seen in the "0" and lowest dose categories." The rationale for this explanation is not clear, and the suggested explanation is implausible.

Coexposure to butadiene among synthetic rubber industry workers is another issue that complicates the interpretation of results pertaining to styrene in the UAB study. We found little evidence of an association between butadiene and NHL in the UAB study, but butadiene was associated positively with CLL. Thus, butadiene must be considered as a potential confounder (or, possibly, as an effect modifier) of any association between styrene and CLL or CLL-NHL.

On balance, the UAB study results suggest a positive but statistically imprecise relation between styrene and NHL but no association between styrene and CLL. Thus, if the association with NHL is real, it may be limited to forms of NHL other than small lymphocytic lymphoma. No study has examined this possibility, as it has not been feasible to obtain systematic retrospective data on histopathologic subtypes of NHL in the occupational cohort studies.

The Background document and material from the NTP include fairly extensive comments about the limitations of the epidemiologic information on styrene. These opinions are, for the most part, reasonable. The document notes that data on specific histopathologic subtypes of leukemia and lymphoma, including NHL, are sparse (page 158), that coexposure to butadiene is a potential confounder in the studies of synthetic rubber industry workers and that lack of data on incident cases of lymphoma and leukemia is problematic in most of the available studies of occupational groups. The document's treatment of the issue of DMDTC as a potential confounder also is reasonable.

The document emphasizes the inevitable misclassification of exposure to styrene in the epidemiologic studies, repeatedly stating that such misclassification "…would be expected to

be nondifferential and to bias any measures of association towards no effect" (page 161). This statement is not necessarily true in analyses of exposure-response in which RRs are calculated for each exposure category, compared to a nonexposed or lowest exposure referent category. More importantly, a presumably underestimated exposure-response association is not tantamount to evidence supporting a true causal relationship. At most, it may be reasonable to argue that lack of an association in a study with an unusual amount of misclassification does not constitute strong evidence against the existence of a true causal relationship, when there are other studies that support such a relationship.

In the case of styrene and NHL, such supportive epidemiologic evidence is not sufficient for a conclusion of causality. The epidemiologic studies, including the UAB study, are, at best, weakly supportive. The Background document downplays the fact that studies of reinforced plastics industry workers do not provide clear support for a causal relationship between styrene and NHL, citing exposure misclassification, short follow-up, large proportions of short-term employees, etc., as explanations. However, reinforced plastics industry workers on average experienced styrene exposure concentrations much higher than those in the synthetic rubber industry. Even short-term workers in the reinforced plastics industry could have had cumulative styrene exposures similar to, or above, the median cumulative exposure of 17 ppm-years estimated for all styrene-exposed decedents (or the median of 30 ppm-years among NHL decedents) in the UAB study (Delzell et al., 2006). Thus, the lack of a clear association between styrene and NHL in the studies of reinforced plastics industry workers is an important shortfall of the evidence for the hypothesis that styrene causes NHL. The Background document's argument that the study of Kogevinas et al. (1994a) is supportive of this hypothesis is unconvincing because the Kogevinas et al. study reported that cumulative exposure to styrene was not associated with NHL (only average intensity of exposure displayed a positive association).
[End of Dr. Delzell quotation.]

Corrective Action: The Background Document (pages 113-126 and Table 3-4) should be revised so that the discussion of Delzell et al. (2006) is consistent with the study authors' conclusion finding no association of styrene exposure with NHL and CLL. Exemplary language follows:

> The multi-plant study included 17,924 male workers employed for at least one year during 1944-1991 at seven SBR plants in the USA and one plant in Canada (Delzell et al., 2006). Analyses were limited to the 16,579 workers for whom quantitative exposures were developed. Those excluded had an employment history considered to be inadequate for exposure estimation. External analyses of major work areas and job groups were limited to the 15,612 workers employed in the SBR-related operations at the eight plants, and analyses of work area and job subgroups were limited to the 14,273 workers employed in SBR-related operations at the six plants who had detailed work histories. Eighty-four percent of the workers were exposed to styrene, with median cumulative exposure of 13 ppm-years, and 57% were exposed to styrene peaks. The Spearman rank correlation coefficient between cumulative exposure to 1,3-butadiene and styrene was 0.79, that between styrene and DMDTC was 0.63.

> During mortality follow-up from 1944 to 1998, 6237 deaths occurred (SMR 0.86; 95% CI 0.84-0.88), including 1608 cancer deaths (SMR 0.92; 95% CI 0.88-0.97). The SMR for NHL was 1.00 (95% CI 0.75-1.30; 53 deaths), that for leukemia 1.16 (95% CI 0.91-1.47, 71 deaths). Analyses of leukemia subtypes revealed a non-significantly increased mortality from chronic myeloid leukemia (SMR 1.67; 95% CI 0.83-2.99; 11 deaths) and chronic lymphocytic leukemia (SMR 1.51; 95% CI 0.87-2.47; 16 deaths) and a non-significantly decreased mortality from acute lymphocytic leukemia (SMR 0.42; 95% CI 0.01-2.34; one death). Internal analyses were conducted on leukemia risk (including an additional 10 cases with leukemia mentioned on the death certificate), according to cumulative exposure to 1,3-butadiene, styrene, and DMDTC. A dose-risk relation was present when styrene alone was included in the regression model, which was reduced when either 1,3-butadiene or DMDTC was added to the model. Given the correlation between the exposures to the three agents and the unavoidable exposure misclassification, statistical adjustment might not allow adequate control for confounding. However, an analysis of styrene exposure stratified by 1,3-butadiene or DMDTC exposure did not indicate a consistent pattern of risks for styrene exposure in any category of exposure to the other agents. Analyses including a 10-year lag yielded similarly inconclusive results, and analyses of leukemia subtypes did not reveal subtype-specific associations with styrene exposure. The analysis of styrene exposure and NHL risk revealed a non-significant trend across increasing cumulative styrene exposure categories.

5. *AML, CML, and ALL Development*

The final Background Document maintains that AML, CML, and ALL develop from the same stem cell, but the data used to support that statement does not demonstrate the same origin.

The draft Background Document states (page 134):

> A possible causal effect between styrene and leukemia is only expected for subgroups originating from a common stem cell, but only a few studies have assessed specific sub-diagnoses of leukemia (Flodin *et al.* 1986, Graff *et al.* 2005, Kogevinas *et al.* 1994a, Kolstad *et al.* 1996, Sathiakumar *et al.* 2005).

This approach was replaced with distinctly different perspective in the final Background Document, which states (page 159, lines 1-7):

> Only a few studies have assessed specific sub-diagnoses of leukemia (Delzell *et al.* 2006, Flodin *et al.* 1986, Graff *et al.* 2005, Kogevinas *et al.* 1994a, Kolstad *et al.* 1996, Sathiakumar et al. 2005). AML, CML, and adult ALL arise from the same pluripotential stem cell, based on observations of specific genetic re-arrangements in these 3 subtypes of leukemia, which comprise about 80% of adult leukemias. For example, the blast crisis of CML, 90% of which have the Philadelphia chromosome, cannot be distinguished from AML. An estimated 10% of adult ALL cases have the Philadelphia chromosome, which

> suggests a common stem-cell origin for these leukemias (Bloomfield *et al.* 1978, Jacobs 1989, Yunis 1983).

This change was drawn from the Expert Panel report.[59]

The sentence "AML, CML, and adult ALL arise from the same pluripotential stem cell" is making a statement of fact based only on varying degrees of correlation. However, the final Background Document does not provide any direct evidence that these types of leukemias arise from the same stem cell, nor does the Expert Panel. The approach taken in the final Background Document does not employ "sound statistical and research methods," and is thus unreliable.

Corrective Action: The language on pages 158-159 concerning AML, CML and adult ALL should be removed, or data should be supplied to support it beyond observation and suggestion regarding AML, CML and adult ALL arising from "same pluripotential stem cell." The best approach would be to reinstate the language in the draft Background Document:

> A possible causal effect between styrene and leukemia is only expected for subgroups originating from a common stem cell, but only a few studies have assessed specific sub-diagnoses of leukemia (Flodin *et al.* 1986, Graff *et al.* 2005, Kogevinas *et al.* 1994a, Kolstad *et al.* 1996, Sathiakumar *et al.* 2005).

6. *Unbalanced Discussion of Coyle et al. (2005)*

The Background Document (pages 148-149) summarizes Coyle et al. (2005) as associating styrene exposure with breast cancer. The IQA guidelines require that the Background Document "specify . . . (iv) each significant uncertainty in the process of the assessment of [health] effects and the studies that would assist in resolving the uncertainty; and (v) peer reviewed studies known to the [agency] that support, are directly relevant to, or fail to support any estimate of

[59] RoC Styrene Expert Panel Report, Part A – Peer Review of the Draft Background Document on Styrene, at 10 (July 21-22, 2008), http://ntp.niehs.nih.gov/files/Styrene_Panel_Report__A_BD_final_Rdtd1.pdf.

[health] effects . . . ."[60] Despite this obligation, the summary of Coyle et al. ignores other literature and factors that bring into question those authors' conclusions.

Coyle et al. (2005) concluded that "styrene was an important breast carcinogen" because the rate of breast cancer in Texas counties correlated with TRI emissions from those counties. Burns et al. (2006) reviewed Coyle et al. (2005) and noted that these results are likely to be an example of an ecological fallacy, and to be an incidental one for several reasons.

Ecologically speaking, the association of ambient styrene exposure in Texas and breast cancer is not supported by breast cancer rates in that state. Furthermore, there are several cohort studies of styrene workers that show no elevation of breast cancer. Air monitoring of styrene in the Houston area demonstrates that exposures are very low, especially compared to occupational exposure. Ambient styrene exposures in the Houston, TX area average 0.018 ppb. Industrial exposures are about 3 million times greater, but no excess risk of breast cancer has been found in these populations.

The authors assert that, because Texas ranks first among all US states for the amount of styrene released, there is support for their finding of a relationship between styrene and breast cancer incidence. If this were true and if styrene releases were a cause of breast cancer, one would expect that the rates of breast cancer in Texas would be among the highest in the nation. However, this is not the case. In fact, Texas has the lowest.[61] The overall average annual age-adjusted incidence rate for breast cancer for the period between 1997 and 2001 for Texas was much lower than the national rate (110 vs. 127 per 100,000). Similarly, the rates per 100,000 among Whites (110 vs. 130), Blacks (104 vs. 107) and Hispanics (77 vs. 87) were lower in Texas than the nation.[62]

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[60] 67 Fed. Reg. at 8457-58. The NIH Guidelines also commit NIH to "mak[ing] every effort to ensure that the presentation . . . of information about environmental health is comprehensive . . . ." NIH Guidelines, *supra* note 4, at § V.2.d.

[61] National Cancer Institute, U.S. Cancer Statistics: 2001 Incidence and Mortality, http://apps.nccd.cdc.gov/uscs/index.asp?Year=2001 (accessed on 9/13/2005).

[62] *Id.*

The hypothesis generated by the ecological data analyses of Coyle et al. is not supported by other studies of styrene exposure. The studies referenced by Coyle et al.[63] do not contain any information on exposure, only on the industrial branch in which individuals worked. Conversely, the cohort studies for which breast cancer rates were reported, representing more than 100,000 men and women with occupational exposure to styrene (Table 1), show no elevation of breast cancer. Given the stronger study design (cohort vs. ecological) of those studies, the hypothesis has been tested and not supported.

Furthermore, the authors' conclusions are inconsistent with dose-response principles. Based on ongoing monitoring, ambient styrene monomer exposures in the Houston area average 0.018 ppb,[64] whereas exposures in reinforced plastics workers have been 50,000 ppb or greater.[65] In other words, industrial exposures are approximately 3 million fold higher than environmental exposures in the area of Texas. It is not biologically plausible that styrene could increase breast cancer rates among individuals living in areas with extremely low exposure potential without evidence of risk among those highly exposed in the industrial settings.

Coyle et al. dismissed the negative results of the occupational cohort studies because women were under-represented. However, breast cancer cases among men (which do occur) are also counted in the occupational studies, and if styrene caused breast cancer as Coyle et al. maintained, some elevated incidence in men should have been observed; it was not.

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[63] Johanni Hansen, *Breast Cancer Risk Among Relatively Young Women Employed in Solvent-Using Industries*, 36 Am. J. Indus. Med. 43-47 (1999); P.R. Band, et al., *Identification of Occupational Cancer Risks in British Columbia: A Population-Based Case-Control Study of 995 Incident Breast Cancer Cases by Menopausal Status, Controlling for Confounding Factors*, 42 J. Occuptl. Envtl. Med. 284-310 (2000).

[64] Houston Area Source Toxic Emissions (HASTE) Project; Health Effects Evaluation, Texas Natural Resource Conservation Commission, Office of Air Quality/Toxicology & Risk Assessment Section (SFR-33) February 1996.

[65] *See* studies cited in n. 63 and L.G. Solionava & V.B. Smulevich, *Mortality and Cancer Incidence in a Cohort of Rubber Workers in Moscow*, 19 Scand. J. Work Envtl. Health 96-101 (1993).

The statistical models in Coyle et al.'s study predicted less than 15% of the variability seen in the study. In light of the low breast cancer rates in Texas (to the US), lack of supporting evidence in occupation cohort studies, and evidence of low styrene levels from air monitoring, the current study is a textbook example of the ecological fallacy. This was the clear conclusion of the European Union, which dismissed the study.[66]

The Coyle study thus hardly meets NIH's undertaking to use the "best available science . . . conducted in accordance with sound and objective scientific practices."[67] It is also unreliable and not useful as a means of evaluating (or communicating) the carcinogenicity of styrene.

Summary of Studies of Breast Cancer and Styrene

Number of Breast Cancer Cases/cohort	**Rate ratio**	**Design**	**Exposure**	**Authors**
54,487 cases (577 males)	1.11 (66.2/59.8)	Ecological	Residence in counties with reported release	Coyle, et al. (2005)
29,009 cases vs., 101,254 controls	1.38 (Exposure level 3)	Case-control	Occupation listed on death certificate	Cantor, et al (1995)
4 cases among 7,949 workers	0.46	Cohort	Employment in reinforced plastic industry	Coggon, et al. (1987)
13 cases among 40,688 workers	0.52	Cohort	Employment in reinforced plastics industry	Kogevinas, et al. (1994)
6 cases among 2492 female workers	0.57	Cohort	Employment in rubber plant	Solionova and Smulevich (1993)

[66] *See* EU Risk Assessment Report, *supra* note 50, at 271:

> It is considered that no toxicological significance should be attributed to this statistical association because of a number of limitations identified in the design and conduct of the study. First of all, there is no information in the study on the actual magnitude or length of exposure to styrene that the individual breast cancer cases may have experienced before they took up residence in the various Texas counties where they were diagnosed with breast cancer, as well as how long they lived in these counties. Secondly, although the study took account of age and ethnicity, no attempt was made to control for several important breast cancer risk factors which are normally found associated with more industrialised areas (nulliparity, age at first child birth, use of contraceptives, obesity, etc..). Furthermore, it should be noted that this positive association, which was found for very low levels of environmental exposure to styrene, stands in contrast to the negative findings of several studies conducted in occupational settings where exposures to styrene are a lot higher.

[67] NIH IQA Guidelines, *supra* note 4, at § V.2.d.

3 cases among 36,691 workers	0.79	Cohort	Employment in rubber industry	Sorahan, et al.(1989)[68]
14 cases among 15,826 workers	0.62	Cohort	Employment in reinforced plastics and composites industry	Wong, et al. (1994)

Corrective Action: The Background Document (pages 148-149) discussion of Coyle et al. (2005) should be deleted or replaced with the following statement:

> A review of Coyle et al. (2005) by Burns et al. (2006) noted that these results are likely to be an example of an ecological fallacy. Ambient styrene exposures in the Houston, TX area average 0.018 ppb. Industrial exposures are about 3 million times greater, but no excess risk of breast cancer has been found in these populations.

7. *Improper Characterization of Lymphohematopoietic Malignancies*

The Background Document (page 192) states:

> In the styrene monomer and polymer industries, the risk of lymphohematopoietic malignancies was also increased in most of the studies (as well as the total number of observed cases across studies), but these workers might also have been exposed to benzene.

This is misleading and not accurate. The 12 studies discussed in the Background Document cover the RPC, SBR and monomer/polymer industries, not just monomer/polymer.

There are four studies of workers in the styrene monomer/polymer industries. None had a significant increase in total lymphohematopoietic cancers; only one (Bond et al. (1992)) even had a nonsignificant increase. And that increase was not among those most highly exposed to styrene, raising further doubt about its validity.

An examination of Table 3-8 (page 171) reveals five entries under monomer/polymer for "all LH," none of which is statistically significant. Similarly, of the 14 entries of subcategories of "all LH" for the five studies, only two are statistically significant: all lymphoma by SMR and all lymphoma by SIR, both of which are finding in Hodgson and Jones (1985). As Boffetta et al. (2009) discuss:

[68] Sorahan *T, Parkes H, Veys C, Waterhouse J, Straughan J, Nutt A: Mortality in the British Rubber Industry* 1946-85, Brit. J. Indus. Med. 46:1-11 (1989).

> A study in a UK plant included 622 workers employed for at least one year between 1945 and 1974, and followed until 1978 (Hodgson and Jones, 1985). A total of 34 deaths were observed (43.1 expected), of which three were from lymphoma (0.56 expected, p=0.02). An analysis of cancer incidence identified four cases of LHP neoplasms (1.6 expected, p=0.08). This study also reported three cases of laryngeal cancer (0.5 expected, p=0.04). There was no apparent association between length of service in the styrene exposed jobs and the incidence of LHP neoplasms. All four cases worked less than seven years and for two of the cases, the time between first exposure and death was four and eight years, which are relatively short intervals. Two of the four cases were reticulum cell sarcomas, one was chronic lymphocytic leukemia (entities currently part of NHL) and one was Hodgkin's lymphoma.
>
> A cohort study included 2904 workers employed for at least one year in four US plants between 1937 and 1971, who were followed up between 1940 and 1986 (Ott et al., 1980; Bond et al., 1992). Workers were potentially exposed to a number of agents including styrene monomer, benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes and pigments, polymer dusts and extrusion fumes. Among the styrene based cohort, 687 deaths occurred (standardized mortality ratio [SMR] 0.76 (95% confidence interval [CI] 0.70–0.82), of which 162 were from cancer (SMR 0.81; 95% CI, 0.69–0.95). There was one death from laryngeal cancer (2.9 expected), five deaths from pancreatic cancer (10.3 expected) and 3 deaths from esophageal cancer (4.6 expected) and 28 deaths from LHP neoplasms (SMR 1.39; 95% CI, 0.92–2.08). The excess mortality was confined to workers exposed less than five years (SMR 2.35, 95% CI 1.22-4.11) while among workers with higher exposure (>5ppm) there were 4 deaths observed and 3.0 expected (SMR 1.33, 95% CI 0.36-3.41) with no significant trend with increasing duration of exposure.
>
> In summary, studies of styrene production workers, while limited by small size, do not provide evidence for a causal association between styrene exposure and cancer.

Corrective Action: The statement in the Background Document on page 192 should be revised to read, consistent with Boffetta et al. (2009):

> In the styrene monomer and polymer industries, studies of styrene production workers, while limited by small size, do not provide evidence for a causal association between styrene exposure and cancer, including lymphohematopoietic malignancies.

8. *Improper Characterization of Pancreatic Cancer*

The Background Document (page 192) states:

> Among the highest styrene-exposed group in the reinforced-plastics industry, there was an excess in the total number of observed cases of pancreatic cancer across the four cohort studies compared with the total number of expected cases [corresponding to an SMR of 1.77 (95 % CI = 1.23 to 247)]. Increases in pancreatic cancer risk were observed in three of the four reinforced-plastics industry cohorts (one of which was statistically significant [Kolstad et al. 1995], and the other two of which were nonsignificant [Kogevinas et al. 1994a, Ruder et al. 2004]).

This statement and its supporting tables are rife with error:

- The statement conflicts with NTP's presentation of the data in Table 3-8 (page 169), which characterizes the results of the four studies of reinforced plastics industry workers as being statistically insignificant, including Kolstad et al. (1995). This statement is thus inaccurate.
- Table 3-9 lists "expected cases" of pancreatic cancer among RPC workers in the Kolstad study as 34.2; that number does not appear in the Kolstad publication.
- Table 3-10 indicates that 7.7 pancreatic cancers were expected in the Kolstad study; the Kolstad manuscript indicates that 12.7 were expected.
- The footnote to Table 3-9 says "* Note that in the Kolstad *et al.* studies, high-styrene exposure groups were defined as those who worked in plants where 50% to 100% of the workers were laminators." This is incorrect. Kolstad says that 50 to 100% of the employees were involved in RPC; only an unknown fraction were laminators (probably between 4% and 9%; see pp. 28-29 above).
- Footnote d of Table 3-9 states the Kogevinas study included "[l]aminators, excluding the Danish workers included by Kolstad *et al*." The Danish workers from the Kolstad study were not included in the category "laminators" in the Kogevinas study; therefore, there should have been no need to exclude them in evaluating the laminators in the Kogevinas study.
- On page 174, the text refers to the "high" exposed group in the Kolstad study as a group in which 50-100% of employees were laminators. This is wrong; only an unknown fraction were laminators.
- The Background Document says that in the Kolstad study "a slightly higher risk was seen among long-term than among short-term workers and earlier start dates," but the differences in incidence rates and 95% confidence intervals are so slight and overlapping that this statement is not supportable. For <1 yr employment, IRR was 3.1(0.9-10.3), and for >1 yr employment IRR was 3.4 (1.0-11.4); for employment starting >1970, IRR was 1.4 (0.4-4.7) and for employment starting <1970, IRR was 2.0 (0.6-6.3).

- The data from Kolstad et al., 1995 cannot be combined in a meta-analysis with the other three studies, as was done in Table 3-8 and 3-9, because Kolstad et al. reported incidence data, while the other three studies reported mortality data.
- Tables 3-8 and 3-9 contain numerous values for "expected deaths," but the original article does not contain those figures. The source of those figures needs to be identified.

For the Background Document to have utility, its statement regarding pancreatic cancer should be revised to state that the studies in Table 3-8 do not provide evidence of a causal association between styrene and pancreatic cancer.

Corrective Action: The statement on page 174 (Section 3.8.2) should be revised to read:

> Incidence of pancreatic cancer. Among Danish companies where more than 50% of the workers were employed in RPC manufacture (Kolstad et al., 1995), there was no measurable increase in incidence among workers who were employed in those companies more than 1 year compared to those employed less than 1 year and no difference between those first employed before 1970 compared to those first employed after 1970. There were no significant increases in death from pancreatic cancer among the other three studies of RPC workers (Kogevinas et al., 1994; Ruder et al., 2004; Wong et al., 1994).

The statement on page 192 of the Background Document should be revised to read:

> No statistically significant increases in death from pancreatic cancer were observed in the reinforced-plastics industry cohort studies.

9. *Characterization of Non-Statistically Significant Data*

As noted in the preceding section and elsewhere, the Background Document finds trends and draws conclusions based on non-statistically significant data. There is no discussion in the Background Document related to this use of statistics, in violation of the IQA requirement that analytic results "be developed using sound statistical . . . methods."

As commonly understood, a result is deemed statistically significant if it is unlikely to have occurred by chance. As Ronald Fisher explained: "Critical tests of this kind may be called tests of significance, and when such tests are available we may discover whether a second sample is

or is not significantly different from the first."[69] Statistical significance denotes statistical evidence that there is a difference.

Stated more formally, hypothesis testing statistics are inferential statistics that are used to evaluate the accuracy of a statistical hypothesis concerning the value of a parameter. One step in this evaluative process is testing the null hypothesis, which may be considered a guess concerning the value of a parameter that suggests the absence of some pattern or relationship of interest.[70]

When results are not statistically significant at some chosen level of statistical confidence, it denotes the absence, to that degree of confidence, of a pattern, relationship or difference that may be taken as evidence of a causal effect. Here, the absence of statistical significance means the absence of a difference sufficient enough for a conclusion to be adopted as to the occurrence of a causal effect.[71] Non-statistically significant differences in data could well be due to chance, or could be statistical artifacts resulting from an inadequate sample size, etc.

We recognize that the literature includes debate about appropriate and inappropriate uses of statistical significance.[72] We do not, for example, consider it inappropriate to evaluate non-statistically significant data in exploring areas for future research. However, the Background Document is a highly influential scientific document that serves as the basis for formal governmental decision making and resultant regulatory obligations. It purports to draw conclusions, moreover, that convey substantial degrees of certainty (i.e., "known" or "reasonably

[69] R. A. Fisher, *Statistical Methods for Research Workers* 43 (Oliver and Boyd, 1925).

[70] These descriptions are drawn from R.E. McGrath, *Understanding Statistics: A Research Perspective* 155-156 (Longman, 1997).

[71] Strictly speaking, a statistically significant difference only justifies rejection of the null (i.e., by chance) hypothesis; it does not demonstrate the correctness of any other hypothesis. But the concordance of several statistically significant findings, combined with other factors like biological plausibility of proposed mechanism of action, collectively can rise to a level of sufficiency that justifies a judgment that some other hypothesis is indeed correct.

[72] *See generally* D.N. McCloskey & S. T. Ziliak, *The Cult of Statistical Significance: How the Standard Error Costs Us Jobs, Justice, and Lives* (U. Mich. Press, 2008).

anticipated") – as opposed to less certain labels like "possible" or "potential." With these purposes and consequences in mind, it is not "sound statistical . . . method[]" or "sound . . . scientific practice[]" for NTP to used non-statistically significant data as a basis for finding trends in the Background Document or other documents related to the Report on Carcinogens.

Corrective Action: Delete any conclusions or inferences based on non-statistically significant data, unless accompanied by a statement that the data are statistically insignificant or that the data do not support a finding of an effect. This correction request applies to the entire Background Document and is not limited to human data.

Examples:
p. 173, line 2; line 10
p. 174, line 5; line 14, 15 (p= 0.068 not significant)
p. 175, line 6, line 16
p. 176, line 10
p. 177, line 4, line 17, line 27 (additionally, SIRC cannot find any mention of analysis by leukemia subtype in the Kogevinas manuscript or complete report, as indicated at this point in the Background Document)
p. 181, line 9, line 14, 15 (p values greater than 0.05)
p. 183, line 1, line 8, 9, line 27
p. 184, line 9, line 18, line 23, 24
p. 190, line 11
p. 191, line 28 (It is not sound and objective scientific practice for the Background Document to claim an increase in multiple myeloma because 4 cases occurred when only 3.4 were expected. If there had been only 3, would that be a deficit?)
p. 192, line 6, line 10, line 25, line 29
p. 193, line 9, line 13

C. IQA Deficiencies for Animal Data

The Background Document's analysis and presentation of the animal data fails to meet IQA requirements in several respects:

- It found "increased" incidence in mouse tumors in two National Cancer Institute (NCI) studies that NCI itself said provide, respectively, "no more than suggestive evidence" and "no evidence" of tumors. It did so by substituting historical control data from other laboratories for the historical control data contained in the NCI (1979a) study, in violation of sound and objective scientific practices, and by introducing interpretive bias into its characterization of the NCI (1979a) study.
- It combined data on fibroadenomas and adenocarcinomas, even though doing so does not represent sound and objective scientific practice and is misleading.
- It deleted references in the draft to decreased incidence of tumors on the basis of unsupported statements about historical experience, in violation of NTP's own policy on use of historical controls.

Most troublesome, many of these flaws – and the resulting bias of the Document – are due to the NTP authors of the final Background Document following recommendations of the Expert Panel that consistently skewed the analysis and presentation of data to deemphasize negative or ambiguous findings in favor of apparent findings of carcinogenicity.

As a result of this persistent biasing of the animal data section of the Background Document, the draft Substance Profile that is based on it reaches the conclusion that there is "sufficient" evidence of styrene's carcinogenicity – when an objective analysis and presentation of the data would conclude that: (i) tumors among animals are seen only in the mouse lung; and (ii) those tumors are caused by a mechanism of action that is not relevant to humans.

1. *NCI Oral Study*

In the final Background Document, NTP developed and used a new historical control analysis to evaluate the NCI (1979a) study, in which NCI concluded that its tumor data were within the historical control range and provided no more than suggestive evidence of cancer. NTP's new historical control analysis used animals from a laboratory different than that used in NCI (1979a) in a purported attempt to obtain a sufficient number of controls for studies that used corn oil as the vehicle, even though NTP itself in its earlier study had concluded that: (i) corn oil had no impact on the incidence of lung tumors; and (ii) historical controls for mouse tumor studies should be drawn only from studies conducted by the same laboratory. NTP's creation and use of this new data base for the final Background Document was thus not in accordance with sound

and objective scientific practices, and its reinterpretation of the NCI study is unreliable. NTP's decision to go counter to its own, earlier study suggests an attempt to bias the interpretation of the NCI study to support a preferred hypothesis.

In its 1979a study, NCI administered styrene in corn oil by gavage to 20 male and 20 female control mice and 50 male and 50 female mice in each of the two treated groups, 150 and 300 mg/kg day (NCI, 1979a). There was an increased trend for lung tumors in males and the high dose was significantly elevated compared to the concurrent control (0, 12 and 18% at 0, 150 and 300 mg/kg/day styrene in corn oil).

The NCI report identifies two studies of chemicals dissolved in corn oil at the same laboratory (Litton), including the styrene study. NCI concluded that this was insufficient to assess the historical control range, and so it relied on the incidence in control mice from several diet studies performed at the same laboratory at about the same time. The historical control level of cancer incidence in the studies NCI selected averaged 12%, with a range up to 20%. The primary reviewer of the NCI report recommended that the study provided suggestive evidence of carcinogenicity, while the secondary reviewer recommended that the study was negative. The NCI concluded that the styrene incidence was within the historical range and the styrene difference from control provided no more than suggestive evidence.

Without explanation of any rationale for doing so, other then an implied disagreement with the NCI authors' conclusions, for the final Background Document, NTP developed a novel historical control data base using corn oil controls from the two studies at Litton, plus 12 additional studies at nearby Hazleton Laboratories from the first 110 technical report (TR) numbers. The Hazleton studies included nearly all NCI studies done at Hazleton with a lower TR number than the styrene study (TR185). This new NTP control database had an average incidence of lung tumors of 4%. Therefore, the Background Document concludes that: (i) the control incidence in the Litton styrene study was not low; (ii) the incidence in the styrene study was outside the historical control range; and (iii) the NCI study provided clear evidence of carcinogenicity of styrene by oral gavage.

Although the Background Document posits that corn oil controls were needed because of the potential for this vehicle to influence tumor outcome, NTP's own, earlier analysis of the NTP historical control database concluded that corn oil specifically did not impact lung tumor incidence in B6C3F1 mice in NCI-NTP carcinogenesis bioassays (Haseman et al., 1985). Further, that analysis reported that the incidence of mouse lung tumors exhibited significant interlaboratory variability and recommended that use of historical control tumor incidence values to facilitate bioassay interpretation should be restricted to values developed within the same testing laboratory (Haseman et al. 1985). Remarkably, the Background Document does not even reference Haseman et al. 1985, despite that fact that the lead author was listed as a contributing consultant on the Background Document (page iii).

The Background Document fails to assess whether there was a difference in lung tumor rates in male B6C3F1 mice at Litton versus Hazleton. As noted earlier, NCI used a group of diet studies from Litton as a comparison, with an average incidence of 12% and range to 20%. The Background Document instead compares the NCI styrene study to the incidence in 14 control groups administered corn oil from Litton (2 studies) and Hazleton (12 studies). SIRC examined not only all the corn oil control studies at Hazleton, but also all the diet studies at Hazleton conducted about the same time as the styrene study. SIRC found 2 additional corn oil studies not included in the NTP database (total 14) and 14 diet studies of approximately 91 weeks. The average tumor incidence (and range) for the corn oil studies was 4% (range 0-18%), based on 10/256 male mice, and for the diet studies was 2%, based on 6/260 male mice (0-11%). These data confirm the conclusion of Haseman et al. (1985) that the use of corn oil has little impact on the incidence of tumors in the NCI-NTP carcinogenicity studies, and particularly so in B6C3F1 mice.

A laboratory difference in lung tumor incidence was further supported by examining 104-week studies at the two labs. The incidence at Hazleton was 11% (7 studies, range 2-18%) and at Litton was 19% (40 studies, range 0-45%). Thus, actual historical control group incidence was

quite different between Litton and Hazelton labs and was much higher than the incidence used by NTP for its reanalysis of the NCI styrene results.

The negative findings of NCI 1979a should also be compared with NCI (1979b), a gavage study of commercial β-nitrostyrene (TR170) in B6C3F1 mice. Commercial β-nitrostyrene is 30% β-nitrostyrene and 70% styrene (Background Document at page 212). Therefore, the mice that received β-nitrostyrene received 2.3 times as much styrene as they did β-nitrostyrene. The β-nitrostyrene doses of were 87.5 and 175 mg/kg 3 days per week for 78 weeks, followed by 14 weeks of observation. The styrene doses were 204 and 408 mg/kg/day 3 days per week. Note that the daily doses of styrene in 1979b (TR170) were greater than those of styrene (by itself) in 1979a (TR185). Again, the incidence of alveolar/bronchiolar adenomas or carcinomas in the control male mice was 0 of 20. 11 of 50 male mice dosed at 204 mg/kg/day styrene developed lung tumors, while only 2 of 36 males dosed at 408 mg/kg had lung tumors. Fourteen male high-dose mice died during week 40, which was attributed to a handling accident. The average weekly doses of styrene in TR170 and TR185 were: low dose - 87.5 vs. 107 mg/kg/day averaged over 7 days/week, and high dose – 175 vs. 214 mg/kg/day averaged over 7 days/week. (Calculated by daily dose x days/week dosed/ 7 days/week.) Thus, both studies demonstrate that at similar doses, styrene (dosed with β-nitrostyrene) did not increase lung tumors in male mice.

In the Background Document, NTP comments on NCI (1979b): "However, because of poor survival of the high-dose male mice there were substantially fewer animals at risk for late-occurring lung tumors." Background Document at page 212. This statement is not complete and reflects interpretative bias. It implies there was poor survival because of toxicity and that the maximum tolerated dose was exceeded. It is clear, however, that 36 healthy animals were tested and that the NCI staff and reviewers agreed that a sufficient number survived. If NTP is going to include interpretive statements in a Background Document, they must be balanced.

All in all, there are four gavage studies of styrene that provide conflicting data and no more than suggestive evidence of carcinogenicity. The results are summarized below:

Animal	Doses	Observations	Reference
Styrene in B6C3F1	0, 150, 300	Increase in high dose males within historical control range NCI: Suggestive evidence	NCI, 1979a
Styrene/β-nitrostyrene in B6C3F1	0, 204, 408	Increase in low dose males NCI: no convincing evidence	NCI, 1979b
Styrene in O20	0, 1350	Increased lung tumors in males and females Severe lung toxicity 50% mortality by week 20	Ponomarkov, 1978
Styrene in C57Bl	0, 300	No increase in lung tumors	Ponomarkov, 1978

Basically, the only tissue that demonstrates any tumor potential, and this is itself limited, is lung tissue.

Corrective Action: Several conclusions can be drawn from this discussion and should be reflected in the discussion of NCI (1979a) on pages 203-216 of the Background Document:

- The overall incidence of alveolar/bronchiolar adenomas or carcinomas in male control mice at Litton in studies TR000-TR190 of ~91 weeks duration was 9.1%. The incidence in the high dose styrene exposed males (18%) was lower than the incidence in the control males of 2 of 16 Litton studies in this number range.
- The incidence of alveolar/bronchiolar adenomas or carcinomas in male mice at Litton was much greater than at Hazleton (overall 9.1% vs., 3.1% for 91 week studies; 19% vs. 11% for 104 week studies). Therefore, the control data at Hazleton is not a valid comparison for Litton studies.
- The NCI's original conclusion that TR185 provides suggestive evidence is the correct interpretation of the study and the Draft Substance Profile's conclusion of clear evidence is not valid. The original conclusion of the NCI should be retained; the study provides no more than suggestive evidence of carcinogenicity of styrene.

2. *Inappropriate use of Huff et al. (1984)*

The Background Document states (page 204):

> However, Huff et al. (1984) reexamined these data and reported specific mammary tumor incidences for fibroadenoma, adenoma, adenocarcinoma, and combined mammary tumors (Table 4-4). The authors reported marginal increase in combined mammary gland tumors (fibroadenoma, adenoma, and adenocarcinoma) in female rats. Incidences were 49 of 96 (51%) in controls, 18 of 30 (60%) in the low-dose group and 40 of 60 (66.7%) in the high-dose group. Huff reported that there was a significant dose-related trend (P = 0.032), and the incidence in the high-dose group was significantly higher than the control group (P = 0.039, Fisher's exact test).

The analysis by Huff et al. (1984) combining various types of mammary tumors is not appropriate because fibroadenomas are not related to adenocarcinomas. McConnell et al. (1986)[73] demonstrate that mammary fibroadenomas should not be combined with malignant mammary tumors unless a continuum has been demonstrated within a given study. No such continuum was demonstrated in the Beliles et al. (1985) drinking water study that Huff et al. (1984) was reanalyzing. Therefore, combining them, as Huff et al. (1984) does not represent "sound . . . and objective scientific practice[]" and is misleading. Discussions of Huff et al. (1984) or such combinations should be removed from the Document.

Corrective Actions: The Background Document should be revised as follows:

- Remove the reference to Huff et al. (1984) from lines 18-26 on page 204 of the Background Document.
- Remove the following language from page 216 (lines 7-9):
  > however, an independent review of this study noted that there was a marginal increase in combined mammary tumors (adenocarcinoma, adenoma, and fibroadenoma) in female rats.
- Remove other erroneous references to Huff et al. (1984), such as pages 195, 205 including Table 4-4, 208 and 209.

3. *Use of Historical Controls*

The draft Background Document (page 173, Table 4-4) included the following description of rat studies by Maltoni et al. (1982) and Conti et al. (1988):

> No increase in tumors, decreased incidence of total benign and malignant tumors and total mammary tumors in females in high dose group
> *[Limitations:* High mortality rate in high-dose females, limited reporting, less than lifetime exposure duration]

With regard to Table 4-4, the Expert Panel recommended that

> The entry "decreased incidence of total benign and malignant tumors and total mammary tumors in females in the high-dose group" should be stricken from the Results/Comments column. The decreased incidence is likely related to the poor survival in that group and has no significance. The depression of mammary tumors in the high-dose group (P =

[73] McConnell E.E., *Solleveld H.A., Swenberg J.A., Boorman G.A. Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies* J. Nat'l Cancer Inst. 76: 283-289 (1986).

> 0.040) in this strain for which mammary tumors are common, highlights the problem of study interpretation in the absence of analysis that accounts for intercurrent mortality.

Based on this recommendation, NTP deleted the observation. However, in using historical control data, the Expert Panel also recommended (at page 13) that NTP use as a guide for the styrene review the general approach to historical controls followed in current NTP reports and by the International Agency for Research on Cancer (IARC).

Quoting "Preamble to the IARC Monographs (amended January 2006)" on page 38:

> Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals

Quoting from the May 2008 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 (Gavage) on page 38:

> However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar.

Historical data can be used when controls are not used in an experiment, for whatever reason. If a control group is used, then often the control data is used for comparison rather than historical data. Further, the group used as the historical control must be comparable to the group(s) tested. This idea is supported by the two quotes from IARC and NTP that were quoted by the Expert Panel.

The change in Table 4-5 (4-4 in the Draft Background Document) ignores the data generated by the researcher and the statistical significance between the decreases in tumors from the control group to the high dose group. Instead, the Expert Panel states that the data does not fit what is known about the strain. The comment does not reference what the historical occurrence of the tumors is for the strain, nor does the Panel discuss whether the cited study was comparable to the historical data.

The Expert Panel's approach raises serious questions. SIRC believes it is not sound scientific practice to omit a finding of a study that has significance ($P = 0.040$) when the data is contrary to what is desired, or to use (unspecified) animal historical data only when it suits the desired outcome. NTP's adoption of the Expert Panel's recommendations has produced a biased and incomplete presentation of the data.

Correction request: In Table 4-5 of Background Document (p. 206), the language from the draft Background Document (page 173, Table 4-4) should be restored and the discussion of the study should be consistent with these findings. Regardless of the Expert Panel's opinion, if NTP now disagrees with the finding of the study authors, then NTP must articulate the rationale for its disagreement, rather than just ignoring the data.

4. *Temporal Observation of Tumors*

The following sentence on page 198 of the Background Document does not make sense.

> Lung tumors were reported to occur at an earlier age in the styrene-treated progeny than in control progeny, [but this may be the result of higher mortality in the styrene-treated mice rather than an effect of styrene. Information necessary to interpret the significance of this observation (whether the lung tumors were incidental or fatal) was not reported.]

In contrast, the Draft Background Document (page 166) read: "Lung tumors occurred at an earlier age in the styrene-treated group." The Expert Panel commented that this sentence was "problematic because the limited reporting does not enable conclusions regarding differences in tumor onset among the dose groups." NTP adopted the Expert Panel's suggestion, but the new wording does not make inherent sense.

The language in the final Background Document leaves unclear whether the intent of the sentence is to note that, since animals were dying earlier in the styrene-treated group, the ability to examine the bodies of the deceased occurred earlier in the study, and that the tumors seen in the styrene group may have also been present in the untreated group, but went unseen at that time point because the animals remained alive. If so, then the document should state this clearly. In the absence of comparable or contemporaneous examination, the sentence should be deleted.

Corrective Action: The following sentence on page 198 of the Background Document should be deleted or revised to be both clear and relevant to the study summary and consistent with the informational goals of the Background Document.

> Lung tumors were reported to occur at an earlier age in the styrene-treated progeny than in control progeny, [but this may be the result of higher mortality in the styrene-treated mice rather than an effect of styrene. Information necessary to interpret the significance of this observation (whether the lung tumors were incidental or fatal) was not reported.]

5. *Effects in Experimental Animals Are Species-Specific and Not Applicable to Humans*

As explained in Part III.H, the information quality defects of the Background Document will carry through to subsequent documents and produce similarly flawed, and ultimately unsupportable, conclusions. That result has happened in the case of the NTP's draft Substance Profile. Relying on the unreliable, incomplete and biased analysis and presentation of data contained in the final Background Document, the draft Substance Profile for styrene states: "There is sufficient evidence for the carcinogenicity of styrene in experimental animals based on the induction of tumors in multiple studies in mice exposed to styrene by two routes of exposure." Contrary to this conclusion, these tumors are confined only to the lung, and there is inconsistency in the tumor incidence among different strains of mice. Each study that reports lung tumors in mice also suffers from limitations, as described below.

In the only chronic inhalation study of styrene in mice, increased incidences of bronchioalveolar adenomas (benign tumors) were observed in male and female CD-1 mice, but only at the end of the 24-month study period and with no dose-response pattern (Cruzan et al., 2001). Females exposed to the highest dose also had an increased incidence (14%) of bronchioalveolar carcinomas (malignant tumors) at the end of the study. The historical control incidences of lung tumors for female CD-1 mice ranged from 0-4% for the laboratory, based on five different studies, and from 0-13.5% for the animal supplier (Charles River), based on nine different studies (Cruzan et al., 2001). The adenomas and carcinomas did not differ in tumor morphology between control and treated mice, and histopathologic changes were observed in the terminal bronchioles at all exposure concentrations in a dose-dependent manner at 12-, 18-, and 24-month

time points. These changes included decreased eosinophilic staining of Clara cells and epithelial hyperplasia that extended into the alveolar ducts.

In a chronic oral gavage study, male B6C3F1 mice treated with the highest dose of styrene showed an increased incidence (18%) of combined bronchioalveolar adenomas and carcinomas (NCI, 1979). The tumors were only observed at the end of the 21-month study period, and there was no increase in tumor incidence in females in any dose group. No lung tumors were observed in the 20 vehicle controls, even though the historical control incidence for untreated controls at the laboratory ranged from 0-20%. The historical control data were insufficient for vehicle-treated controls. Because of the discrepancy in control incidence and the tumor response at only one site and in one sex, the authors concluded that "under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in . . . B6C3F1 mice of either sex."

In other chronic oral gavage studies, an increased incidence of combined adenomas and carcinomas was observed in the lungs of male and female O20 mice, but only at a very toxic dose of styrene associated with early mortality (Ponomarkov and Tomatis, 1978). Lung tumors were observed in 20/23 (87%) of styrene-treated males and 32/32 (100%) of styrene-treated females, compared to 8/19 (42%) of male and 14/21 (67%) of female vehicle controls, after adjusting for early mortality. Lung tumors were also observed in 34/53 (64%) of male and 25/47 (53%) female untreated controls, indicating that O20 mice are very susceptible to the development of lung tumors. The incidence in the styrene-treated males was significantly higher than the vehicle controls only, whereas in styrene-treated females, the incidence was significantly higher than either control group. The authors concluded that "the increased incidence and early appearance of lung tumors could possibly indicate a carcinogenic effect for styrene in O20 mice. This experiment, however, has severe limitations, since the dose used was obviously very high, causing severe toxic effects and an early mortality . . . . Results from additional studies are needed before a final evaluation of the carcinogenicity of styrene in rodents can be made." The same group, using a similar study design, reported no increased incidence of

tumors of any type in styrene-treated male and female C57Bl mice (Ponomarkov and Tomatis, 1978).

In addition to the inhalation and oral gavage studies in mice, intraperitoneal injection of styrene into A/J mice for seven weeks did not result in an increased incidence of lung tumors at sacrifice 20 weeks later (Brunnemann et al., 1992).

There were no increased tumor incidences reported in seven chronic rat studies of styrene by various exposure routes, including inhalation (Cruzan et al., 1998; Conti et al., 1988; Jersey et al., 1978), oral gavage (Conti et al., 1988; NCI, 1979; Ponomarkov and Tomatis, 1978), and drinking water (Beliles et al., 1985). The NTP draft profile for styrene states: "Data from experimental cancer studies with rats are insufficient for reaching a conclusion." Thus, there is a lack of generality of the tumorigenic response among rodent species.

Although the data are inconsistent, there is evidence that styrene causes an increased incidence of lung tumors, but only in certain strains of mice. With the exception of the Ponomarkov and Tomatis (1978) study in which a highly toxic dose of styrene was administered to O20 mice, the tumors occurred at the end of the chronic study period and were not life-shortening. In addition, the tumors were observed in the presence of lung toxicity. The mouse lung is an organ with a high background incidence of tumors (Cruzan et al., 2001). Neither lung toxicity nor lung tumors have been observed in humans exposed to styrene, and as described in Part IV.D below, the proposed mode of action for styrene-induced lung tumors in mice is not applicable to humans, or even to other rodent species. Taken together, the animal data for styrene do not meet the NTP criteria for "sufficient" evidence of carcinogenicity in animals.

While the "sufficient" characterization is a policy choice by NTP, it must be based on a rational interpretation of the underlying data, which must be generated, analyzed and presented objectively. When that analysis is unsound and unreliable and its presentation is biased and incomplete, the resulting characterization can become arbitrary and capricious – as has occurred here.

Correction Request: The Background Document (page 214-216) summary of the animal studies should be revised to note that the effects are "limited," not "sufficient," and are species-specific.

D. IQA Deficiencies for Mechanistic Data

The Background Document's analysis and presentation of the available data on styrene's mechanisms of action violates the IQA Guidelines in three principal areas:

- The critical question of styrene metabolism is treated in a very biased and incomplete fashion in the Background Document. First, it presents two competing theories on how styrene metabolites cause mouse lung tumors as if both were still plausible, when in fact the theory that genotoxic events from styrene-7,8-oxide (SO) lead to lung tumors has been discredited in favor of the theory that cytotoxic styrene metabolites generated by cytochrome P450 2F2 (CYP2F2) cause cell proliferation that leads to tumors. Second, it continues the draft's failure even to cite the finding of Hofmann et al. (2005) that levels of SO in rat lungs at eight times the tumorigenic level in mice do not produce tumors in rats, and other data that indicate mouse lung tumors are unrelated to SO. Third, it does not describe the crucial role played by CYP2F2 in catalyzing the ring oxidation of styrene to 4-vinylphenol in the mouse lung. Finally, it fails to note the concordance of studies on the much greater proliferative effect of 4-vinylphenyl compared to SO.
- The Background Document's genotoxity discussion is neither objective nor useful. On the topic of chromosomal aberrations and sister chromatid exchanges, it omits criticisms of the cited human studies, fails even to note the many contrary studies, and does not address the lack of increases in chromosomal aberration or micronuclei in controlled experiments in laboratory animals. The discussion of DNA adducts does not explain that adducts are biomarkers of exposure, not indicators of adverse health effects. The discussion of DNA strand breaks does not describe the severe limitations of the COMET assays employed.

Taken together, the first two of these shortcomings skew the Background Document into an endorsement of the now discredited genotoxicity theory, when a complete and unbiased analysis and presentation of the available data would show that styrene is carcinogenic only in mouse

lungs due to cytotoxic effects that are not relevant to humans. Such a profoundly misleading document has no utility to government decision makers or the public.

While we explain these points summarily below, a more thorough discussion of these mode of action issues is contained in G. Cruzan et al., *Mouse Specific Lung Tumors from CYP2F2-Mediated Cytotoxic Metabolism: An endpoint/toxic response where data from multiple chemicals converge to support a mode of action*.[74] A prepublication copy was previously submitted to NTP, and is incorporated here by reference.

1. *Styrene Metabolism*

a. Biased description of the state of the science

The Background Document inaccurately espouses the theory that the metabolism of styrene to styrene 7,8 oxide can then lead to genotoxic effects and cancer. On page 383, it states:

> The proposed mechanisms for the carcinogenicity of styrene include both genotoxic and epigenetic pathways. These mechanisms, which are not necessarily mutually exclusive, include: (1) metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue and (2) cytotoxic effects of styrene metabolites in the mouse lung.

The above proposes that any genotoxic MOA is through styrene-7,8-oxide (SO) and that a cytotoxic MOA may also occur because of the formation of SO. By treating these mechanisms as equally plausible and not mutually exclusive, the Background Document does not objectively present the data. As a short discussion of the evidence mechanism of action reveals, the first hypothesis is no longer plausible; the plausible mechanism is cytotoxic effects of styrene metabolites in the mouse lung.

The following comments evaluate whether the data on styrene and styrene oxide support the hypothesis that lung tumors in mice exposed to styrene occur as a result of the formation of SO:

[74] 55 REG. TOX. & PHARM. 205-218 (2009) (available online July 7, 2009), available at http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WPT-4WPJ64J-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=6bf41fe1f4fad6d5eb016af9b023acd4

*Hypothesis***:** Styrene-7,8-oxide resulting from the metabolism of styrene causes genotoxic events, leading to cancer.

Facts that support this hypothesis:

a. There is ample evidence that styrene is metabolized to styrene-7,8-oxide in liver and lung (IARC, 1994, 2002).

b. Styrene inhalation exposure in animals and humans results in circulating levels of styrene-7,8-oxide. (Cruzan et al., 1998, 2001; reviewed in IARC, 2002)

c. Low levels of styrene oxide-DNA adducts have been reported in animals and humans exposed to styrene (reviewed in IARC, NTP draft document).

d. *In vitro* genotoxicity studies of styrene-7,8-oxide are positive, including bacterial mutagenicity and chromosomal aberrations (IARC, 1994; NTP draft document).

Facts that contradict this hypothesis:

a. Genotoxic carcinogens normally cause tumors at multiple sites in multiple species. **This is not true for styrene.** The only tumor type increased in 8 rat studies and 5 mouse studies is mouse lung tumors (IARC, 2002; EU, 2007, Cohen et al., 2002).

b. **Administration of styrene 7,8 oxide (SO) (up to 550 mg/kg) to mice did not result in increased lung tumors.** Lijinski et al., 1985 administered SO to rats and mice at 275 and 550 mg/kg/day. Severe necrosis of the forestomach and forestomach tumors were found. In low-dose males, there was an increase in liver tumors. There was no increase in lung tumors. Dose-response for cell proliferation paralleled dose response for forestomach tumors; cell-proliferation plateaued at 200 mg/kg (Dalbey et al., 1996). Lutz and coworkers (1992) found very low level of DNA adducts, and proposed that genotoxicity did not explain the forestomach tumors.

c. **Increased mouse lung tumors are not related to the level of SO in the lungs.** Inhalation of styrene at 40 ppm resulted in increased mouse lung tumors (estimated SO level in terminal bronchioles = 4.38 nmoles/mL); gavage of SO at 550 mg/kg/day did not cause increased lung tumors (estimated SO level in terminal bronchioles = 5.56 nmoles/mL). Increased mouse lung tumors are thus not related to the level of SO in lungs. (Sarangapani et al., 2002)

d. **Lung levels of SO do not explain rat/mouse differences (Cohen, 2002).** Hofmann et al., 2005 demonstrated 8-fold higher levels of SO in rat lung *ex vivo* exposed to 1000 ppm (2.05 nmole/ml) than in mouse lung exposed to 40 ppm (0.25 nmole/ml). They concluded that mouse lung tumors were not related to the presence of SO.

e. **There is no increase in DNA adducts in target tissues.** Levels of SO-DNA adducts are very low (<1 in 10^7 nucleotides) and are not higher in mouse than rat or in mouse lung than mouse liver.

f. **Genotoxicity studies in mouse lung are negative**. There was no increase in chromosomal aberrations in the lungs of mice exposed to 125, 250 or 500 ppm styrene for 2 weeks (Kligerman et al, 1992). There was no increase in lung tumors after the 20 weeks observation period when styrene was administered intraperitoneally for 6 weeks in a lung tumor initiation in A/J mice (Brunnemann et al., 1994).

g. **CYP2E1 metabolism does not affect styrene lung toxicity**. CYP2E1 metabolizes styrene primarily to *S*-SO (Green et al., 2001).[75] A role for CYP2E1 metabolism of styrene in liver toxicity was demonstrated using both a CYP2E1 inhibitor and CYP2E1-knockout mice. However, inhibition of CYP2E1 did not reduce the lung cytotoxicity from styrene exposure; furthermore, the lung cytotoxicity of styrene was not diminished in CYP2E1-knockout mice compared to wild-type.

Conclusions of others:

- Cohen et al., 2002: Differences in lung tumor response are not explained by differences of SO in rat and mouse lung.
- Cruzan et al., 2002: Lung tumors are not related to total SO.
- Hofmann et al., 2006: Mouse lung tumors are unrelated to the level of SO in the lung.

The European Union's recent evaluation of styrene objectively presents the state of the science regarding the likely cause of mouse lung tumors:

> On the question of the relevance of the mouse lung tumours for human health, consideration of the available toxicokinetic information and data from single and repeated inhalation exposure studies in experimental rodents suggests the following as the most plausible toxicological mechanism for the mouse lung tumours. Styrene is metabolised by cytochrome P450 enzymes in the metabolically active Clara cells (non-ciliated bronchiolar epithelial cells involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium) of the bronchiolar epithelium of the mouse, producing cytotoxic metabolites of styrene including styrene 7,8 oxide (SO) and oxidative metabolites of 4-vinylphenol (4-VP). These metabolites cause early Clara cell toxicity/death and sustained regenerative bronchiolar cell proliferation which, in turn, leads to compensatory bronchiolar epithelial hyperplasia and ultimately tumour formation. Clara cell toxicity could also be a consequence of the long term depletion of glutathione, because of conjugation with SO. Genotoxicity of SO (an EU-category 2 and IARC group 2A carcinogen) or other reactive styrene metabolites is unlikely to be involved in tumour development as minimal binding

[75] *S*-SO refers to the *S*-enantiomer of styrene oxide. In stereochemistry, an enantiomer is one of two stereoisomers that are non-superimposable, complete mirror images of each other.

of styrene metabolites to DNA has been detected in mouse lung with no species- or tissue-specificity.[76]

Corrective Action: The Background Document (page 383) should be revised to state that, while two hypotheses have been considered, cytotoxic effects of styrene metabolites in the mouse lung is the plausible mechanism of action.

b. Failure to Reference Hofmann et al. (2006)

SIRC's comments on the draft Background Document cited and discussed Hofmann et al. (2006), which, as noted above, demonstrated that rat lungs exposed to 1,000 ppm styrene produced 8 times as much SO as mouse lungs exposed to 40 ppm SO.[77] However, the final Background Document continues to ignore the Hofmann paper and does not address its conclusion that SO is not responsible for producing mouse lung tumors. The failure to address this paper conflicts with the objectivity criteria of the IQA related to completeness, lack of bias and comprehensive and transparent analysis.

Corrective Action: Particularly because the Background Document attempts to construct a contrary position, section 5.5 (pages 368-384) should integrate Hoffman et al. (2006) into the discussion. The findings in Hoffman et al. (2006) should be supported.

c. The Role of CYP2F2-mediated metabolites (alternative hypothesis to SO-mediated tumorigenesis)

The tissues that are high in CYP2F are the primary tissues that experience cytotoxicity from styrene and a series of similar chemicals. CYP2F2 is expressed primarily in mouse lung Clara cells and nasal olfactory epithelium. These are the primary tissues of cytotoxicity from styrene. In rats, CYP2F4 is expressed primarily in the nasal olfactory epithelium; this is the only tissue in rats that experiences cytotoxicity from styrene exposure. Inhibition of CYP2F2 inhibits both the nasal and lung cytotoxicity from styrene exposure. CYP2F2 catalyzes the ring oxidation of styrene to 4-hydroxystyrene (also called 4-vinylphenol). Inhibition of CYP2F2 inhibits the

[76] EU Risk Assessment Report, *supra* note 50, at 253.

[77] Hofmann, C., Putz, C., Semder, B., Faller, T.H., Csanady, G.A., Filser, J.G. *Styrene-7,8-Oxide Burden in Ventilated, Perfused Lungs of Mice and Rats Exposed to Vaporous Styrene,* Toxicol. Sci. 90, 39-48 (2006).

formation of 4-hydroxystyrene. Furthermore, 4-hydroxystyrene is further metabolized to 4-hydroxystyrene-7,8-oxide and to 3, 4-dihydroxystyrene. Inhibition of CYP2F2 inhibits this metabolism and inhibits the cytotoxicity from 4-hydroxystyrene. The necessity of oxidation of the aromatic ring is further supported by the lack of lung cytotoxicity or increased lung tumors in mice exposed to 4-methylstyrene.

Similar cytotoxicity in lung of mice and nasal olfactory epithelium in mice and rats has been reported for several other chemicals of similar structure including coumarin, naphthalene, ethylbenzene, cumene, and divinylbenzene. Lung tumors in mice, but not rats, have been reported for all of these. For several, metabolism by CYP2F2 to cytotoxic metabolites has been demonstrated, and analogs that block the CYP2F2 metabolism are not cytotoxic or do not produce lung tumors in mice. A manuscript discussing these findings was previously submitted to NTP and the article has now been published.[78] And yet the Background Document does not present a complete discussion of this issue, rendering its conclusions unreliable.

Corrective Actions: On page 383, delete lines 19-23. These statements are speculative. Styrene genotoxicity studies *in vitro* and in laboratory animals are nearly all negative. The human studies present a mixed picture and do not correspond to the animal studies. Replace with:

> It has been proposed that styrene-7,8-oxide, a major metabolite of styrene, is responsible for any genotoxicity and tumorigenicity from styrene. However, oral administration of styrene-7,8-oxide to mice did not produce lung tumors. SO levels in rat lungs up to 8 fold higher than the level that produces tumors in mice do not produce tumors in rats.

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[78] George Cruzan, James Bus, Marcy Banton, Ralph Gingell and Gary Carlson, *Mouse specific lung tumors from CYP2F2-mediated cytotoxic metabolism: An endpoint/toxic response where data from multiple chemicals converge to support a mode of action*, Regulatory Toxicology and Pharmacology, vol. 55, issue 2, pages 205-218 (Nov. 2009)(available online at http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WPT-4WPJ64J-2&_user=10&_coverDate=11%2F30%2F2009&_rdoc=13&_fmt=high&_orig=browse&_srch=doc-info%28%23toc%236999%232009%23999449997%231528278%23FLA%23display%23Volume%29&_cdi=6999&_sort=d&_docanchor=&_ct=16&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=b2728d7b159d8ac17ef769721217b722).

On page 384, line 7 add:

> Cruzan et al., (2002) concluded that mouse lung tumors were unrelated to the total SO lung levels. Hofmann et al. (2006) likewise concluded that mouse lung tumors were not caused by SO.

On page 384, line 14 add:

> Similar toxicity and tumors in the lungs of mice but not rats, and CYP2F metabolism in a series of related compounds, add weight to this hypothesis. Lack of tumorigenicity by analogs of these compounds that cannot be metabolized to the same metabolites further strengthens this mode of action for styrene.

d. Unbalanced Discussion of 4-vinylphenol vs. SO

Pages 252-253 of the Background Document discuss the finding of Kauffman et al. (2005) that injections of 4-vinylphenol into CD-1 mice caused substantially greater proliferation in lung cells than did injections of styrene-7,8-oxide (up to 15.1 fold for 4-vinylphenol vs. 7.5 fold for styrene-7,8-oxide in large/medium bronchi) and (up to 19.7 fold for 4-vinylphenol vs. 10.5 fold for styrene-7,8-oxide in terminal bronchioles). But the Background Document failed to note that this study is consistent with the findings of Gamer et al. (2004), which reported that 4-vinylphenol produced nearly double cell proliferation (19x) when compared with styrene oxide (10x), and at one-third the dose of 4-vinylphenol compared with styrene oxide.

Corrective Action: The Background Document's discussion of the effects of styrene metabolites should be expanded to note the concordance between Kauffman et al. (2005) and Gamer (2004).

2. *Styrene Genotoxicity*

The Background Document's discussion of genotoxicity contains incomplete and biased summaries of the experimental data regarding chromosomal aberrations (CA) and sister chromatid exchanges (SCE), DNA adducts, and DNA strand breaks. These discussions vitiate the utility of the document to the Expert Panel, the Board of Scientific Counselors, and other policy-makers at NTP, and render it not objective.

The Background Document (pages 275-310) lists the human studies of CAs and SCEs as generally positive. Critical reviews of the individual studies, however, have reported deficiencies in many of the studies that report increases, including inappropriate control populations (either by size or characteristics) and non-standard assay conditions. Although Bonassi et al. (1996), included in the Background Document (page 308, 330), concluded there were increased CAs in workers exposed to greater than 30 ppm styrene, many others have disagreed (Scott and Preston, 1994; Speit and Henderson, 2005; Nestmann et al., 2005; IARC, 2002)(Background Document page 264, 372). As stated by IARC (2002): "Inconsistent results have been reported for chromosomal aberrations, micronuclei, and sister chromatid exchange in approximately 30 studies of workers exposed to styrene in various industries."

The Background Document (page 330) discounts Scott and Preston (1994) on the grounds that it did not consider later studies included in the review by Bonassi et al. (1996). However, the Background Document fails to give the same consideration to Speit and Henderson (2005), Nestmann et al. (2005), and IARC (2002), all of which were six to eight years after Bonassi et al. (1996). The selective use of the one review that found increased CAs while disregarding or not discussing later reviews to the contrary is a failure to present information in a complete and unbiased way. It is also a failure to present "peer-reviewed studies known to the agency that support, are directly relevant to, or fail to support any estimate" of health effects. It is also a failure of sound and objective scientific practice because it ignores the current state of the science in favor of a single review that found the sought-for effect. The genotoxicity discussion in the Background Document must be corrected and presented with balance and transparency.

DNA adducts are discussed in section 5.4.1 of the Background Document (pages 264-272). DNA adducts have been reported in humans, rats and mice exposed to styrene. The Background Document fails to note that these are measures of exposure; they do not necessarily demonstrate genetic damage likely to lead to the development of tumors. In mice, Boogaard et al. (2000) found lower levels of DNA adducts in lung than in liver and found no greater levels in mice than in rats, even though tumors are observed in mice rather than rats, and in mouse lungs rather than mouse livers. The adducts found in animals and humans were at less than 1 in 10^7 nucleotides,

and were largely N-7 adducts which are quickly repaired. In serial human studies reported by Vodička et al., there was no accumulation in workers over several years. To have utility, the Background document must make clear the exposure measurement function of these DNA adduct studies and their limitations and qualifications.

The Background Document (page 264) reports styrene-related DNA strand breaks. The actual studies are Comet or DNA unwinding assays. These are notably subject to false positive results and are not sufficiently sensitive to distinguish between genotoxic or non-genotoxic modes of action. One COMET assay conducted by the inhalation route of exposure (Kligerman et al., 1993), the route of exposure of most relevance to humans, showed no genotoxic effect of styrene in female Fischer rat peripheral blood lymphocytes following exposure at 125 to 600 ppm for 6 hours/day for 2 weeks. Vodička et al. (2001) reported equivocal results for a COMET assay for styrene in which mice were exposed by inhalation for up to 21 days at 175 to 350 ppm for 6 hours/day. Overall, the results for styrene in COMET assays conducted by relevant routes of exposure present no substantive evidence of a clear genotoxic effect in vivo. Again, NTP states that the Background Document is intended to assist NTP policy-makers in their review of styrene. Without noting that these types of studies are subject to false positive results and are not able to distinguish between genotoxic or non-genotoxic modes of action, the Background Document does not meet the utility and objectivity requirements of the IQA.

There are two assays of styrene genotoxicity on mouse lung. Kligermann et al. (1993), discussed at page 285 of the Background Document, found no increase in CAs in the lungs of mice exposed for 14 consecutive days at 125, 250 or 500 ppm styrene by inhalation. Exposure at 250 or 500 ppm resulted in lethality in 30 to 50% of the mice. These exposure levels were greater than those of the chronic inhalation studies (20-160 ppm). Thus, it is unlikely that increased CAs were a factor in the formation of lung tumors in mice exposed to styrene by inhalation. Furthermore, in a study to examine carcinogen initiators from cigarette smoke, Brunnemann et al., (1992)(Background Document at page 203) injected styrene intraperitoneally for 7 weeks in A/J, a strain designed to be sensitive to the formation of lung tumors in mice, followed by 20 weeks of observation. Styrene did not cause an increase in lung tumors, indicating no tumor

initiating potency. In summary, the genotoxicity data for styrene are not convincing of a genotoxic mode of action.

Corrective Action: The above-noted corrections should be incorporated into the Background Document and the following language added:

> The bacterial mutation assays were nearly all negative, but positive results were reported for chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in *in vitro* assays (summarized in Cruzan et al., 2009). The positive in vitro genotoxicity studies of styrene occurred at concentrations of styrene not achievable in humans, under conditions of metabolism of styrene, but of inhibited downstream metabolism of styrene oxide. This is in contrast to the in vivo situation, where styrene oxide is rapidly removed. Cruzan et al. (2009)
>
> The *in vivo* genotoxicity assays of styrene in rodent assays are overwhelmingly negative. Five of seven micronucleus (MN) assays were negative. One study reported increased MN at 7 days, but not at 1 or 21 days of exposure to styrene; a subsequent publication by this laboratory indicated the positive results could not be duplicated. Eleven of twelve studies of CA in experimental animals were negative, including one in the lungs of mice exposed to styrene by inhalation at concentrations that caused lethality in some mice. *In vivo* assays have indicated that exposure to styrene results in increased SCE (summarized in Cruzan et al., 2009).

3. *Additional Corrective Actions for "Other Data" Section of Background Document*

Page 222, lines 21-24: Luderer et al. (2005) is a review, not a research paper. SIRC could not locate the recalculation on dermal uptake that is cited in the NTP document. NTP should confirm the source and cite it or else delete the sentence.

Page 226, Table 5-1: Table 5-1 shows phenylglycine as a metabolite. Phenylglycine in the figure is not mentioned by anyone other than Manini et al. (2002b), to whom the figure is attributed. Manini's paper notes that this is a hypothesized metabolite that has never been identified in either animals or humans; therefore, it would be more accurate to delete phenylglycine or use a different diagram of metabolites.

Pages 229-230: The discussion of Boogaard et al. (2000b) is misleading. The summary of the study should indicate that it was an inhalation study followed by isolation of the cells for analysis. The last

sentence of this section states: "Clara cells are the predominant cell type in mouse lung, while type II cells predominate in rat lung." This does not make much sense because type II cells and Clara cells are found in different parts of the lung (alveolae versus bronchioles), which has significant implications for proper interpretation and application of the study. The sentence should be deleted or a fuller and correct discussion added.

Page 235, line 1: The Background Document references "total CYP450" being measured in Wenker et al. (2001c). SIRC could not locate this information in the cited study. The reference to total CYP450 should be deleted.

Page 235, line 20: The Background Document states: "Carlson (2003), (see Table 2 in Carlson 2003) reported that styrene metabolism by pulmonary microsomes in Cyp2e1-knockout mice is about ***one-half*** that in wild-type mice" (emphasis added). The correct figure is about 73%, not one-half. 73% should be substituted for one-half.

Page 237: The discussion and summary of Arand et al. (1999) should be revised to note that the study involved mutant forms of the enzyme, and the relevance of the study to styrene metabolism in an intact organism is unclear.

Page 246, lines 8-10: The Background Document states (emphasis added):

> There was suggestive evidence that occupational exposure to styrene was associated with ***increased serum prolactin*** and depletion of peripheral blood dopamine-metabolizing enzyme activities, but the clinical relevance of these findings was unclear.

The prolactin levels of the workers were within the normal human range. Thus, this observation is mistaken or, at best, its significance is unclear. The reference to prolactin should be deleted or contextually clarified.

Page 259: The discussion of Cohen et al. (2002) should be modified by adding a citation to Filser et al. (1999), a key study that Cohen relied upon and the source of the data that the Background Document is presenting.

Page 261, lines 3-4: The work by Linhart (2001) is presented in a misleading way because it appears to present original data. Linhart (2001) is a review of the work of Carlson et al. and not original data. The distinction between reviews and original research should be expressly noted here (and elsewhere in the Background Document).

Page 280, lines 8-28: The Background Document discusses DNA adducts in NMRI mice resulting from exposure to styrene. The Background Document should be amended to note that the significance of DNA adducts in NMRI mice exposed to 175 or 350 ppm is not clear because exposure to these levels is lethal to some CD-1 and B6C3F1 mice (up to 50% at 250 ppm). Morgan et al., (1993) and Cruzan et al. (1997).

Page 328, lines 4-28: The Background Document lists human chromosomal aberration (CA) studies and indicates that positive results were observed in studies with higher levels of exposure, but that statement is inaccurate, as shown in the following table. In the table, the human CA studies are arranged by exposure. Note that the proportion of positive and negative studies does not change with exposure concentration, and that the study with the highest exposure (Fleig 1978) was considered as negative by IARC. In the RoC Document there were 8 studies with reported exposures above 50 ppm; 5 were + and 3 – (IARC did not report one of these studies, which was considered negative by the RoC Document). If one considers the highest 15 exposure studies (above 25 ppm), there were 10+, 5-; in the lower half, there were 9+ and 6-. Note that 3 of 4 studies with the lowest exposures were reported as +. A more accurate statement is that there are mixed results for CA studies in humans.

Human CA Studies by Exposure

Author	Date	E/C	PPM	Urinary	Results	IARC
Fleig	1978	14/20	50-300		+	-
Camurri	1983/4	2-7/2-7	<90		+	+
Anderson	1980	36/37	(75)	1204	+	+
Maki-Paakanen	1991	17/17	70		+	+
Jablonica	1988	11/11	58		-	-
Theiss	1980	24/31	58		-	-
Forni	1988	40/40	<57		+	+
Vodicka	2004a	86/42	(50)	798	-	NR
Somaroska	1999	44/19	46		+	?

Author	Date	E/C	PPM	Urinary	Results	IARC
Sorsa	1991	109/54	43		-	-
Hogstedt	1979	6/6	11-92		+	+
Meretoga	1977	10/5	(45)	721	+	NR
Watanabe	1981	16/13	(41)	650	-	-
Meretoga	1978	26/6	(35)	570	+gaps	+
Tomanin	1992	50/54	26		+	+
Dolmerski	1983	30/2	<23		+gaps	NR
Oberheitman	2001	14/7	<23		-	NR
Maki-Paakanen	1987	21/21	23 (8-60)		-	-
Watanabe	1983	18/16	(22)	350	-	-
Tates	1994	46/23	20		+	NR
Artuso	1995	46/51	(20)	319	+high group	NR
Anwar	1995	18/18	(20)	328	+	NR
Pohlova	1985	2 plants	(14)	<226	+ at one	-
Hansteen	1984	18/9	13		+gaps	-
Hagmar	1989	11/14	13		-	-
Migliore	2006	72/89	8.5 (1-123)		-	NR
Vodicka	2004c	84/16	<3		+	NR
Van Sittert	1985	200/135	<1.5		+	NR
Lazutka	1999	97/90	<1.4		+	NR
Biro	2002	10/25	NR		-	NR

Page 360, lines 17-18: The Background Document states: "However, most of the studies published prior to 1994 were negative while most of the studies published after 1994 were positive." This statement is not complete or transparent. The most important aspect of the temporal observation is that the earlier studies included periods when worker exposure levels were much higher. Thus, most of those conducted since 1994 were reported as showing *increase* sister chromatid exchange (SCE) when worker exposures were at least four-fold *lower* than in earlier studies. To be complete, additional discussion should attempt to explain this curious result and its implications for attributing SCE to styrene exposures, particularly given the inconclusive meta-analysis by Bonassi et al. (1996) that is cited.

Page 367, Table 5-18: This table does not match its description in the text of the Background Document. The text says mutation studies in humans are "inconclusive to weakly positive," while the table says "weakly positive." The text says "results of clastogenic effects are inconclusive" while the table indicates CAs "weakly positive," and SCE and micronucleus formation (MN) had "equally positive and negative results." On page 346, lines 4-6, the

Background Document cites Cohen et al. (2002) for the conclusion that "there is no compelling evidence in humans that exposure to styrene was associated with micronucleus formation." This is not the same the "equal numbers of positive and negative studies" characterization in Table 5-18. The terminology in Table 5-18 should be revised consistent with the text of the Background Document in this regard.

Pages 368-369: The quoted statement by Melnick (2002) is inappropriate for SO: SO is not highly reactive; it has a half-life in blood *in vitro* of ~30 minutes. There is no indication of increases in "liver, harderian gland, and circulatory system neoplasms in mice," "Zymbal's gland and brain tumors in rats" or "mammary gland tumors in both rats and mice" from exposure to SO. There were only forestomach tumors in rats and mice, and liver tumors in the low dose of mice, as stated in the next sentence. There is no reason to include this sentence and it indicates that SO is NOT like other epoxides described by Melnick (2002).

Page 375, lines 22-25: The Background Document states: "Studies by Gadberry et al. (see Section 5.2.2.3) showed that styrene-7,8-oxide administered by i.p. injection caused pulmonary toxicity in mice, suggesting that styrene-7,8-oxide is responsible for the pneumotoxicity and that systemically available styrene-7,8-oxide can enter the lung cell." The studies by Gadberry do not necessarily indicate that SO is responsible for the lung cytotoxicity of styrene. Those studies also showed that 4-vinylphenol was toxic at 5 times lower concentration than was SO. It is likely that a further metabolite of both of them is responsible for the cytotoxicity. Studies by Bartels et al. (2005)[79] indicated that 3,4-dihydroxystyrene and 4-hydroxystyrene-7,8-oxide could be trapped with excess GSH from incubation of styrene or 4- vinylphenol using lung microsomes.

Page 376-377: The discussion of Cohen et al. (2002) is incomplete in light of the described model and later literature. Although Cohen et al. (2002) identified CYP2E1 as important in the cytotoxicity of styrene, later studies in Carlson's lab have demonstrated that CYP2E1 does not play an important role in lung cytotoxicity. The Cohen model assumes that all metabolism of

[79] M. Bartels et al., "In vitro metabolism of 4-vinylphenol and styrene in mouse, rat and human microsomes." 84 THE TOXICOLOGIST: abstract 1563 (2005).

styrene occurs in the liver and does not include lung metabolism. Thus, it cannot explain mouse and rat differences. The conclusions of Cohen et al. about styrene oxide lead logically to the conclusion that SO is not responsible for the cytotoxicity from styrene in mouse lung terminal bronchioles. These observations should be added to the discussion to put the review by Cohen et al. (2002) in proper context.

E. Failure to Discuss Inconsistencies or Lack of Concordance Among Human, Animal and Mechanistic Data

As noted previously, the Background Document is the basis for NTP's classification determinations. While the division of its review into human, animal and other data is common and perfectly appropriate, such a stovepiped presentation that goes unreconciled results in the Background Document failing to present inconsistencies or lack of concordance among the conclusions or observations reached in the different sections. For example, there is no discussion or even mention of the conflict between the Document's interpretation of the epidemiologic and the mechanistic data. Comments by two members of the Expert Panel help illuminate this failing:

> Dr. Yost: I have a paradigm that I'd like to use in looking at risk assessment and things, I've done this for a number of years for the National Academy of Sciences so I know a little bit about this process. I am not an epi person, but I think reality checking is a good idea. And for us to make an assumption for the higher category, the stronger category based upon one or two studies in an epidemiology literature, that flies in the face of any mechanistic work that I've seen on styrene gives me some pause. I simply cannot override the data that we have on mechanisms in animals and in in vitro and human cells and everything else to say well, you know, here an end point, in lymphomas that it may be real maybe important maybe you know valid. But it doesn't fit mechanistically with what we know about how this chemical causes cancer. We are talking about DNA adducts; we're talking about epigenetic mechanisms; we're talking about bioactivating to an epoxide to other epoxides, cytotoxicity, et cetera, et cetera. And none of these things, I don't think, there's evidence occurring in the cells that we're talking about that are the precursors for Non-Hodgkin's Lymphoma. Maybe there are some DNA Adducts. I don't know that might be I think there was evidence, right? For adducts in lymphocytes, is that correct? Yeah, but anyway that would be like the only piece of evidence and so I think in the face of that I have a hard time listing as reasonably anticipated, let alone a stronger category.
>
> Dr. Que Hee: Yes, Shane Que Hee, I'd like to also add to what Dr. Yost just said by consideration of the levels of exposure. The plastics industries have concentrations of a high of 2320?? part per million if you look at the constant. Whereas the high in--the--now I'm not talking about part per million here, I'm talking about absolute air concentrations.

> And the high in the butadiene, the styrene butadiene industry, is about 1.8 per million; so that's consideration number one. Consideration number two is that the small number, because of the short latency period in the most of the plastics including, what's his name, the Kogevinas, whatever it is study it might be premature to actually make the leap than actually go the minimal kind of level and then reserve that leap to when there's more data to see, if in fact, the Epi studies hold true. And there's not an artifact that we haven't thought of might be causing some of the results, a synergism or whatever. So that's what I think.

(Transcript Part 31.)

As a result of its failure to reconcile the inconsistencies between the human, animal and mechanistic data, the Background Document presents a fundamentally inaccurate picture of their synthesis:

> Although quantitative differences in styrene disposition exist across species, there are no demonstrated qualitative differences between humans and laboratory animals that contradict the relevance of the rodent cancer studies for evaluations of human hazard. The detection of styrene-7,8-oxide-DNA adducts at base-pairing sites and chromosomal aberrations in lymphocytes of styrene-exposed workers supports the potential human cancer hazard from styrene through a genotoxic mode-of-action (NTP, 2009).

This statement is incorrect for several reasons. There is no concordance among the human, rodent, and mode-of-action data on the effects of styrene. The styrene-induced lung toxicity and tumor formation observed in mice are species- and strain-specific, as they are not observed in rats, humans, or certain other mouse strains exposed to styrene. The mechanistic data strongly suggests that these tumors are the result of mouse-specific lung toxicity that depends upon the localized metabolism of styrene by mouse CYP2F2. In humans, CYP2F2 does not appear to metabolize styrene. In addition, the metabolic pathway resulting in the formation of 4-vinylphenol, which appears to be the substrate for the CYP2F2-dependent cytotoxic metabolite in mouse lung, is a very minor pathway in humans compared to mice. We further note that while increased chromosomal aberrations have been reported in some studies of workers exposed to styrene, increased chromosomal aberrations have not been reported in mice. The document ignores this important difference.

The epidemiological data as a whole do not suggest that styrene exposure is associated with any specific cancer type in humans, either within or among studies. The NTP Background Document,

however, interprets these data as suggesting that styrene exposure increases the incidence of lymphohematopoietic cancers, and possibly pancreatic and esophageal cancers, in humans (NTP, 2009). Even if one accepts this interpretation, there have been no corresponding responses observed in animals, as no increased incidences of lymphohematopoietic, pancreatic, or esophageal tumors have been reported in styrene-exposed animals. The animal data also indicate that orally-administered styrene does not induce tumors systemically, as an increased incidence of tumors was observed specifically in the mouse lung.

The studies in which SO-DNA adducts and chromosomal aberrations were detected in styrene-exposed workers do not support a carcinogenic role for styrene. These studies are limited by their small size and lack of controlling for potential confounders. In addition, styrene-exposed rodents form similar DNA adducts, with higher levels observed in rats than in mice, suggesting that these adducts are not associated with an increased incidence of tumors (Boogaard et al., 2000; Cruzan et al., 2002). Furthermore, agents that are known or expected to cause lymphohematopoietic cancers in humans are believed to act through immune dysregulation and not through DNA damage (Alexander et al., 2007).

A genotoxic mode of action for styrene, either in animals or in humans, is not plausible. The genotoxicity data for styrene are simply inconsistent. Styrene is not carcinogenic in humans, and the animal and mechanistic data support a non-genotoxic mode of action for styrene. Styrene exposure has been associated with an increased tumor incidence in only one species and one site, the mouse lung. The late onset and mostly benign lung tumors observed in mice were accompanied by lung cytotoxicity and cell proliferation and were dependent upon lung-specific metabolism of styrene. These data suggest a nongenotoxic mechanism of action attributable to increased cell proliferation in the lung resulting from the cellular damage induced upon styrene metabolism.

Taken together, the human, animal, and mode-of-action data for the effects of styrene do not support a conclusion even of reasonably anticipated human carcinogenicity for styrene. The epidemiology data do not suggest that styrene exposure is associated with an increased incidence

of any tumor type. The lack of correspondence of tumor incidence and tumor type among rodents, even within the same species, and humans indicates that there has been no particular type of cancer consistently observed among all available studies and renders the argument for the human carcinogenicity of styrene as implausible. The various data indicate that the only plausible mechanism for styrene-induced carcinogenesis is a nongenotoxic mode of action that is likely strain-specific in the mouse lung. Thus, there is no evidence in the scientific literature that adequately supports the classification of styrene as reasonably anticipated to be a human carcinogen.

Corrective Action: The Background Document's discussion of concordance warrants extensive revision so that it completely and objectively presents the lack of concordance among human, animal and mechanistic data. Corrective language appears in the revised Executive Summary, which appears below.

V. THE EXPERT PANEL'S PEER REVIEW OF THE DRAFT BACKGROUND DOCUMENT DOES NOT IMMUNIZE THE FINAL BACKGROUND DOCUMENT FROM THIS CORRECTION REQUEST

When OMB issued its IQA Guidelines, it recognized the central role that peer review plays in the scientific process. As OMB explained subsequently, "[p]eer review is one of the important procedures used to ensure that the quality of published information meets the standards of the scientific and technical community. . . . [P]eer reviews can filter out biases and identify oversights, omissions and inconsistencies."[80] Accordingly, the OMB, HHS and NIH IQA Guidelines establish a presumption that material subjected to formal, independent, external peer review "may generally be considered to be of acceptable objectivity." This presumption provides important protection of agency resources and credibility.

[80] OMB, Information Quality Bulletin for Peer Review, 70 Fed. Reg. 2664, 2665 (Jan. 14, 2005).

The presumption is not absolute, however, and "is rebuttable based on a persuasive showing to the contrary by a complainant in the particular instance."[81] Unfortunately, the Background Document for styrene is one of those instances, given both: (i) the numerous ways in which the Expert Panel actively degraded the quality of the draft Background Document; and (ii) NTP's issuance of the final Background Document well before the close of the public comment period on the Expert Panel's report.

Finally, and in any event, peer review does not create any presumption of utility, and the Background Document violates that IQA requirement as well.

A. The Expert Panel Consistently Made Recommendations that Bias the Background Document Toward a Finding of Cancer Causation

As explained in more detail in numerous places throughout Part III above, the Expert Panel:

- Urged NTP to switch from RPC workers to SBR workers as the most relevant exposed population, even though the RPC worker population had higher cumulative levels of exposure to styrene and was as numerous as the SBR worker population. Notably, there is no reliable evidence of elevated cancer incidence in RPC workers. *See* pp. 24-27.
- Misconstrued the Delzell study to reach conclusions about cancer causation that Delzell herself disputes. This included developing and relying on a new analysis of exposure groups that is not documented anywhere in the record. *See* pp. 33-40.
- Originated the spurious argument that all three forms of leukemia arise from the same stem cell. *See* pp. 40-41.
- Urged NTP to ignore or suppress uncertainties associated with Coyle et al.'s claim that ambient levels of styrene are associated with breast cancer, in violation of the IQA requirement to identify significant uncertainties and peer-reviewed studies that are relevant to resolving them. *See* pp. 41-45.
- Urged NTP to revise Tables 4-1 and 4-3 to count benign tumors in rodents even where there is no evidence of progression to malignancy. *See* pp. 55-56.
- Created a revised Table 4-3 that concealed time series data that showed absence of trends in cancer causation. *See* pp. 58-59.

[81] NIH Guidelines, *supra* note 4, at § V.2.a.

- Instructed NTP to delete references to statistically significant decreases in tumor incidence in studies by Maltoni et al. (1982) and Conti et al. (1988) because the decreases did not match unidentified control experience. See pp. 56-58.

This consistent pattern of skewing the data to meet Panel members' evident biases is ample reason to conclude that the "objectifying" effect of formal, external, independent peer review did not occur here – in fact, the opposite occurred. SIRC cannot know for sure whether this bias was the conscious goal of one or more of the Expert Panel members, although that is a fair inference from the actions and statements of several, as reflected in the transcript of the Expert Panel's meeting. A primary reason for inferring intent is that it is difficult to understand otherwise why Panel members would make recommendations that produced a less transparent and complete document. The result in any event is that the final Background Document is far less objective and scientifically sound than the draft was. The presumption of objectivity should not attach to the final Background Document, as it has been rebutted here.

B. NTP Finalized the Background Document Before Considering Comments on the Expert Panel's Report

The Expert Panel is given three tasks under the NTP's RoC Review Process:

> (1) Provide peer review comments on the draft Background Document;
>
> (2) Apply the RoC listing criteria to the available scientific evidence and recommend whether to list the substance, and if so, under what characterization; and finally
>
> (3) Provide the scientific justification for its listing recommendation.[82]

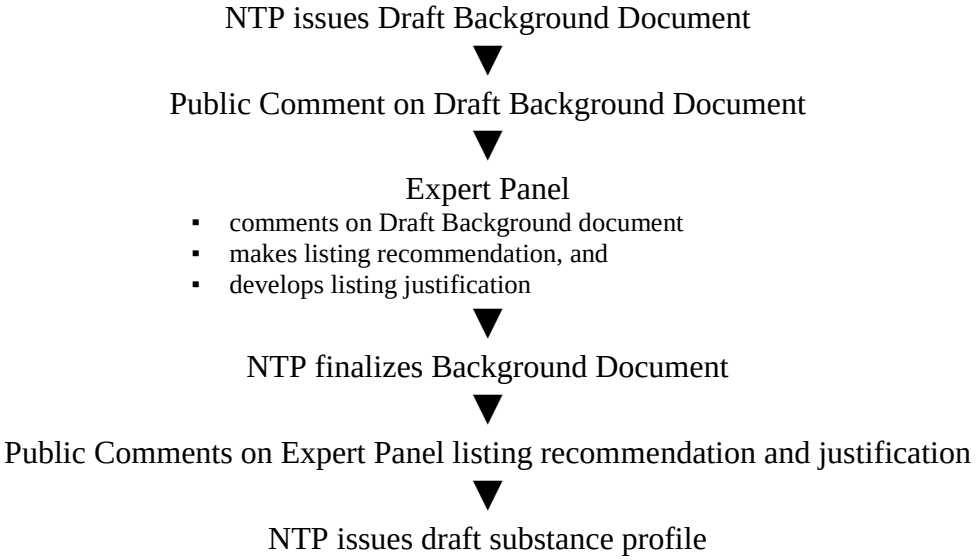
In mandating public comment on the work of the Expert Panel, the RoC review process lists only the second and third tasks as being open to public comment. The Panel's peer review comments on the draft Background Document, and any related changes to the document that the Panel recommends, are, therefore, inexplicably not subject to public comment. The apparent consequence of this omission is that NTP feels free to finalize the Background Document *before*

[82] *NTP Report on Carcinogens Review Process*, "Expert Panel Meeting," available at http://ntp.niehs.nih.gov/index.cfm?objectid=FA93DE50-F1F6-975E-7DA225D6FBE45515.

the NTP receives comments on either (i) those topics or (ii) the Panel's listing recommendation and its scientific justification.

The fundamental flaw of this approach was evident in the styrene review. The Expert Panel recommended numerous changes to the draft Background Document so that it would express the new interpretation of the data promoted by the Panel – a new interpretation that underlay the Panel's recommendations for listing and its justification. Without these changes, the draft Background Document would not support the Panel's recommended listing as explained in the Panel's justification. In short, in the case of styrene, the three functions of the Expert Panel were inextricably tied to and relied on each other. An error in the first function (i.e., recommended changes to the Background Document) would be propagated to the next two. Unfortunately, NTP chose not to wait for public comments on the validity of the changed interpretations of the underlying studies that the Expert Panel proposed for incorporation into the Background Document, and prematurely finalized the Background Document to incorporate the Expert Panel's recommended changes. Then, when NTP was asked to incorporate the public comments on the Expert Panel's recommendations into the Background Document, NTP asserted that the final Background Document was complete and would not be changed, despite the critical nature of the public comments on the whole of the Expert Panel's work. NTP's actions between issuance of the draft and final Background Document thus vitiate the credibility of the presumption of objectivity in this case.

The illogic of this approach can be further appreciated by considering a portion of the RoC process time line.



NTP's approach assumes that no public comment on the Expert Panel's recommendations regarding the draft Background Document will ever prompt NTP to reconsider those recommendations. Put another way, NTP's approach assumes that the Expert Panel's recommendations will always be right and that NTP will ultimately choose to follow them in every case, no matter what errors public comments may identify in those recommendations, or what questions the comments may raise about them. As can be seen from the case of styrene, this assumption is clearly erroneous.

In this case, NTP released the final Background Document on September 30, 2008, more than three weeks before the end of public comment period on the draft Expert Panel report (Oct. 23, 2008). SIRC and others filed comments on the Expert Panel report on or around the due date. SIRC, in particular, filed extensive comments (17 pages, plus two attachments) on October 23 criticizing the Expert Panel's report and flagging many of the biased statements and actions identified above. One of the attachments to SIRC's report was comments of Dr. Delzell specifically objecting to the Expert Panel's reinterpretation of her work.

Because NTP finalized the Background Document to incorporate the recommendations of the Expert Panel before it had received comments on those recommendations, the Background

Document cannot enjoy the presumption of objectivity that the peer review should otherwise have provided it.

C. There Is No Presumption of Utility

As noted earlier, the IQA requires federal agencies to establish guidelines regarding "the quality, objectivity, utility, and integrity of information . . . disseminated by Federal agencies," and the OMB, HHS and NTP IQA Guidelines have interpreted this mandate by establishing requirements regarding objectivity, utility and integrity. (They define "quality" as being determined by the other three requirements.) Information like the Background Document must meet all three types of requirements. The IQA Guidelines of the three agencies establish a presumption that a document meets the "objectivity" requirement if it has been properly peer reviewed. But there is no similar presumption regarding the independently applicable requirements of utility and integrity. As shown repeatedly in Part IV above, the final Background Document is not useful to NTP policymakers, other federal agencies or the public because it is so fundamentally unreliable and misleading.

VI. CORRECTION OF THE BACKGROUND DOCUMENT'S EXECUTIVE SUMMARY

NTP states that the Background Document was "prepared to assist in the review of styrene" (Background Document, page i). This is a gross understatement. Background documents are viewed as NTP's expert summary of the literature, are the basis for drafting the substance profile, and are the basis for listing and classification in the Report on Carcinogens. With this important role in mind, the Background Document is and must be more than a listing of abstracts, and needs to contain a coherent overview of the data that NTP policy makers, other federal agencies and the public can use. This is particularly true for substances, such as styrene, that have been the subject of such a rich and daunting array of studies. A Background Document fails its purpose and is not clear, complete or useful without a coherent overview that addresses the significance or consequence of the studies. From an IQA perspective, the absence of clear or complete assessment, and a pattern of IQA errors, reflects poorly on NTP and suggests a consistent bias in interpretation and presentation.

NTP's Review Process for the 12th RoC states that "NTP staff reviews and considers the expert panel's peer review comments and any public comments as it finalizes the background document on the candidate substance."[83] This statement, and its counterpart on page i of the Background Document for styrene, is inaccurate in the case of the 12th RoC, given the process that NTP actually followed. As explained previously, NTP finalized the Background Document for styrene well before the Expert Panel report was finalized.

With these points in mind, and in addition to correction requests noted separately, a revised Executive Summary appears below.

Note: Underlining denotes additions and strikeout denotes deletions. Brackets [] are in the original NTP Background Document and do not indicate any change or suggestion by SIRC.

Executive Summary

Introduction

Styrene is a viscous, highly flammable liquid used worldwide in the production of polymers, which are incorporated into products such as rubber, plastic, insulation, fiberglass, pipes, automobile parts, food containers, and carpet backing.

Styrene was nominated for possible listing in the *Report on Carcinogens* by a private individual based on its widespread use and exposure and evidence of carcinogenicity from studies in humans and experimental animals.[1] *See*

Human Exposure

The primary use of styrene is in the manufacture of polystyrene, which is used extensively in the manufacture of plastic packaging, thermal insulation in building construction and refrigeration equipment, and disposable cups and containers. Styrene also is used in styrene-butadiene rubber, other polymers, and resins that are used to manufacture boats, shower stalls, tires, automotive parts, and many other products. U.S. production of styrene has risen steadily over the past 70 years, with 11.4 billion pounds produced in 2006 (domestic production capacity for 2006 was estimated at 13.7 billion pounds).

Styrene and styrene metabolites in blood and urine, and styrene-7,8-oxide–DNA adducts and styrene-7,8-oxide–hemoglobin adducts are generally accepted biological

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http://ntp.niehs.nih.gov/go/29353.

indices of exposure to styrene. The primary source of exposure to the general public is inhalation of indoor air; however, exposure can also occur from inhalation of outdoor air, ingestion of food and water, and potentially from skin contact. Tobacco smoke also can be a major source of styrene exposure for both active smokers and individuals exposed to environmental tobacco smoke. Outdoor and indoor air levels (including air levels in most other occupational settings) are generally below 1 ppb [0.001 ppm], although higher levels have been reported. Workers in certain occupations, including the reinforced plastics, styrene-butadiene, and styrene monomer and polymer industries, are potentially exposed to higher levels of styrene than the general public. Air levels in the reinforced plastics industry are generally lower than 100 ppm, [although much higher levels have frequently been measured in older evaluations], while levels in the styrene-butadiene industry and the styrene monomer and polymer industries have rarely been reported to exceed 20 ppm. Numerous Federal agencies have established regulations for styrene, including the Department of Homeland Security, DOT, EPA, FDA, and OSHA, and both ACGIH and NIOSH have established guidelines to limit occupational exposure to styrene.

Human Cancer Studies

Numerous epidemiological studies have evaluated the relationship between styrene and cancer in humans. Most of the studies are cohort studies of workers in three major industries: (1) the reinforced-plastics industry, (2) the styrene-butadiene rubber industry, and (3) the styrene monomer and polymer industry. Two additional cohort studies (one on biomonitored workers, and the second on environmental exposure to styrene butadiene), several case-control studies, and an ecological study have also been published.

We found no consistent increased risk of any form of cancer among workers exposed to styrene. A large study of reinforced plastic workers reported an association between average estimated styrene exposure and risk of total lymphohematopoietic cancers ($p=0.05$), but no trend was observed with increasing duration of exposure. None of the studies of reinforced plastics workers found increased risk from NHL. In two US studies of reinforced plastic workers, esophageal cancer mortality was increased, but these findings were generated in a background of multiple comparisons.[84] Results for

[84] *Comment*: In statistics, the multiple comparisons (or multiple testing) problem occurs when one considers a set, or family, of statistical inferences simultaneously. The term "comparisons" in multiple comparisons refers to comparisons of two groups, such as a treatment group and a control group. Multiple comparisons arise when a statistical analysis encompasses a number of formal comparisons, with the presumption that attention will focus on the strongest differences among all comparisons that are made. Failure to compensate for multiple comparisons can have important consequences. Errors in inference, including confidence intervals that fail to include their corresponding population parameters, or hypothesis tests that incorrectly reject the null hypothesis, are more likely to occur when one considers the family as a whole. Several statistical techniques have been developed to prevent this from happening, allowing significance levels for single and multiple comparisons to be directly compared. These techniques generally require a stronger level of evidence to be observed in order for an individual comparison to be deemed significant, so as to compensate for the number of inferences being made. *See* Miller, R.G. SIMULTANEOUS STATISTICAL INFERENCE (2nd ed. 1981). While it has been argued that

other cancers were unremarkable. The available epidemiologic evidence does not support a causal relationship between styrene exposure and any type of human cancer.

The limitations of these studies include potential misclassification of styrene exposure and disease, small numbers of long-term workers, inadequate follow-up, and the potential for co-exposure to other chemicals. ~~Thus, although m~~More than a hundred thousand workers have been studied to assess a possible carcinogenic effect of styrene exposure~~, only a small fraction of well-characterized, high-level, and long-term styrene-exposed workers have been followed for a sufficiently long time~~. In addition, most of the available studies of occupational cohorts have focused only on male workers (who constitute the majority of exposed workers) or have not performed gender-specific risk analyses. [Thus, comparatively few data are available on cancer incidence or mortality among exposed female workers, limiting the ability to evaluate breast cancer or cancers at tissue sites specific for females.] Workers in the reinforced-plastics industry have the highest levels of exposure and few other potentially carcinogenic exposures, but many of the workers in this industry have short-term exposure, often of less than a year. Cancer mortality or incidence was studied in the following four populations of reinforced-plastics workers: (1) in Washington state in the United States (Ruder *et al.* 2004), (2) in 30 manufacturing plants in unspecified U.S. locations (Wong *et al*. 1994), (3) in Denmark (Kolstad *et al.* 1994), and (4) in Europe (Denmark, Finland, Italy, Norway, United Kingdom, and Sweden) (Kogevinas *et al.* 1994a). (The Danish and the European populations were partly overlapping, as 13,682 Danish male workers were included among the 36,610 male workers making up the European cohort.)

In the styrene-butadiene industry, the ~~cohort studies are among the largest, with the longest follow-up times. The~~ principal methodological challenge is to separate the potentially independent or synergistic effects of butadiene, a known human carcinogen, which is highly correlated with styrene in this industry. Two independent (non- overlapping populations) are available, a small cohort of 6,678 male workers at a rubber tire manufacturing plant (a subset of the workers were engaged in the production of styrene-butadiene and other rubbers) (McMichael *et al.* 1976a), and a larger cohort established by Delzell and colleagues (Delzell *et al.* 1996, 2006) of 13,130 to 16,610 styrene-butadiene rubber industry workers from multiple plants in the United States and Canada. The cohort established by Delzell includes most (but not all) of the workers from two cohorts — a 2-plant cohort (Texas) (Meinhardt *et al.,* 1982) and an 8-plant cohort originally established by Matanoski and colleagues (United States and Canada) and reported in a series of previous publications (7 of the 8 plants were included in the Delzell cohort). Thus, there is considerable overlap between these populations. Two nested case-control studies (Matanoski *et al.*1997, Santos-Burgoa *et*

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multiple comparison adjustments are not necessary when there is a strong basis for expecting the result to be true, that is not the case here. Rothman, K.J. "No Adjustments Are Needed for Multiple Comparisons" 1 EPIDEMIOLOGY (1): 43-46 (1990). It does not appear that NTP or the study authors applied appropriate statistical techniques in reaching the conclusion that the study data support a causal relationship.

al., 1992) of a single group of cases with lymphohematopoietic cancers were available from the Matanoski cohort. The Delzell cohort expanded the previous cohorts to include workers employed from 1943 to January 1, 1991 and followed to 1998, whereas the earlier cohort included workers employed until 1976 and followed until 1982. In addition, the individual study populations were established by different procedures and exclusion criteria (which may partly explain the lack of complete consistency in the number of study subjects across the published studies) and often used different exposure assessments, selection of study subjects, and types of analysis. Two types of analyses were conducted on the Delzell cohort: external analyses reporting on standardized mortality ratios (SMRs) for the total cohort or subsets of the cohorts for multiple cancers sites (Sathiakumar *et al.* 1998, 2005); and secondly, internal analyses of relative risk (RR) estimates for quantitative exposure to styrene and lymphohematopoietic cancers (Delzell *et al.* 2001, 2006, Macaluso *et al.* 2006, Graff *et al.* 2005). (Dimethyldithiocarbamate [DMDTC] was also included as a potential confounder in some analyses of lymphohematopoietic cancer in the Delzell cohort, according to the authors, because of its potential immunosuppressant activity in CD4+ lymphocytes, although its carcinogenicity has not been evaluated outside of this series of studies). Workers in the styrene monomer and polymer industry may be exposed to a variety of chemicals, including benzene, toluene, ethylbenzene, and various solvents, and the cohorts are smaller, with many short-term workers, and few cancer outcomes.

The potential effect of styrene on lymphohematopoietic cancers has been studied most extensively. Findings for lymphohematopoietic cancer and other tumor sites of interest are discussed below.

Lymphohematopoietic cancers

Statistically significant increases were observed for all lymphohematopoietic cancers combined and leukemia among rubber-tire manufacturing workers (McMichael *et al.*1976) and statistically nonsignificant increases were observed for combined lymphohematopoietic cancers and some specific lymphohematopoietic cancers in the Meinhardt and Matanoski cohorts, but the potentially confounding effects of butadiene and other exposures were not analyzed. Two nested case-control studies (using different types of analyses and exposure assessments and the same group of cases) from the Matanoski cohort attempted to evaluate the relative contribution of styrene and butadiene to lymphohematopoietic cancer mortality. Santos-Burgoa *et al.* (1992) found no significant excess risks for combined and specific lymphohematopoietic cancers and mean exposure after controlling for butadiene exposure. Matanoski *et al.* (1997) calculated risks for both average and cumulative exposure to styrene. Taking into account butadiene exposure, and demographic and employment variables in step-down regression analyses, these models found, for an average exposure of 1 ppm vs. no exposure, significant associations for all lymphohematopoietic cancers combined, lymphomas, and myeloma, but not leukemia. For cumulative exposure, significant positive associations between styrene

exposure and combined lymphohematopoietic cancers, leukemia, and myeloma were found, with butadiene exposure dropping out of each of the final models except for leukemia. Specific lymphohematopoietic cancers have been studied more extensively in the Delzell cohort. With respect to leukemia, statistically significant increases have been reported among subgroups of workers with longer durations of employment and longer latency, with the highest cumulative exposure, and in certain specific job groups (Sathiakumar *et al.* 2005, Delzell *et al.* 2006). Internal analyses by Delzell *et al.* involving single-chemical (styrene only), 2-chemical (styrene and butadiene), and 3-chemical (styrene, butadiene, and DMDTC) models of cumulative exposure have shown increased relative risks of leukemia with increasing cumulative styrene exposure. However, the response was attenuated when controlling for exposure to butadiene and was no longer apparent (RRs were less than or equal to one) after additionally controlling for DMDTC. Elevated risks for leukemia were also observed with increasing exposure to styrene peaks in single-chemical, 2-chemical and 3-chemical models (although it was attenuated somewhat in the 2- and 3-chemical models) (Graff *et al.* 2005, Delzell *et al.* 2006).

No statistically significant increased risks were found for other lymphohematopoietic cancers in all employees of the Delzell cohort, but statistically significant risks of NHL and CLL combined were found among workers with higher exposure in an external (SMR) analysis, and in internal analyses among ever-hourly workers, ever-hourly workers with 10+ years of employment and 20 to 29 years or 30 years since first hire, and among specific job groups. Risks of NHL or NHL and CLL combined appeared to increase with increasing cumulative styrene exposure; the risks increased when butadiene was added to the model, and were somewhat attenuated in models that included DMDTC. Exposure to butadiene did not appear to be related to NHL and CLL combined or NHL risk. However, it should be noted that no trend analyses relative to styrene exposure were performed on these data and the SMR in the unexposed group was 0.25. (Graff *et al.* 2005, Delzell *et al.* 2006). No associations were found for other types of lymphohematopoietic cancers and styrene exposure in the Delzell cohort.

In the reinforced-plastics industry, among the highest-exposure groups, the total number of observed versus expected deaths or cases across the four cohorts were comparable for all lymphohematopoietic (52 observed vs. 52.8 expected), lymphomas (14 vs. 15.1), leukemia (19 vs. 19.8) or multiple myeloma (4 vs. 3.4), and were slightly, but not significantly, higher than expected for Hodgkin's disease (11 observed vs. 7.9 expected). Significantly increased risks for leukemia incidence were reported in the Danish study among workers with earlier first date of exposure, and who had worked at least 10 years since first employment, but not for workers employed for 1 year or more (Kolstad *et al.* 1994). No attempt was made to determine if any of the 32 cases was actually exposed to styrene. Based on this methodology and data, it is not reasonable to conclude that this study provides evidence of increased cancer from styrene exposure. In the European multi-country cohort (which overlaps with the Danish study), no excess of leukemia mortality was found, and no exposure-response relationships with cumulative or average exposure were observed, although a non-

significant trend was observed with time since first exposure (Kogevinas *et al.* 1994a). With respect to other lymphohematopoietic cancers, no significantly increased risk for non-Hodgkin's lymphoma was found in the Danish and European multi-country cohorts. Significant exposure-response relationships with average styrene exposure and time since first exposure was observed for total lymphohematopoietic cancers (P = 0.019 and 0.012, respectively) and non-significant trend for malignant lymphoma (P = 0.052 and 0. 072, respectively) in the European multi-country cohort, but no relationship with cumulative exposure was observed (Kogevinas *et al.* 1994a). There was no significant increased risk for non-Hodgkin lymphoma. The authors concluded that: "These findings leave open the possibility of an excess risk of neoplasms of the lymphatic and hematopoietic tissues among workers exposed to styrene." NTP concurs with the authors that the study does not demonstrate a causal association but does not exclude that possibility.

A US study included 15,826 workers employed 6 months or more in areas with exposure to styrene between 1948 and 1977 in one of 30 manufacturing plants and followed up to 1989 (Wong, 1990; Wong et al., 1994). Of these workers 23% were employed for less than one year, and 27% for more than 5 years. Individual exposure levels were estimated based on a job-exposure matrix including individual work histories and time-weighted average job-specific exposure levels. There were 1628 deaths from all causes (SMR 1.08; 95% CI, 1.03–1.13), of which 425 were from cancer (SMR 1.16; 95% CI, 1.05–1.27). The SMRs were 0.82 (95% CI 0.56-1.17; 31 deaths) for LHP neoplasms, 0.72 (95% CI 0.39-1.48, 10 deaths) for NHL and 0.74 (95% CI 0.37-1.33; 11 deaths) for leukemia. Excess mortality was observed for esophageal cancer (SMR 1.92; 95% CI, 1.05–3.22; 14 deaths); lung cancer (SMR 1.41; 95% CI, 1.20–1.64; 162 deaths); cervical cancer (SMR 2.84; 95% CI 1.36–5.21; 10 deaths), and cancer of other female genital organs (SMR 2.02; 95% CI 1.07–3.45; 13 deaths). There was, however, no upward trend in mortality with increased duration of employment for any cause of death. Indeed, most of the increases occurred among employees who worked for only six months to a year, with no significant increase in mortality for the highest cumulative exposure group. Race information was not available for the cohort, and therefore all were assumed to be white for the analyses. However, the death certificates indicated that 7.6% of the decedents were non-white. The authors speculated that some of the SMRs could have been overestimated due to the inability to adjust for race and that lifestyle factors such as smoking may have also confounded the risk estimates. In internal Cox regression analyses including sex, age, duration of exposure and cumulative exposure, neither indicator of exposure was associated with risk of LHP neoplasms or any other cancer. In particular, there was no relation between cumulative exposure to styrene and LHP neoplasm mortality (SMR 1.05, 0.55, 0.76, 0.93 for less than 10, 10-29.9, 30-99.9 and 100 or more ppm-years).

No excesses in mortality from any lymphohematopoietic cancers were observed in ~~the two smaller cohort studies (~~Ruder *et al.* 2004~~ and Wong *et al.* 1994)~~.

In the styrene monomer and polymer industries, the risk of lymphohematopoietic malignancies was also increased in most of the studies (as well as the total number of

observed cases across studies), but these workers might also have been exposed to benzene.

Pancreatic cancer

Among the highest styrene-exposed group in the reinforced-plastics industry, there was an excess in the total number of observed cases of pancreatic cancer across the four cohort studies compared with the total number of expected cases [corresponding to an SMR of 1.77 (95 % CI = 1.23 to 247)]. Increases in pancreatic cancer risk were observed in three of the four reinforced-plastics industry cohorts (one of which was statistically significant [Kolstad *et al.* 1995], and the other two of which were nonsignificant [Kogevinas *et al.* 1994a, Ruder *et al.* 2004]). The risk of pancreatic cancer was slightly higher among the Danish workers with longer term employment and earlier start date, and increased with cumulative exposure in the multi-plant cohort. No indications of exposure-response relationships were found in the smaller U.S. cohorts. Statistically nonsignificant increased risks were also observed in one study in the styrene monomer and polymer industry (Frentzel-Beyme *et al.* 1978), and among biomonitored workers (10 years after the first measurement) (Anttila *et al.* 1998). However, no increased risk of pancreatic cancer was reported among styrene-butadiene workers (Sathiakumar *et al.* 2005).

Esophageal cancer

In two US studies of reinforced plastic workers esophageal cancer mortality was increased, but these findings were generated in a background of multiple comparisons.[85] Among workers with high potential exposure to styrene, increases in esophageal cancer risk were reported in three of the four cohorts (statistically significant increases in mortality were observed among all exposed workers in the two U.S. studies of reinforced-plastics workers [Ruder *et al.* 2004, and Wong *et al.* 1994] and a statistically nonsignificant increase among a subset of laminators in the European cohort [Kogevinas *et al.* 1994a]). Risks were not elevated among the Danish reinforced-plastics workers (Kolstad *et al.* 1994). Across the industry, an approximately 2-fold excess of esophageal cancer was observed among high-exposed groups (laminators and others). A nonsignificant trend with cumulative exposure was reported in the European multi- country study. No increases in risk were reported among styrene-butadiene rubber workers or among styrene monomer and polymer workers.

Other sites

Findings were less consistent for cancer at other sites. Significantly increased risks were observed for cancers of the lung, larynx, stomach, benign neoplasms, cervix and other female tumors, prostate, rectum, and urinary system in either a single study or two studies. There were some supporting exposure-response data for cancers of the urinary system and rectum. A significant increase in breast cancer mortality was observed in a case-control study of occupational exposures among adult females (Cantor *et al.* 1995), although there was no evidence of increased risk between low- and high-

[85] *See* note 84, *supra*.

exposure categories. An ecological study reported a significant increase in the risk of invasive breast cancer in the general population, but exposure estimates were based on environmental releases of styrene, which are the least precise measures of exposure.

In summary, no consistent evidence of an increased risk of LHP neoplasms overall, or of lymphoma or leukemia, emerged from occupational cohort studies. An association between average level of styrene exposure and total LHP risk was suggested in the multicenter European study (Kogevinas et al., 1994b), but no trend for increased NHL was evident either with duration of exposure to styrene (the SMR of NHL for 5 or more years of employment was 1.01 [95% CI 0.27-2.57]) or with cumulative exposure (Figure 2). In that study, the proportion of short term workers was higher among laminators, who had the highest exposure to styrene, than among other workers (Kogevinas et al., 1994a). However, short-term workers are known to experience an increased mortality from many causes, likely due to lifestyle factors and exposures in other occupations (Boffetta et al., 1998). In this respect the excess in the risk of tobacco-related cancer observed in some of the studies of reinforced plastic workers (Kolstad et al., 1993; Wong et al., 1994) is notable, since it suggests confounding by tobacco smoking (information on tobacco smoking is not available in most occupational studies).

The excess leukemia mortality in the SBR industry is in line with what would be expected from exposure to the established carcinogen 1,3-butadiene (IARC, in press), with no evidence of an amplified effect from the co-exposure to styrene. Studies in styrene manufacture and polymerization are less informative because the level of styrene exposure experienced in these industries is lower. These studies, however, provide no evidence of an association with lymphoma, leukemia or other neoplasms. Furthermore, case-control studies conducted in the general population, and studies based on environmental exposure, provide no evidence for an increased risk of LHP neoplasms or specifically, NHL.

Given the relatively large number of studies of styrene, it is predictable that an increased risk of a few cancers would be found in some studies. An association with esophageal cancer was evident in two US studies of reinforced plastic workers (Wong et al., 1994; Ruder et al., 2004), but not in the European studies of such workers (Kogevinas et al., 1994a; Kolstad et al., 1994), or in studies of other groups of styrene exposed workers. A meta-analysis of the results on esophageal cancer (Table 3) resulted in a summary RR (based on random-effects models) of 1.21 (95% CI 0.84-1.73) with evidence of heterogeneity (p-value for heterogeneity 0.01). The lack of available results by level of exposure or cumulative exposure limits the interpretation of the overall excess risk, which can be considered, at most, as suggestive. Results for other cancers show no consistent patterns and the occasional positive findings are probably due to chance.

Several studies showed low levels of DNA adducts in lymphocytes of workers exposed to styrene. Several issues should be considered in the interpretation of DNA adduct data on NHL risk, however. Following styrene exposure, rats and mice form adducts similar to those found in humans. Although levels are higher in rats, no excess cancer incidence has been detected (IARC, 2002). Furthermore, agents known or suspected to cause NHL

in humans are believed to act through immune disregulation rather than through DNA damage (Alexander et al., 2007).

We conclude that the suggestion of a carcinogenic effect of styrene in humans mostly comes from an association of borderline statistical significance between average level of exposure and NHL risk in a large European study of reinforced-plastics workers. However, this suggestion is not supported by results on duration of exposure in the same study, nor by results on NHL risk from other studies. The excess mortality from esophageal cancer in two studies has not been confirmed in other studies. Overall, the available data do not convincingly support an increased risk of cancer, and notably NHL and esophageal cancer, following exposure to styrene.

The evidence for human carcinogenicity of styrene is inconsistent and weak. Based on the available evidence, one cannot conclude that there is a causal association between styrene and any form of cancer.

Studies in Experimental Animals

The carcinogenicity of styrene in rats and mice has been investigated by several routes of exposure. Other relevant studies in experimental animals include studies of mixtures (ß- nitrostyrene and styrene) and studies of the major metabolite of styrene, styrene-7,8-oxide (styrene oxide).

Mice

Three strains of mice were exposed to styrene by gavage. In male $B6C3F_1$ mice, exposure to styrene for 5 days per week for 78 weeks was associated with a significantly increased incidence of alveolar/bronchiolar adenoma and carcinoma (combined) in high-dose (300 mg/kg) animals, and a significant positive dose-response trend was observed (NCI 1979a). NCI questioned the significance of these lung tumors because the incidence in the control group was unusually low compared with historical untreated controls, and only small numbers of vehicle historical controls were available from the same testing laboratory. There also was a significant dose-response trend for hepatocellular adenomas in female $B6C3F_1$ mice, but no significant pair-wise comparisons were observed. The other gavage study included a single dose of styrene administered to pregnant dams on gestation day 17 and weekly exposures of the pups after weaning (Pomomarkov and Tomatis 1978). O20 mice (a strain with a high spontaneous incidence of lung tumors) were dosed at 1,350 mg/kg and C57Bl mice were dosed at 300 mg/kg. A significantly higher incidence of lung tumors (adenoma and carcinoma combined) occurred in both male and female O20 mice compared with vehicle controls. Tumor incidence was not significantly increased in C57Bl mice.

Significantly increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred in male CD-1 mice at inhalation exposure concentrations of 40 to 160 ppm over a period of 104 weeks and in female mice at exposure concentrations of 20, 40, and 160 ppm over a period of 98 weeks (Cruzan *et al.* 2001). Female mice in the high-dose (160-ppm) group also had increased incidences of alveolar/bronchiolar carcinoma.

No increased incidences of tumors were observed in female A/J mice (also a strain susceptible to lung tumors) treated with 20 intraperitoneal injections of styrene over 7 weeks (total dose of 200 µmol [approximately 100 mg/kg b.w.]) and evaluated 20 weeks after the last injection (Brunnemann *et al.* 1992).

Rats

Several of the studies in rats were limited because of short duration, high mortality, incomplete histopathology, or incomplete reporting. None of the carcinogenicity studies reviewed in rats showed evidence of lung tumors, and none of the gavage (NCI 1979a, Pomomarkov and Tomatis 1978, Conti *et al.* 1988), or intraperitoneal or subcutaneous injection studies (Conti *et al.* 1988) reported an increased incidence in any tumor type.

An oral gavage study in F344 rats (NCI 1979a) and an inhalation study in Sprague-Dawley rats (Cruzan *et al.* 1998) were the most robust and most completely reported carcinogenicity studies. Neither study showed an increase in tumor incidences in styrene-exposed rats, although Sprague-Dawley rats exhibited a negative trend in pituitary and mammary gland tumors and a positive trend for testicular interstitial-cell tumors. In another inhalation study in Sprague-Dawley rats, there was a dose-related increase in the incidences of malignant mammary gland tumors; treatment-related and statistically significant incidences of these tumors were seen in the top three dose groups (Conti *et al.* 1988). A drinking-water study did not report any dose-related carcinogenic effects (Beliles *et al.* 1985). However, statistical reanalyses of study data indicated a marginal increase in the incidence of mammary fibroadenoma in high-dose female rats and a significant dose-related trend. Another inhalation study (Jersey *et al.* 1978) [unpublished but reviewed in several published reports] indicated that styrene was associated with a statistically significant increase in incidence of mammary adenocarcinoma in the low- (600-ppm) but not high-dose (1000-ppm) group and a significant increase (when compared with historical but not concurrent controls) in the combined incidence of lymphosarcoma and leukemia in female rats in both the 600-ppm and 1000-ppm dose groups. The authors did not consider the mammary adenocarcinomas to be causally associated with styrene exposure because the incidence of mammary adenocarcinoma was low compared with historical controls and there was no incidence of mammary adenocarcinoma in the high-dose group. Elevated incidences of leukemia/lymphosarcoma were observed in both treatment groups of female Sprague-Dawley rats in this inhalation study.

Mixtures and Metabolite Studies

No increase in tumor incidence was observed in rats exposed by gavage (3 days per week) to a mixture of 70% styrene and 30% ß-nitrostyrene over 78 weeks (NCI 1979b), but an increased incidence of lung tumors was observed in male mice in the 175 mg/kg dose group, but not in the 350 mg/kg dose group exposed to this styrene/ß-nitrostyrene mixture. [However, because of poor survival of the high-dose male mice there were substantially fewer animals at risk for late-occurring tumors.] The styrene metabolite, styrene-7,8-oxide, was previously evaluated for carcinogenicity and is listed in the Report on Carcinogens [first listed in the 10th Report on Carcinogens,

2002] as *reasonably anticipated to be a human carcinogen* based on forestomach tumors in rats and mice and liver tumors in male mice.

Absorption, Distribution, Metabolism, and Excretion

Styrene can be absorbed through inhalation, ingestion, or skin contact, but the most important route of exposure in humans in occupational settings is by inhalation, which results in rapid absorption and distribution of approximately 60% to 70% of inhaled styrene; the highest tissue concentrations are in subcutaneous fat. Food is also an important source of exposure for the general population. Metabolic activation of styrene results in formation primarily of the genotoxic metabolite styrene-7,8-oxide, which can be detoxified by glutathione conjugation or conversion to styrene glycol by microsomal epoxide hydrolase. Styrene is metabolized in both the liver and the lung, and the Clara cells in the lung are regarded as the major cell type in styrene activation following inhalation exposure. The initial step in styrene metabolism is catalyzed by cytochromes P450; CYP2E1 and Cyp2f2 are the predominant enzymes in humans and experimental animals. In animals, CYP2E1 predominates in liver, while Cyp2f2 is the primary enzyme in mouse lung. CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially expressed in the lung compared with liver in humans, and the human CYP2F1 has been shown to be capable of metabolizing styrene when expressed *in vitro*. Because styrene- 7,8-oxide contains a chiral carbon, this and some subsequent styrene metabolites can exist as either *R*- or *S*-enantiomers. A second metabolic pathway results in formation of 4-vinylphenol, which has been detected in humans, rats, and mice *in vivo*,. Almost all absorbed styrene is excreted as urinary metabolites, primarily mandelic acid and phenylglyoxylic acid.

Species differences exist among rats, mice, and humans in the metabolism and toxicity of styrene, which may be related, at least in part, to interspecies differences in the stereochemistry of metabolism. The *R*-enantiomer, which has been suggested by some reports to be more toxic than the *S*-form, has been reported to be produced in relatively larger amounts in mouse lung than in rat lung, but the difference was less pronounced when microsomal preparations were used. In mice, the *R*-isomer of styrene-7,8-oxide was significantly more hepatotoxic than the *S*-isomer; the toxicity of the *R*-isomer also was greater in the lung, but the difference was not statistically significant.

Toxicity

Styrene exposure has been associated with numerous health effects in humans and laboratory animals. The acute toxicity of styrene is low to moderate with an oral LD_{50} of 320 mg/kg and an inhalation LC_{50} of 4,940 ppm (4-hour exposure) in mice and an oral LD_{50} of 5,000 mg/kg and an inhalation LC_{50} of 2,770 ppm (2-hour exposure) in rats. The primary effects of acute exposure to styrene in experimental animals and humans include irritation of the skin, eyes, and respiratory tract and CNS effects. Drowsiness, listlessness, muscular weakness, and unsteadiness are common signs of systemic styrene intoxication. Several studies have reported effects on color vision, hearing threshold, reaction time, and postural stability following long-term occupational

exposure to styrene at concentrations ranging from about 20 to 100 ppm. Reports of ischemic heart disease and hepatic, renal, hematological, and immunological effects have been inconsistent. Human data are insufficient to determine whether styrene is a reproductive or developmental toxicant, but effects of styrene to increase serum prolactin levels within the normal range in humans have been reported.

Styrene toxicity in experimental animals is similar to that reported in humans. Exposure to styrene vapors can cause eye and respiratory tract irritation, CNS depression, and death, except in mice. Clara cells are the main target of styrene pneumotoxicity in mice, but pneumotoxicity is not seen in rats, Glutathione depletion as a result of styrene exposures has been reported to be associated with damage to lung, liver, and kidney tissues. The cytotoxicity of styrene in the mouse lung, a tissue high in CYP2F isoforms, could be prevented by CYP2F inhibitors. Some studies have reported reproductive and developmental effects, but these effects were seen mostly at doses associated with maternal toxicity. Reported effects have included embryonic, fetal, and neonatal death, skeletal and kidney abnormalities, decreased birth weight, neurobehavioral abnormalities, and postnatal developmental delays. The possibility of polystyrene dimer and trimer extracts from food containers mimicking the physiological effects of estrogen have also been investigated, but with a mixture of positive and negative results.

Genetic Damage

In vitro studies show that styrene-7,8-oxide forms DNA adducts and causes positive results in Comet assays in a dose-related manner. DNA adducts, primarily N7- and O^6-adducts, were reported in white blood cells in all studies of styrene-exposed workers employed mainly in hand-lamination plants. Several human studies have shown a correlation between Comet assay and DNA adducts and indicate that the strand breaks, which are not generally regarded as significantly lethal or mutagenic lesions, are efficiently repaired within several hours after exposure has stopped. Adducts are formed primarily at the N7-, N^2-, and O^6-positions of guanine. N7-adducts are formed in the greatest amount but are the least persistent, while O^6-adducts are formed in the least amount but are the most persistent. Styrene-7,8-oxide was mutagenic without metabolic activation in all *in vitro* mutagenicity test systems reported and caused mutations in some studies in the presence of metabolizing enzymes. Both styrene and styrene-7,8-oxide caused cytogenetic effects (sister chromatid exchange [SCE], chromosomal aberrations, and micronuclei) in human lymphocytes or other mammalian cells *in vitro*. DNA adducts have been detected in liver and lung cells of mice and rats exposed to styrene *in vivo*, although the levels were not increased in target organs of mice or in mice compared to rats.. The majority of studies in experimental animals demonstrated an effect of both styrene-7,8-oxide and styrene exposure on Comet assay, while mostly negative results for cytogenetic or clastogenic effects of styrene were reported. . The limited data on mutation frequencies in *HPRT* and *GPA* in styrene-exposed workers are inconclusive. More than half the studies measuring chromosomal aberrations have reported an increase in chromosomal aberrations in styrene-exposed workers (or subgroups of workers), and several studies have reported a positive exposure-response relationship with styrene air levels or urinary metabolites. A

meta-analysis of 22 studies found a positive association between styrene exposure level and chromosomal aberration frequency when exposure levels were dichotomized as greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted average, although other reviewers of these studies did not find an association between styrene exposure and chromosomal aberrations. Studies of other cytogenetic markers in humans are conflicting. About half of the studies that evaluated micronucleus and SCE frequency in styrene workers were positive, and a few studies have reported significant dose-response relationships with styrene exposure. A meta-analysis of 10 micronucleus studies was inconclusive, and a meta-analysis of 14 SCE studies indicated a slight increase in SCE frequency but, again, was too small to be conclusive. A number of studies have been published on the possible modulating role of genetic polymorphisms, mainly in xenobiotic metabolism enzymes and DNA-repair genes, at the level of various biomarkers. Some authors have suggested that genetic susceptibility (probably at many loci) may be important in styrene-mediated genotoxicity.

Mechanistic Data

The proposed mechanisms for the carcinogenicity of styrene include both genotoxic and epigenetic pathways. These mechanisms, which are not necessarily mutually exclusive, include: (1) metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue and (2) cytotoxic effects of styrene metabolites in the mouse lung.

It is unlikely that genotoxicity from styrene-7,8-oxide (SO) plays a role in the development of mouse lung tumors because administration of SO to mice did not increase lung tumors, despite producing an equivalent level of SO as metabolism of styrene in the lung, non-tumorigenic doses of styrene produced eight times greater concentration of SO in rat lung explant studies than did tumorigenic doses in mice, genotoxicity studies in mouse lung are negative, CYP2E1 metabolism (which produces mostly S-SO) does not affect lung toxicity from styrene, and the level of DNA adducts was not greater in mice than in rats and was not greater in mouse lung than liver, nor in mouse Clara cells than in the remainder of the lung. The Harvard Center for Risk Analysis (Cohen *et al.* 2002) considered three factors as possible explanations for the greater susceptibility of mouse lung than rat lung to development of hyperplasia leading to tumors with exposure to styrene are: (1) the presence of the styrene-metabolizing cytochromes in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-7,8-oxide, and (3) the susceptibility of mouse lung tissue to glutathione depletion. However, they concluded that although toxicokinetic models generally predict higher rates of metabolism by mice and rats than by humans, the models do not consistently predict a difference between the rodent species. IARC concluded that the styrene 7,8-oxide mode of action hypothesized by Cohen et al. (2002) was not likely in humans.

An alternative mechanism is that interspecies differences in styrene toxicity are most likely explained through CYP2F-generated metabolites (2f2 in mice, 2F4 in rats, and 2F1 in humans) in the mouse lung. This is based on data showing that the effects of

> cytotoxicity (included focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia, and bronchiolo-alveolar hyperplasia) and tumor formation were seen in mouse respiratory tissues, which are high in CYP2F isoforms, and that CYP2F inhibitors prevented cytotoxicity. Moreover, metabolites formed from ring oxidation, including 4-vinylphenol, are about 6-fold higher in mice compared with rats, and 4-vinylphenol is more potent than styrene-7,8-oxide as a pneumotoxicant. Furthermore, compoundsthat cannot be oxidized in the 4 position of the ring, such as 4-methylstyrene or 3,4-dimethylstyrene, do not cause cytotoxicity or lung tumors in mice.

VII. CONCLUSION

When the epidemiologic evidence is considered as a whole, there are no consistent associations between styrene exposure and mortality or incidence of any cancer type, either within or among studies. These data show low numbers of observed cases, a lack of an exposure-response relationship, and concerns regarding co-exposures to known carcinogens and confounding. Thus, a causal interpretation is not credible, and the standards of "limited" evidence are not met.

The evidence for styrene-induced carcinogenesis in experimental animals does not meet the standards of "sufficient" evidence. Increased incidences of mostly benign tumors have been observed in certain strains of only one species (mice) and at one tissue site (lung). This tumor type is common in mice, and the tumors developed late in life in the presence of chronic cytotoxicity.

The mechanistic data on styrene suggest a non-genotoxic mode of action for styrene that is based on local cytotoxicity and subsequent cell proliferation and is highly species-specific. Thus, this mode of action is not applicable to humans or other animals and does not support the classification of styrene as a human carcinogen.

The rationale for using rodent bioassay results as indicators of possible human carcinogenicity rests on the broad similarity among mammals in anatomy, physiology, and biochemistry; the applicability of a rodent response as an indicator of potential human risk amounts to hypothesizing that, owing to this underlying commonality, the carcinogenic processes responsible for the animal results could also plausibly occur in humans. That is, one is

hypothetically generalizing the phenomenon from the particular animal species showing the response to other mammals, including humans.

For styrene, however, it is clear that the processes responsible for the tumorigenesis observed in mice do not occur in rats. Not only do rats not show a tumor impact from styrene inhalation (the hypothesized generality of which across mammals is the basis for inferring its relevance to humans), but also the specific mode of action – the tissue-specific metabolic activation – is not present. In short, the proposed generalization of effects across mammals is contradicted. Moreover, there is no indication that the mode of action would be present in humans, either.

We have shown that the animal evidence is not "sufficient" according to NTP's standards of interpretation. Beyond this, however, it is clear that the animal evidence for a carcinogenic effect of styrene applies only to mice and not to rats, so any "sufficiency" of animal evidence does not apply to all animals – and to the degree that it doesn't, its applicability to humans is also questionable, since one would have to propose why, against all available evidence, humans should be like mice and not like rats in their response to styrene.

We have also argued that the human studies on styrene and cancer do not support a conclusion of "limited" evidence. There are no consistent responses among the human data of the kind that one would expect if there were true biological causation. The diversity of proposed tumor endpoints in human studies raises more questions than it answers – why is it that styrene would affect some tumor responses in some studies and other responses (requiring other modes of action) in other studies? If there were truly a mode of action of sufficient generality and broadness to cause such a variety of tumor responses in humans, why is there no sign of it – and why is there no indication of hematopoietic cancers – in rats or in mice?

To bring together animal, human, and mode-of-action data into an overall weight of evidence conclusion about the potential for human carcinogenicity, one seeks to characterize the likelihood of a common thread that ties together the evidence from the different sources and proposes a biologically plausible line of reasoning as to why a potential hazard in humans is

indicated. For styrene, there is no such commonality. The mouse tumor responses are best interpreted as a species-specific phenomenon that does not apply to rats and for which there is evidence against its applicability to humans. There is no consistent response among human studies, and the hypothesized human effects have no counterpart in the animal data. No mode of carcinogenic action has been identified that would apply beyond the mice. All together, these data do not support the Profile's characterization of styrene as "reasonably anticipated to be a human carcinogen."

While corrective actions have been suggested for each IQA deficiency noted, collectively they lead to major problem for NTP. The final Background Document served as the basis for NTP's draft substance profile and NTP's classification recommendations. Because the Background Document needs substantial revision, so does the current draft version of the substance profile. And any final listing and classification decision that NTP issued on the basis of the Background Document would be similarly unreliable – and arbitrary and capricious.

Collectively, the data discussed above support the following conclusions relative to NTP's criteria for "reasonably anticipated to be a human carcinogen:"

1. There is no evidence of a causal relationship between styrene exposure and cancer in humans. The Draft Substance Profile's characterization of the human data as "limited" is not consistent with the NTP classification criteria.
2. The animal studies provide at best only limited evidence of carcinogenicity – clear evidence by one route of administration and no more than suggestive evidence by another. The draft profile's classification of the animal data as "sufficient" does not conform to the NTP classification criteria.
3. The available data do not support genotoxicity through styrene-7,8-oxide as the mode of action for mouse lung tumors.

The discussion in the Background Document should be revised consistent with these points. Because the draft substance profile relies on the defective Background document, NTP must withdraw and correct it as well.

* * *

For the above-stated reasons, the Background Document of September 29, 2008, must be withdrawn and, if reissued, corrected as indicated. Similarly, any subsequent NTP documents based on the flawed Background Document, such as the draft substance profile issued in December 2008, should be withdrawn and, if reissued, revised consistent with the corrected Background Document.

SIRC and its members would welcome the opportunity to meet and discuss these issues or provide clarifications to assist the review and correction of the Background Document. Please do not hesitate to contact me for any further information.

Very truly yours,

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FINAL

Report on Carcinogens Background Document for

Styrene

September 29, 2008

U.S. Department of Health and Human Services
Public Health Servces
National Toxicology Program
Research Triangle Park, NC 27709

Abbreviations

ABS: acrylonitrile-butadiene-styrene

ACGIH: American Conference of Governmental Industrial Hygienists

ADH: alcohol dehydrogenase

ALDH: aldehyde dehydrogenase

AlO: aldehyde oxidase

ALL: acute lymphocytic leukemia

AML: acute myelogenous leukemia

ANOVA: analysis of variance

ASPEN: Assessment System for Population Exposure Nationwide

ATSDR: Agency for Toxic Substances and Disease Registry

BCF: bioconcentration factor

BEAM: Boston Exposure Assessment in Microenvironments

BEI: biological exposure indices

BLS: Bureau of Labor Statistics

BRCA1: breast cancer 1, early onset gene

b.w.: body weight

C: control

C+: centromere positive

C–: centromer negative

CA: chromosomal aberrations

Cal/OSHA: California Division of Occupational Safety and Health

CBI: covalent binding index

CC1b: Clara-cell specific protein

CDC: Centers for Disease Control and Prevention

CEH:	*Chemical Economics Handbook*
CERHR:	Center for Evaluation of Risks to Human Reproduction
CHO:	Chinese hamster ovary
CLL:	chronic lymphocytic leukemia
cm:	centimeter
CML:	chronic myeloid leukemia
CNS:	central nervous system
CO:	cyclohexene oxide
CPBI:	cytokinesis proliferation block index
CR:	creatinine
CREST:	calcinosis-Raynaud's phenomenon-oesophageal dismobility-sclerodactyly-telangiectasis syndrome of scleroderma
CYP:	cytochrome P450
Cyt-B:	cytochalasin B
d:	day
Da:	Dalton
DAPI:	4',6-diamidino-2-phenylindol·2HCl
DC:	decarboxylase
dm:	decimeter
DMDTC:	dimethyldithiocarbamate
DMSO:	dimethylsulfoxide
DNA:	deoxyribonucleic acid
DOT:	Department of Transportation
E:	exposed
EPA:	Environmental Protection Agency
EPHX:	epoxide hydrolase

ETS:	environmental tobacco smoke
E.U.:	European Union
F:	female
FDA:	Food and Drug Administration
FISH:	fluorescence *in-situ* hybridization
g:	gram
GGT:	gamma-glutamyl transpeptidase
GI:	gastrointestinal
GPA:	glycophorin A
GSH:	glutathione
GSTM1:	glutathione S transferase M1
GSTT1:	glutathione S transferase T1
γ-GT:	gammaglutamyl transpeptidase
h:	hour
HA:	hydroxylapatite
HazDat:	Hazardous Substances Release and Health Effects Database
HE:	human erythrocytes
HEL:	human embryonic lung
HFC:	high-frequency cells
HIC:	highest ineffective concentration
HID:	highest ineffective dose
HPRT:	hypoxanthine phosphoribosyltransferase
HSDB:	Hazardous Substances Data Bank
Hz:	Hertz
IARC:	International Agency for Research on Cancer

ICD: International Classification of Diseases

i.p.: intraperitoneal

IRR: incidence rate ratio

JEM: job-exposure matrix

K+: kinetochore-positive

kg: kilogram

K_{oc}: soil organic carbon-water partitioning coefficient

K_{ow}: octanol-water partition coefficient

L: liter

LC: liquid chromatography

LD_{50}: lethal dose for 50% of the population

LEC: lowest effective concentration

LED: lowest effective dose

LH: lymphohematopoietic

LHC: lymphohematopoietic cancer

LWAE: lifetime weighted average exposure

M: male

m^3: cubic meter

MA: mandelic acid

mEH: microsomal epoxide hydrolase

mfg.: manufacturing

mg: milligram

mL: milliliter

MM: multiple myeloma

MN: micronuclei

MNBC: binucleated lymphocytes

MNMC: mononucleated lymphocytes

mol wt: molecular weight

MS: mass spectrometry

N: sample size

NA: not available

NA-AAF: *N*-acetoxy-2-acetylaminofluorene

NAcT: *N*-acetyltransferase

NADPH: nicotinamide adenine dinucleotide phosphate, reduced form

NAP: not applicable

NCEs: micronucleated normochromatic erythrocytes

NCHS: National Center for Health Statistics

NCI: National Cancer Institute

ND: not detected

NDMA: *N*-nitrosodimethylamine

NDT: not determined

NHANES: National Health and Nutrition Examination Survey

NHL: non-Hodgkin's lymphoma

NI: not identified

NIEHS: National Institute of Environmental Health Sciences

NIOSH: National Institute for Occupational Safety and Health

ng: nanogram

NLM: National Library of Medicine

NNK: 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone

No.: number

NQ: not quantified

NR: not reported

NRC: National Response Center

NS: not significant

NT: not tested

NTP: National Toxicology Program

OH: hydroxyl

OR: odds ratio

OSHA: Occupational Safety and Health Administration

PAH: polycyclic aromatic hydrocarbon

PAMA: phenacylmercapturic acid

PBL: peripheral blood lymphocytes

PBPK: physiologically based pharmacokinetic model

PC: personal computer

PCEs: micronucleated polychromatic erythrocytes

PEL: permissible exposure limit

PGA: phenylglyoxylic acid

PHA: phytohemagglutinin

PHEMA: phenylhydroxyethyl mercapturic acids

PWN: pokeweed

ppb: parts per billion

ppbv: parts per billion by volume

ppm: parts per million

r: correlation coefficient

REL: recommended exposure limit

RoC: Report on Carcinogens

RR: relative risk

RTECS: Registry of Toxic Effects of Chemical Substances

RV: recreational vehicle

s.c.: subcutaneous

S_B: styrene in blood

SBR: styrene-butadiene rubber

SCE: sister chromatid exchange

SD: standard deviation

SDH: sorbitol dehydrogenase

SE: standard error of the mean

SIR: standardized incidence ratio

SIRC: Styrene Information and Research Center

SO: styrene oxide

SOC: Standard Occupational Classification

SOCMI: Synthetic Organic Chemical Manufacturing Industry

SSB: single-strand breaks

STEL: short-term exposure limit

TDS: Total Diet Study

TK: thymidine kinase

TLV: threshold-limit value

TRI: Toxics Release Inventory

TWA: time-weighted average

U_B: styrene in urine

UDS: unscheduled DNA synthesis

USITC:	United States International Trade Commission
µg:	microgram
VOC:	volatile organic chemical
VPT:	vinylphenol
WHO:	World Health Organization
XO:	xanthine oxidase
XPC:	xeroderma pigmentosum, complementation group C
XPD:	xeroderma pigmentosum, complementation group D
XPG:	xeroderma pigmentosum, complementation group G
XRCC:	X-ray repair cross-complementing group
yr:	year