A Collaboration Project between International Agency for Research on Cancer (IARC) and National Occupational Research Agenda (NORA)

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Identification of research needs to resolve the carcinogenicity of high-priority IARC carcinogens

Views and Expert opinions of an IARC/NORA expert group meeting

Lyon, France: 30 June – 2 July 2009

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Disclaimer
The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of any United States Federal agency to which any author is affiliated.

Disclaimer
Dr. Ruth Lunn did not participate in the discussions on cobalt metal with tungsten, formaldehyde, or styrene.

Introduction

History of the NORA process and framework for the meeting
by Paul A. Schulte

This is a concerted effort to identify means of reducing the insufficiency of available data for classifying particular agents in the International Agency for Research on Cancer (IARC) system by identifying information needs and gaps and the research to address them for 20 selected agents generally, in IARC categories 2A, 2B, and 3.

This project originated as part of the National Institute for Occupational Safety and Health's (NIOSH) National Occupational Research Agenda (NORA) to enhance occupational cancer research. The project ultimately involved collaboration with IARC, the National Institute of Environmental Health Sciences (NIEHS), the American Cancer Society (ACS), and the National Cancer Institute (NCI). The planning group consisting of Kurt Straif and Vincent Cogliano (IARC), Paul Schulte, Tania Carreón-Valencia, Avima Ruder, Mary Schubauer-Berigan (NIOSH), Ruth Lunn (NIEHS), Nat Rothman (NCI), and Elizabeth Ward (ACS) selected 20 agents, reviewed in previous IARC Monographs, on the basis of potential for workplace or environmental exposure, and importance or interest by a particular agency.

The purpose was two-fold: (1) identify research needs to address the information needs and gaps for a more definitive classification, and (2) Create a report that will be of value to funding agencies in determining possible projects to support.

The selected experts, generally two for each agent (one toxicologist and one epidemiologist), who either had participated in an IARC Monograph working group that addressed a candidate agent or who were knowledgeable about one or more agents, were selected by the planning committee. For each agent, the experts used a systematic approach reviewing the Monograph and the literature published since the Monograph meeting to determine whether potential research gaps and needs still existed, and what research was needed to change the classification. In addition, experts were encouraged to identify research in progress that they were aware of that could possibly affect the gap analysis.

For each agent, a common reporting template was used. All templates had the following categories:

- Summary
- Citation for most recent IARC review
- Current evaluation
- Exposure and biomonitoring
- Cancer in humans
- Cancer in experimental animals
- Mechanisms of carcinogenicity
- Research needs and recommendations
- Selected relevant publications since IARC review / References

The focus of the recommendations was not meant to be a compilation of all useful or interesting research but rather a focus on research that would be critical or important in resolving classification uncertainties. These templates were uploaded and shared among the experts before the meeting, and discussed during the meeting. Afterwards the templates were revised reflecting the discussions in the meetings. The templates vary in length depending on available new cancer research conducted on the carcinogenic agent since the last IARC update. These revised templates are compiled here to make this comprehensive technical report. The results of this meeting are published in this extensive technical report published by IARC, and in summary format as an article in the Environmental Health Perspectives.

Resolving the Carcinogenicity of Agents in IARC Groups 2A and 2B: Identification of High-Priority Research

by Vincent Cogliano

Common interests of IARC and NORA are to identify research that could lead to a more definitive classification for suspected carcinogens whose classification is now unresolved (e.g. in IARC Groups 2A or 2B). This could lead to well-planned epidemiologic or mechanistic studies, followed by a more definitive classification (either higher or lower) for these agents.

The previous NORA–IARC workshop (Sept 2006) was charged with considering current data for agents in IARC Groups 2A, 2B, or 3. These agents were then sorted into three groups:

- Agents that could be in Group 1 now and should be considered for Volume 100 of the *IARC Monographs*, which is updating the assessments of the more than 100 agents classified in Group 1;
- Agents for which new research is likely to change the classification and should be considered for NORA priority list; and
- Agents for which new research is not likely to change the classification and should be dropped as a NORA priority.

Overview of IARC's evaluation process

The *IARC Monographs* are a series of scientific evaluations developed by international working groups of expert scientists. Periodically, IARC convenes advisory groups to advise on priorities for future evaluation or reevaluation (IARC 2003). Agents are selected for evaluation based on evidence of human exposure and some evidence or suspicion of carcinogenicity. Each year, IARC generally convenes three separate working groups on different topics. Meetings are announced on the Internet about one year in advance, accompanied by a Call for Data (IARC 2004). Two principles govern the selection of working group members: to invite the best qualified experts, and to avoid real or apparent conflicts of interest.

There is a standard structure of the Monographs, where the first four sections provide a critical review of the pertinent scientific literature, and the final sections include summaries of the scientific data and the evaluations developed by the working group. The working group develops its evaluations through a series of distinct steps; providing insight into the working group's reasoning by revealing the weight given to each line of evidence.

The evidence of cancer in humans and cancer in experimental animals has four descriptors: "sufficient evidence," "limited evidence," "inadequate evidence," or "evidence suggesting lack of carcinogenicity" (for definitions of these terms, see IARC 2006). These two partial evaluations are combined into a preliminary default evaluation that the agent is "carcinogenic to humans" (Group 1), "probably carcinogenic to humans" (Group 2A), "possibly carcinogenic to humans" (Group 2B), "not classifiable as to its carcinogenicity to humans" (Group 3), or "probably not carcinogenic to humans" (Group 4) (Figure 1).

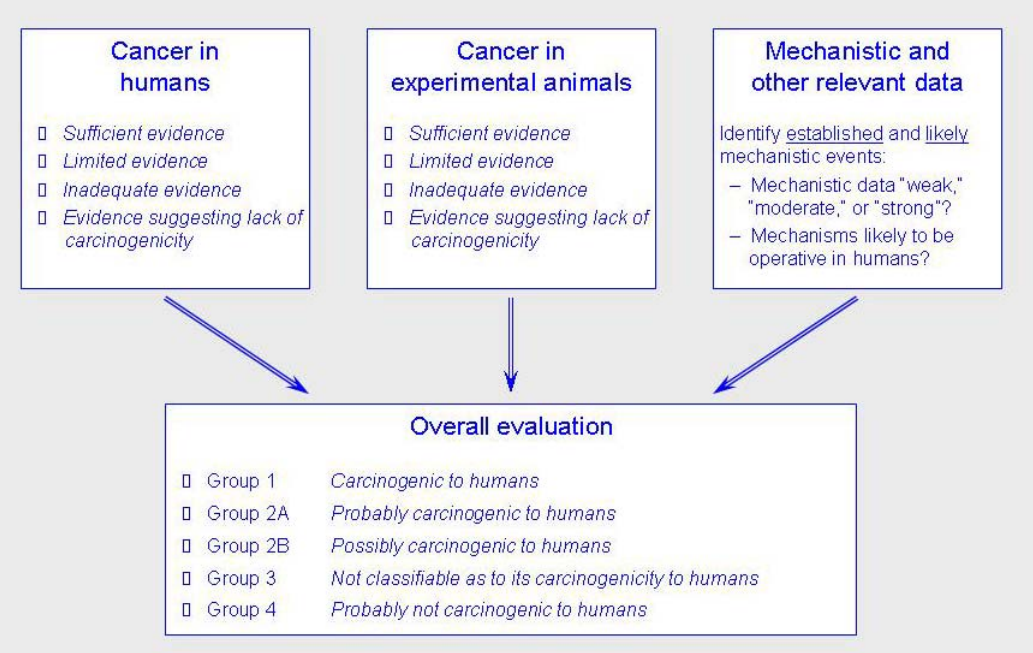

Figure 1 Overview of IARC's evaluation process

For example to reach *sufficient evidence* of carcinogenicity, as stated in the Preamble (IARC 2006): the Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

To reach an evaluation in IARC Group 1: *Carcinogenic to humans*, as stated in the Preamble (IARC 2006): This category is used when there is sufficient evidence of carcinogenicity in humans. "Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity."

The mechanistic and other relevant data are considered to determine whether the default evaluation should be modified (figure 2). This determination considers the strength of the mechanistic evidence and whether the mechanism operates in humans. The final overall evaluation is a matter of scientific judgment, reflecting the weight of the evidence derived

from studies in humans, studies in experimental animals, and mechanistic and other relevant data.

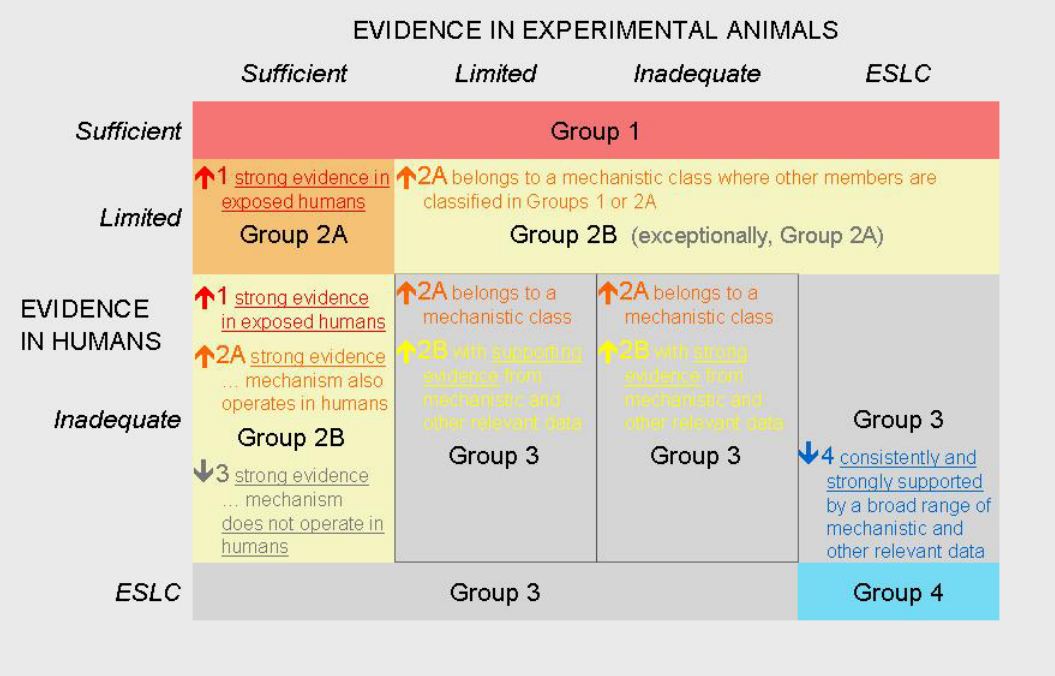

Figure 2. Mechanistic data can be pivotal when the human data are not conclusive

The NORA-IARC workshop 2009 was charged with two tasks:

- for each NORA priority agent where epidemiologic studies may yield sufficient evidence in humans, include human cancer studies in the research plan
- for each NORA priority agent where mechanistic studies may yield "strong evidence in exposed humans," include mechanistic studies in the research plan

This technical report list the agents for review in the following order: Inorganic agents such as metals (lead and lead compounds, indium phosphide, cobalt metal, titanium dioxide), metal fumes (welding fumes), fibers (refractory ceramic fibers), and particles (diesel engine exhaust, carbon black), followed by organic agents such as oxides (styrene, styrene oxide, propylene oxide), aldehydes (formaldehyde, acetaldehyde), chlorinated solvents (trichloroethylene, tetrachloroethylene, dichloromethane, chloroform, PCB), plasticizer (DEHP), and pesticide (atrazine), and lastly, shift work.

IARC-Classified Agents Selected for Review

Agent	Year of Monograph	Volume	Human evidence	Animal evidence	Overall evaluation
Formaldehyde	2006	88	S	S	1
Lead and lead compounds	2006	87	L	S	2A
Indium phosphide	2006	86	I	S	2A
Shiftwork that involves nightwork	2007	98	L	S	2A
Cobalt metal with tungsten carbide	2006	86	L	S	2A
Diesel engine exhaust	1989	46	L	S	2A
Clorinated solvents					
Styrene-7,8-oxide	1994	60	I	S	2A
Trichloroethylene (TCE)	1995	63	L	S	2A
Tetrachloroethylene (PERC)	1995	63	L	S	2A
Polychlorinated biphenyls (PCB)	1987	18	L	S	2A
Styrene	2002	82	L	L	2B
Propylene oxide	1994	60	I	S	2B
Acetaldehyde	1999	71	I	S	2B
Dichloromethane (DCM)	1999	71	I	S	2B
Chloroform	1999	73	I	S	2B
Di-2-ethylhexyl phthalate (DEHP)	2000	77	I	S	3
Atrazine	1999	73	I	S	3

I = Inadequate evidence; L = Limited evidence; S = Sufficient evidence

Lead and lead compounds

by Hartwig Muhle PhD and Kyle Steenland PhD

Citation for most recent IARC review

IARC Monographs 87, 2006

Current evaluation

Conclusion from the previous Monograph:
Inorganic lead and lead compounds are probably carcinogenic to humans (Group 2 A).

Exposure and biomonitoring

The CDC has an ongoing exposure and biomonitoring program for a number of chemicals including lead (CDC, National Exposure Report, 2005) which follows changes in blood lead concentrations over time. More recently, the FLEHS Biomonitoring Study (Ketelslegers et al., 2008) has reported the results of multi-plex genotyping as a biomarker of susceptibility to carcinogenic exposures. These studies, which included lead, reported a positive relationship between lead exposure and the carcinoembryonic antigen BRCA2N^{372}H. Lead associated levels of this antigen were also found to be 1.9 times higher in persons carrying the double variant allele (heterozygous) and 1.6 times higher in persons carrying the wild type. An even greater increase was observed in smokers in response to lead exposure. The importance of these data rests with a better understanding of the relationship between genetic inheritance, lead exposure and risk of cancer.

Cancer in humans

(*limited***,** Vol 87, 2006).

The 2006 evaluation was based primarily on six larger historical cohorts exposed to inorganic lead. Data on the effects of exposure to organic lead was sparse and no conclusions could be drawn. Historical organic lead exposure was largely confined to tetra-ethyl lead used as an additive in gasoline (now discontinued); tetra-ethyl lead was broken down into inorganic lead when gasoline was burned.

Regarding the six cohorts exposed to inorganic lead, stomach cancer was consistently elevated (30-50%) in four of the five cohorts where stomach cancers were reported. Lung, kidney, and brain cancer showed elevation in some studies but were not consistent. Since 2006 there are five studies with new information, although none are large or would change the conclusions of the 2006 Working Group.

Wijngaarden et al. (2006) studied brain cancer in the National Longitudinal Mortality Study, a prospective census-based cohort study of the United States population in 1979-1989 (n=317,968). Using a job-exposure matrix and industry and occupation from the census, the authors found increased risk of brain cancer in jobs likely to have involved lead exposure (RR 1.5, 0.9-2.3)(18% exposed), with indications of an exposure-response trend based on

estimated intensity of exposure. This study is limited due to lack of direct data on exposure, but adds to the positive evidence for brain cancer.

Rousseau et al. (2007) conducted nested-case control studies for 11 types of cancers and estimated lead exposure, with exposure estimated based on work history and expert assessment (Montreal/Siemiatycki data base). 17% of subjects were exposed to inorganic lead, while 3% were exposed to organic lead. Stomach cancer was associated with organic lead (ORs 2.0 (1.1-3.8) and 3.0 (1.2-7.3) depending on control group), but there was no association with inorganic lead, nor were there any notable positive associations for other cancers. Again this study is limited due to lack of direct data on exposure, but the lack of association weakens the IARC 2006 conclusion.

Lam et al. (2006) linked 3192 lead-exposed workers in New Jersey, identified by a NIOSH-sponsored lead surveillance program, to the NJ Cancer Registry. All cohort members had measured blood leads >25 μg/Dl at some point in the past. They found 83 incidence cancer cases, with a deficit of cancer overall (0.51, 0.41-0.62). There were no notable excesses (stomach cancer SIR 1.1, 4 cases). This study is limited by small numbers, and possible under-ascertainment of cases due to out-of-state migration.

Siddiqui et al. (2006) conducted a case-control of breast cancer in India, with 50 malignant and 50 benign cases. Blood lead was significantly higher in the malignant case croups, as were zinc, iron, and calcium. This study is limited as blood lead was present at background environmental levels and reflected exposure in recent past (3 months).

Rajaraman et al. (2006) reported increased rates of brain tumors (gliomas and menigomas) in relation to genetic polymorphisms in the ALAD gene and lead exposure suggesting a need to take genetic inheritance of ALAD polymorphisms into account with respect to lead exposures and development of these types of cancer. The ALAD2 allele was found to increase the risk of menigioma from 1.1 to 5.6 and 12.8 based upon estimated based upon intermediate and longer term cumulative lead exposures respectively. ALAD may play an important role in the bioavailability of lead to sensitive molecular sites which is known to vary as a function of the ALAD2 allele (Scinicariello et al., 2007) and lead inhibition of this enzyme and others in the heme biosynthetic pathway may also contribute to the generation of oxidative stress via generation of reactive oxygen species.

Cancer in experimental animals

(*sufficient***,** Vol 87, 2006)

No new carcinogenicity assays have been reported since the last evaluation since 2002. In contrast, there have been a number of studies reporting the inhibitory effects of lead exposure on sperm development and motility in male experimental animals (Kaspercyk et al., 2008, Wang et al., 2008)

Mechanisms of carcinogenicity

The mechanisms of lead induced cancers in experimental systems are complex appear to involve oxidative stress (Jurczuk et al., 2006) and may involve lead interaction with zinc finger proteins (Jarzecki et al., 2007), induction of apoptosis (Xu et al., 2006, 2008), altered cell signaling pathways (Wang et al., 2008b, 2009) and regulation of lead interactions with cellular genetic machinery by high affinity lead - binding proteins (Fowler et al., 1994, Rajaraman, 2006).

Research needs and recommendations

Additional epidemiologic information can be provided by large cohorts with established exposure above background environmental levels. It appears likely that there will be a mortality study of approximately 50,000 workers with past measured blood lead levels above 25 µg/Dl in the United States (NIOSH ABLES surveillance program), which should prove informative. There are also an additional 50,000 subjects with lower blood leads. Subsequent possible case-control studies of cancers of interest (stomach, brain, kidney, lung) could also provide more information. This study could be strengthened by the addition of two components: 1) measurement of a sample of subjects for bone lead to determine the correlation of the blood lead measurements with cumulative exposure as measured by bone lead, and 2) assessment of whether *Helicobacter pylori* infection has been more common among those with higher blood leads. If so such infection could either be a mechanism by which lead caused higher rates of stomach cancer, or it could be a confounder.

In addition, given positive results from the FLEHS study (Keteleslegers et al., 2008) and the report by Rajaraman et al. (2006) it is clear that future epidemiological studies of relationships between lead exposures and cancer should include evaluation of genetic susceptibility factors if at all possible.

Further experimental research studies are needed to evaluate the complex mechanisms by which lead may cause cancer with particular emphasis on the roles of oxidative stress / apoptosis and the roles of cellular defense mechanisms, signaling pathways and intracellular lead binding patterns in mediating these processes.

Selected relevant publications

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Xu J, Lian LJ, Wu C, et al. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. *Food and Chem Toxicol* 2008; 46: 1488-1494.

Indium phosphide and other indium compounds

includes indium phosphide, indium arsenide, indium tin oxide, CIS, CIGS

by Bruce A. Fowler PhD, Mary Schubauer-Berigan PhD, and Cynthia J. Hines MS.

Citation for most recent IARC review

IARC Monographs 86, 2006

Current evaluation

Conclusion from the previous Monograph:
Indium phosphide is *probably carcinogenic to humans (Group 2A)*. Despite a lack of evidence from human studies, the carcinogenicity was upgraded because of the "extraordinarily high incidences of malignant neoplasms of the lung in male and female rats and mice; increased incidences of pheochromocytomas in male and female rats; and increased incidences of hepatocellular neoplasms in male and female mice." These occurred at very low doses and short exposure periods.

Exposure and biomonitoring

Occupational exposure

Since the publication of Monograph 86, production of indium compounds has increased but appears limited by the low rate of refining indium as a byproduct of zinc and lead-zinc smelting (Hageluken 2006). The most well documented exposed population likely remains the semiconductor industry. Chen (2007) estimates that over 30,000 people were employed in 2007 among 350 semiconductor manufacturing firms in Taiwan. In the United States, 255,000 workers were estimated to be involved in the manufacture or application of semiconductor chips in 2004, possibly including exposure to inorganic indium compounds (http://www.sia-online.org/cs/papers_publications/press_release_detail?pressrelease.id=221). [It is unlikely that all workers in the global semiconductor industry have been exposed to indium compounds and these workers may be exposed to a variety of other Group 1 or 2 human carcinogens].

A burgeoning industry worldwide has developed in optoelectronics (e.g., light-emitting diodes and photovoltaics) and flat panel display technology, in which a variety of indium compounds [indium phosphide, indium tin oxide (ITO), indium arsenide, indium sulfide, copper indium diselenide (CIS), copper-indium-gallium-diselenide (CIGS)] are used. The use of indium in these industries has exceeded that in the semiconductor industry; (Mikolajczak 2009) reports that 80% of indium produced or reclaimed globally is used as ITO in flat panel displays. The next largest application of indium compounds is in the photovoltaic industry, either as a semiconducting material (e.g. CIS or CIGS) or as a transparent conductive top contact (e.g. ITO). (Alsema et al., 1997, Panthani et al., 2008) Many of these technologies also involve the use of indium compounds in research and development settings. For example, nanoscale ITO powder has been proposed as a transparent, conductive coating material in the production of solar panels because of its antistatic and electro-magnetic interference-shielding properties (Cho et al., 2006). Some of these technologies result in products with short useful lives (e.g., 3-5 years for liquid crystal displays); thus indium exposure may occur during disposal or recycling of the component materials (Hageluken 2006, Li et al., 2009). Indium radioisotopes are also widely used in medical research and therapy (Fowler 2007).

Several studies have recently evaluated exposures among semiconductor and optoelectronics workers in Asia. Miyaki et al. (2003) measured indium concentrations in whole blood, serum and urine from 107 workers exposed to water-insoluble indium particles and from 24 unexposed workers. Mean exposures in whole blood, serum, and urine (respectively) were 16.8, 14.6, and 2.45 μg/L among the exposed workers. Among the unexposed workers, most samples were below the detection limit, with a mean concentration of 0.57 μg/L for whole blood. Serum and whole blood concentrations were highly correlated (r=0.987), with a beta regression coefficient very close to 1. Whole blood concentration was also significantly correlated to urine concentration and to creatinine-adjusted urine levels (better for the latter).

Whole blood and urine concentrations of indium were measured among four groups of optoelectronics workers (no specific indium compounds mentioned) in Taiwan (Liao et al., 2004), including an unexposed group of office workers. Blood and urine concentrations (respectively) averaged 0.22 μg/L and 0.02 μg/L among the exposed workers and 0.14 μg/L and 0.02 μg/L among the unexposed workers. Blood and urine indium concentrations were found to be significantly correlated (Pearson's r=0.194); however, the authors commented on the greater sensitivity of blood than urine as a marker of indium exposure (in contrast to gallium and arsenic, the other metals evaluated). Job title was found to be correlated with blood indium concentrations. In addition to this study, several case reports (described below) from Japan have appeared in the literature. One (Taguchi et al., 2006) estimated serum indium concentrations of 40, 99 and 127 μg/L among three workers with interstitial pulmonary disease (and involved in wet surface grinding of ITO) among 115 workers examined.

One recent study evaluated concentrations of indium in inhalable air and urine of workers in two Taiwanese semiconductor manufacturing sites (Chen 2007). This study included 144 exposed workers [72 production workers ("operators") and 72 engineers, with no specific compounds mentioned] and 72 unexposed administrative workers. Personal air samples were collected during a shift, and spot urine samples were collected at the end of the shift. The latter were analyzed using ICP-MS [which is preferable to GF-AAS as an analytic method]. Operators and engineers were found to have similar mean inhalable air exposure concentrations (8.4 and 7.4 $\mu g/m^3$, respectively), but each group had significantly higher air

exposures than administrative workers (2.1 μg/m^3) [We note that ambient outdoor air concentrations are at least 3 orders of magnitude lower than this, per Fowler 2007]. Similar patterns were observed for indium in urine (7.0, 5.9 and 1.2 μg/L, respectively). Air and urine concentrations were found to be significantly correlated. The maximum air exposure concentration was 101 μg/m^3 for an operator—this was the sole exposure above the US NIOSH recommended exposure limit (REL) of 0.1 mg/m^3. By contrast, 71-76% of the "exposed" workers (compared to 7% of the administrative workers) had arsenic exposure levels above the NIOSH REL. The authors use USEPA risk coefficients to derive a lifetime cancer mortality risk of 10% (Chen 2007) at the mean arsenic air concentrations. The authors attribute the highest indium exposures to the manufacture of high-brightness LEDs, telecommunication laser diodes, optical storage lasers, electric devices, and solar cells (Chen 2006, 2007). [We note that urine indium concentrations in the optoelectronics workers (Liao et al., 2004) were more than 2 orders of magnitude lower than those measured among semiconductor workers (Chen 2007); however, it is unclear how representative these two studies are of their respective industries. Also, given the high potential cancer risk from arsenic exposure and the correlation of indium and arsenic exposure, independently evaluating the risk of indium through epidemiologic studies of semiconductor workers may be difficult].

Other than blood and urine measurements, there are no clear biomarkers of exposure to indium compounds. Current methods only analyze for elemental indium and cannot distinguish between specific indium compounds. Promising research has begun on analytic methods to measure indium species in environmental (air) samples (Profumo et al., 2008), which should better characterize the likely toxicity of specific indium compounds. Liao et al. (2006) found that malondialdehyde, a biomarker of lipid peroxidation, was not correlated with indium blood or urine levels among 100 workers in the optoelectronics industry.

Cancer in humans

(*inadequate***,** Vol 86, 2006)

Since the publication of the previous monograph, several new cancer studies have been reported among semiconductor workers. Nichols and Sorahan (2005) updated cancer incidence and mortality in a cohort of 1807 UK semiconductor industry workers that was described in the previous monograph. Small numbers of cancer deaths occurred with the added follow-up. Mortality rates for all cancers combined and for lung cancer were non-significantly elevated among male workers but not among females. Significant elevations in incidence were observed for rectal cancer in men and malignant melanoma and pancreatic cancer in women.

Beall et al. (2005) evaluated cancer mortality among 126,836 workers at 3 facilities owned by a single company; two engaged in semiconductor manufacture, masking and packaging and one in storage device manufacture. Bender et al. (2007) evaluated cancer incidence among workers at two of these three facilities (excluding one semiconductor manufacture site). Overall lung cancer SMRs in this study were 0.61 (95% CI: 0.55, 0.67) among men and 0.98 (95% CI: 0.82, 1.17) among women. SMRs for the person-time associated with longer durations of employment and greater time since first employment were also not elevated. Excluding the storage device production facility reduced the lung cancer SMRs among men and slightly increased them among women, but the values were still close to 1. In the

combined group of semiconductor facilities, women in masking tasks had a higher lung cancer SMR. [The authors note that several smoking-related diseases showed SMRs below unity.] The SIR for lung cancer at the semiconductor manufacturing facility was significantly depressed overall (0.60; 95% CI: 0.51-0.70) and among the "exposed" (i.e., non-office) workers. [Nearly half the workforce was professional and likely had lower smoking rates than the general population or than the "unexposed" office workers.] Significantly elevated SMRs and SIRs were also observed for central nervous system cancer among process equipment maintenance workers at one of the semiconductor facilities and for prostate cancer among certain workers at the storage device manufacturing facility.

Clapp (2006) reported proportionate cancer mortality ratios (PCMRs) among 31,941 decedents who worked at a large U.S. computer manufacturing company. The lung cancer PCMR was significantly depressed. Overall CNS cancer PCMRs were elevated, and PCMRs of skin melanoma and cancers of kidney and pancreas were significantly elevated in male manufacturing workers. Clapp and Hoffman (2008) analyzed death data for workers at one U.S. facility involved most recently in circuit board manufacture and reported elevated PCMRs of 3.67 (95% CI: 1.19, 8.56) for malignant melanoma and 2.20 (1.01, 4.19) for lymphoma in males. A PCMR of 1.03 (95% CI: 0.71, 1.42) was observed for lung cancer in men.

In the United States, the Semiconductor Industry Association has commissioned a study of 85,000-105,000 (numbers conflict) wafer fabrication plant employees of its member companies. A feasibility study was conducted by Johns Hopkins researchers, and the retrospective cohort study is being conducted by Vanderbilt University-Ingram Cancer Center (see http://www.sia-online.org/cs/issues/occupational_health_and_safety). [The main limitation of all the studies described above is the lack of specific information on exposure to indium compounds and on the multitude of other Group 1 or 2 carcinogens to which these employees were potentially exposed. It is also unclear whether these workers had substantial exposure to indium phosphide.]

No cancer incidence or mortality studies were found that focused specifically on indium. One case of lung cancer was reported in a non-smoking ITO worker (Nogami et al., 2008). Beyond this, there is some evidence of pulmonary effects among indium workers, similar to those seen in animal studies. Several case reports in Japan have reported fatal interstitial pneumonia (attributed to ITO particles in lung) among workers producing or using ITO (Homma et al., 2003, 2005, Taguchi and Chonan 2006, Nogami et al., 2008). A more comprehensive study of pulmonary function was conducted among 108 workers from the plant in which several cases were reported (Chonan et al., 2007). Significant interstitial pulmonary changes and reduced lung function were found in 22% of the workers, and these were associated with workplace exposure to indium. In a more recent study (Hamaguchi et al., 2008), 93 exposed and 93 unexposed ITO (and other indium compounds) workers did not exhibit significant differences in prevalence of interstitial or emphysematous pulmonary changes, but significant exposure-response relations were shown between serum indium concentrations and several biomarkers (serum KL-6, SP-D, SP-A) for early interstitial pulmonary changes among the exposed workers. [We note that it is unclear to what extent these case reports and cross-sectional studies overlap].

Cancer in experimental animals

(*sufficient***,** Vol 86, 2006)

An extensive inhalation study of indium phosphide in rats and mice (NTP 2001, Gottschilling et al., 2001) demonstrated clear-cut development of lung cancers in both species of rodents although some differences between genders and species were observed. These data provided the experimental animal data for sufficiency of cancer in animals in the prior IARC review. In addition, other long-term intratracheal instillation studies in hamsters using indium arsenide and indium phosphide (Yamazaki et al., 2000) have demonstrated that both indium arsenide and indium phosphide produced similar pulmonary hyperplastic lesions. Indium arsenide was observed to produce more severe lesions than indium phosphide but this finding may be related to differences in solubility between the 2 compounds (Takanaka 2004). In addition to lung lesions, Omura et al. (2000) reported similar testicular toxic effects in hamsters following a 2 year intratracheal instillation regimen of indium phosphide and indium arsenide. The degree of lesion severity was regarded as more severe for indium arsenide (See Takanaka 2004 for review). Lison et al. (2009) evaluated the lung toxicity of sintered ITO in comparison with indium oxide (In_2O_3), tin oxide (Sn_2O_3) or a mixture of indium oxide and tin oxide (MIX) in rats after a single pharyngeal instillation. They observed the formation of oxygen centered radicals and Fenton chemistry in the presence of ITO but not the other compounds in an acellular system using EPR spectrometry. They did, however, report the formation of OH* radicals with all the compounds tested and the silica controls in the presence of H_2O_2. Taken together these studies indicate that indium phosphide and indium arsenide have similar toxic properties and that indium-induced reactive oxygen species may play a mechanistic role in the observed carcinogenic process.

Mechanisms of carcinogenicity

The studies by Gotschilling et al. (2001) suggested that indium phosphide-induced oxidative stress may play an important role in the pulmonary carcinogenesis of indium phosphide. The prior observation of indium arsenide induced inhibition of the heme biosynthetic pathway with attendant development of increased porphyrin excretion patterns (Conner et al., 1995) suggest that this effect could also contribute to the development of oxidative stress since porphyrins are also capable of catalyzing singlet oxygen formation. This observation coupled with the ability of indium to induce apoptosis in rat thymocytes (Bustamente et al., 1997) suggest a carcinogenic mechanism related to repair-associated cell proliferation. In addition, indium arsenide has also been shown to elicit a specific stress protein response in hamster renal tubule cells following a single intratracheal instillation of InAs which was observed to be attenuated over time and associated with the development of increased proteinuria (Fowler et al., 2005). These data suggest indium-induced suppression of this important cellular mechanism for protection against oxidative stress induced proteotoxicity. The mechanism of inhibition of protein synthesis may be related to loss of ribosomes from the rough endoplasmic reticulum following indium exposure (Fowler et al., 1983). In addition, comparative studies of using male and female hamsters (Fowler et al., 2008) showed marked gender differences in the degree to which indium altered protein expression patterns in hamster renal tubule cells. These data may be useful in helping to explain observed gender differences in indium phosphide carcinogenicity in rodents (NTP 2000).

Research needs and recommendations

The previous monograph on indium considered indium phosphide alone; since then, the use of other indium compounds [indium tin oxide (ITO), CIGS, and others] has burgeoned. More than 75% of indium is now used as ITO in flat panel displays. More than 300,000 workers are employed worldwide in the semiconductor industry, and a large number of workers are also employed in manufacturing flat panel displays and optoelectronics (including photovoltaics), and in reclaiming indium from spent indium-containing materials.

Several studies of workers in the US semiconductor industry, including a large study by NIOSH of circuit board manufacturing workers and an even larger study by a semiconductor industry trade association, are currently underway. While these studies are attempting to characterize risk associated with work in specific departments or operations, they are unlikely to inform on cancer risk of indium compounds, because: 1) little indium exposure may have occurred in past circuit board manufacturing, 2) wafer fabrication workers (those most likely to have indium phosphide exposure) are typically exposed to a wide variety of other carcinogens, including arsenic, trichloroacetic acid, tetrachloroacetic acid, and more than 20 others (Cullen et al., 2001); and 3) little historical exposure monitoring information is likely available to provide estimates of exposure to indium phosphide or other potential carcinogens, which would be necessary to evaluate the contribution of indium to any observed carcinogenicity among wafer fabrication workers.

A better approach may be to conduct (if feasible) epidemiologic studies (e.g., retrospective cohort studies) of workers involved in primary (e.g., zinc smelting) or secondary refining industries. Most primary indium refining occurs in Asia although there are two large secondary refineries in the United States and several elsewhere. Studies in secondary refineries may be more informative because of the presence of cadmium in zinc smelting. Also, the focus of secondary refineries on indium production suggests that exposures to other carcinogenic substances may be lower than those to indium. Analogy exists to the Group 1 carcinogenic metals (e.g., nickel, cadmium, and beryllium), for which the most informative studies have generally been conducted among the refiners and production facilities for these metals and metal compounds (IARC Monograph 100C, Straif et al., 2009). A series of case reports has identified pulmonary effects that may be occurring in indium-exposed workers in Asia. Studies of current exposure and biomarkers of genetic damage (using the metrics described below) of these and other indium-exposed workers may be informative in identifying early precursors of cancer.

Further experimental research is needed into the mechanisms of indium compound induced toxicity and carcinogenesis with particular focus on formation of oxidative stress and inhibition protective protein synthetic mechanisms and DNA damage. Oxidative DNA damage from indium and/or arsenic exposures could be evaluated by measurement of 8-OHdG in accessible cells (e.g., nasal epithelium, buccal cells, and circulating lymphocytes) and also micronuclei micro-RNA profiling, and chromosomal aberrations.

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Cobalt with tungsten carbide

by Bruce A. Fowler PhD and Damien M. McElvenny MSc.

Citation for most recent IARC review

IARC Monographs 86, 2006

Current evaluation

Conclusion from the previous Monograph (IARC 2006)

Cobalt metal with tungsten carbide is probably carcinogenic in humans (Group 2A). A number of working group members supported an evaluation in Group 1 because: (1) they judged the epidemiological evidence to be sufficient, leading to an overall evaluation in Group 1; and/or (2) they judged the mechanistic evidence to be strong enough to justify upgrading the default evaluation from 2A to 1. The majority of working group members who

supported the group 2A evaluation, cited the need for either sufficient evidence in humans or strong mechanistic evidence in exposed humans. Cobalt metal without tungsten carbide is possibly carcinogenic to humans (Group 2B). Cobalt sulfate and other soluble cobalt (II) salts are possibly carcinogenic to humans (Group 2B).

Exposure and biomonitoring

Cobalt is widely distributed in the environment, occurring in the earth's crust mainly in the form of sulfides, oxides and arsenides. Cobalt metal is used to make corrosion- and wear-resistant alloys used in aircraft engines (superalloys), in magnets (magnetic alloys) and in high-strength steels and other alloys for many applications. Cobalt metal is added to metal carbides, especially tungsten carbide, to prepare hard metals (two-phase composites; also known as cement carbides) for metal-working tools. Cobalt is also used to manufacture cobalt-diamond grinding tools, cobalt discs and other cutting and grinding tools made from cobalt metal. Other uses of cobalt include catalysts, batteries, dyes and pigments and related applications. Occupational exposure to cobalt occurs predominantly during the refining of cobalt, in the production of alloys, and in the hard-metal industry where workers may be exposed during the manufacture and maintenance of hard-metal tools and during the use of diamond-cobalt tools.

Exposures

The epidemiological evidence to date comes from hard-metal industry and cobalt production. Exposure to hard-metal dust takes place at all stages of the production of hard metals, but the highest levels of exposure to cobalt have been reported to occur during the weighing, grinding and finishing phases (Reber and Burckhardt 1970, McDermot 1971, NIOSH 1981, Sprince et al., 1984, Hartung 1986, Kusaka et al., 1986, Balmes 1987, Meyerbisch et al., 1989, Auchincloss et al., 1992, Stebbins et al., 1992). For example, in two factories in the USA producing hard metals, peak cobalt concentrations in air during weighing, mixing and milling exceeded 500 $\mu g/m^3$ in more than half of all samples (Sprince et al., 1984), and in powder rooms with poorly-regulated control of cobalt dusts, concentrations of cobalt in air ranged from between 10 $\mu g/m^3$ and 160 $\mu g/m^3$ (Auchincloss et al., 1992).

Cobalt concentrations in air were determined for all stages in the manufacturing process in Japan (Kusaka et al., 1986, Kumagai et al., 1996). The concentrations of cobalt were shown to be lognormally distributed and the geometric means for the different workshops ranged from 2 $\mu g/m^3$ in blasting and electron discharging to 211 $\mu g/m^3$ in rotation in powder prevention to 233 $\mu g/m^3$ in rubber press.

Several studies exist that have examined cobalt concentrations in urine. Notable among these were a study of hard-metal workers in several small factories in northern Italy. Cobalt concentrations in the urine of six operators on machines without aspirators were up to 13 times higher than those in the reference population (Cereda et al., 1992). A British study reported median concentrations of cobalt in urine of 19 nmol/mmol creatinine in workers in the hard-metal industry and 93 nmol/mmol creatinine in workers manufacturing and handling cobalt powders, salts and pigments in the chemical industry (White and Dyne 1992).

Concentrations of different tungsten species (W, WC, WO, WO_4^{2-}) were studied in air and in urine samples from workers in different areas in a hard-metal factory in Germany (Kraus et al., 2001).

Cancer in humans

(*limited evidence***,** Vol 86, 2006)

The current epidemiological evidence falls just short of providing convincing evidence for a carcinogenic effect of exposure to cobalt with tungsten carbide in humans.

There have been no relevant epidemiological studies since the most recent monograph was published. The current epidemiological evidence comes from a small nested case-control in the French hard-metal industry (Moulin et al., 1998) and a smaller study in the Swedish hard-metal industry (Hogstedt and Alexandersson 1990).

Cancer in experimental animals

No new experimental animal studies involving cobalt without tungsten carbide were found since the last IARC review (IARC 2006).

De Boeck et al. (De Boeck et al., 2003) studied the effects of a single intratracheal instillation of tungsten carbide-cobalt (WC-Co) particles on rat type II pneumocytes using the alkaline comet assay and micronucleus tests using a dose response /time course protocol. The comet assay was also conducted on cells recovered by broncho-aveolar lavage (BAL) and on peripheral blood mononucleated cells (PBMC). Direct lung toxicity was evaluated by group of standard toxicity markers. The results of these studies indicated that the WC-Co particle exposure produced a statistical increase in DNA comet tails at 12 hours and in micronuclei at 72 hours in the type II pneumocytes at a dose level that produced only mild lung toxicity. No effects were observed in the PMBC. These data provide evidence of WC-Co particle mutagenic potential in a lung target cell population following in vivo exposure.

Mechanisms of carcinogenicity

Cobalt

An extensive and growing literature on molecular mechanisms of cobalt cellular toxicity has been published since the last IARC review. Cobalt induced formation of reactive oxygen species (ROS) leading to a number of molecular effects related to oxidative stress have been document in cellular test systems. Studies by De Boeck et al. (De Boeck et al., 2003) demonstrated cobalt alone or in combination with tungsten carbide particles induced micronucleated binucleates in human peripheral blood mononucleated cells (PMBC) in a concentration dependent manner. Kang et al. (Kang et al., 2005) demonstrated cobalt-induced Hypoxia inducible factor (HIF) stabilization and IRP-1 activation in human lung carcinoma A459 cells grown in iron- free salt-glucose medium (SGM). If iron was added to the medium, HIF stabilization and IRP-1 activation were reversed. Subsequent studies (Hervouet et al., 2008) focused on the increased formation of ROS and stabilization of HIF-alpha subsequent to cobalt induced decreases mitochondrial oxidative phosphorylation in clear cell renal carcinoma cells. These investigators also noted HIF stimulated increases in ROS formation and mitochondrial manganese superoxide dismutase content. Global gene expression studies (Malard et al., 2007) using human A549 lung cells identified 85 genes

which were either up or down regulated in response to soluble cobalt treatment. These investigators reported that 29 of these genes representing basic cellular functions were evaluated by RT-PCR. And the expression profiles of 6 of these were evaluated by quantitative RT-PCR in a time-dependent manner with confirmation by Western blots. A number of these genes were regarded as putative cobalt carrier proteins, tumor suppressor proteins or transcription factors and genes linked to the stress protein response. The investigators also indentified 9 genes which code for secreted proteins that could serve as possible future biomarkers. The results of these studies are consistent with ROS induction of the stress protein response and alterations of a number of proteins responsible for regulating cellular proliferation. Studies by Witkiewicz and Bal (Witkiewicz-Kucharczyk and Bal 2005) reported damage to zinc finger proteins in DNA repair proteins and suggested that the resultant inhibition of DNA capability may play a role in development of a subsequent carcinogenic response. In addition, recent studies by Li et al. (Li et al., 2009) demonstrated both positive and negative cobalt induced alterations of histone methylation patterns in human lung A549 cells and bronchial epithelial (Beas-2B) cells. These investigators noted the potentially important role of cobalt –induced epigenetic alterations in homeostatic histone methylation patterns in mediating gene expression patterns and the carcinogenic response.

Cobalt with tungsten carbide

Lombaert et al. (Lombaert et al., 2004) studied the apoptotic potential of WC-Co dust, WC and metallic cobalt in human peripheral blood mononucleated cells (PMBC) incubated in vitro with different concentrations of these materials for up to 24 hours. Apoptosis was assessed by Annexin-V staining, flow cytometry (FACS) and ELISA analysis o f DNA fragmentation. Both WC and metallic cobalt particles induced apoptosis and the WC-Co particles appeared to show additivity with respect to this parameter. Interestingly, apoptosis induced by WC particles appeared to be mediated by caspase-9 while that induced by metallic cobalt was mediate by both caspase-8 and caspase-9. The investigators suggest that the apoptosis from WC is due to effects on monocytes while that of metallic cobalt on both monocytes and lymphocytes. WC-Co effects are the result of an additive combination. Subsequent studies by Lombaert et al. (Lombaert et al., 2008) examined the in vitro effects of WC-Co particles on human PMBC gene expression patterns at a 24 hour time point. They observed that the apoptotic, and stress protein pathways were the most markedly up-regulated while the immune response pathways were the most strongly down- regulated in the PMBC. For WC-Co treated monocytes, the nucleosome/chromosome pathways were most up-regulated while the immune response pathways were also most strongly down -regulated. Mateuca et al. (Mateuca et al., 2005) examined the influence of DNA repair enzyme genetic polymorphisms in lymphocytes of workers exposed to cobalt. These investigators found that the incidence of micronucleated mononucleates (MNMC) was elevated in WC-Co exposed workers with the hOGG1(326) genotype base excision enzyme. Multivariate analyses which controlled for a number of potential confounders demonstrated that MNC and comet assay tail DNA were also modified by genetic polymorphisms. In the exposed and total worker populations studied, MNMC frequencies were elevated in those carrying the hOGG1 genotype and XRCC3 double strand break repair enzyme. These data indicate the need for including genotyping in evaluations of workers exposed to these dusts to elucidate those most at risk. The role of glutathione and cysteine SH –group oxidation by WC-Co dust induced ROS formation was studied by Fenoglio et al. (Fenoglio et al., 2008). They reported dust

concentration- dependent generation of thiyl radicals at particle surface sites. It was suggested that this process, in combination with depletion of cellular anti-oxidant defenses, would further exacerbate cellular oxidative damage caused by the WC-Co particle formation of ROS.

Research needs and recommendations

It was noted that there is a proposed study, which is pending a funding decision, to do a cohort (n>10,000) and nested case-control study based at 18 manufacturing sites in the United States, UK, Austria, Germany, Sweden and England. This study is expected to take 4 to 5 years to complete. If it is possible, consideration should be given to this new study incorporating the determination of an appropriate biomarker for exposure. It is recommended that the possibility of updating the French and Swedish studies be explored if they are able to provide sufficient extra data on lung cancer risks, adjusted for potential confounding factors, before the proposed international study. Additional research is also needed to further understand the genetic factors which regulate cellular protective systems in order to better protect sensitive human sub-populations exposed to cobalt with tungsten carbide.

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Titanium Dioxide (TiO_2)

by Eileen D. Kuempel PhD and Avima Ruder PhD

Citation for most recent IARC review

IARC Monograph 93 (in press)

Current evaluation

Conclusion from the previous review:
Titanium dioxide is *possible carcinogenic to humans (Group 2B)* based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.

Exposure and biomonitoring

Occupational exposure

Titanium dioxide is produced from iron titanate or titanium slag by digesting with sulfuric acid or from ores with a high titanium content by heating with coke and chlorine to form titanium tetrachloride, then oxidizing to titanium chloride. Production workers are exposed to sulfuric acid mist or hydrochloric acid and titanium dioxide dust. The occupational

epidemiology studies do not differentiate production of fine titanium dioxide from that of ultrafine (or nano) titanium dioxide. A relative risk assessment ranked manufacture of nano-titanium dioxide at 62 (range 0-100), similar to the assigned rank of automotive lead battery manufacture (Robichaud, et al. 2005)

Four U.S. companies manufacture 1.3 million metric tons/year of bulk titanium dioxide, 25% of global production, with the chloride process at eight sites. The percentage of titanium dioxide manufactured as nanoparticles has been estimated as 2.5% in 2009 and about 10% by 2015 (Robichaud et al., 2009).

Liao and colleagues transformed mass exposure data from two recent epidemiology studies to surface area measurements for nano-titanium dioxide, calculating that 1 g titanium dioxide had 50 m^2 surface. Calculated concentrations were 0.168 m^2 for packers, the highest exposed job, in U.S. production plants and 0.387 m^2 for surface treaters in European plants (Liao et al., 2008).

Workers in industries using titanium dioxide are also exposed. Levels of titanium dioxide exposure in user industries have not been reported. U.S. user industries include paints and pigment (57%); plastics (26%); paper (13%); cosmetics, catalysts, ceramics, printing inks, roofing granules, glass, and welding fluxes (Robichaud et al., 2009).

Environmental exposures

There is conflicting evidence as to whether nanoparticles of titanium dioxide can pass through the skin (Kiss et al., 2008; Wu et al., 2009). If they can, the presence of titanium dioxide in a large variety of cosmetic powders and creams may be a cause of concern.

Cancer in humans

(*inadequate*)

The previous IARC review Monograph (93, in press) evaluated three retrospective cohort studies of titanium dioxide production workers and one case-control study published through 2006 A 2008 re-evaluation of two previously conducted case-control studies found no association with lung cancer (Ramanakumar et al., 2008).

No studies have been conducted of workers using titanium dioxide as a pigment in the manufacture of cosmetics, paints, varnishes, lacquers, paper, plastics, ceramics, rubber, or printing ink.

Cancer in experimental animals

(*sufficient*, Monograph 93, 2006)

Elevated lung cancer was observed in two chronic inhalation studies in rats exposed to fine (Lee et al., 1985) or ultrafine (Heinrich et al., 1995) TiO_2.

Lee et al. ***(1985)***

Rats (female CD Sprague-Dawley-derived) were exposed by whole body inhalation to fine, rutile TiO_2 (aerodynamic mass median diameter of 1.5-1.7 μm) for 6 hr/day, 5 days/week, for

up to two years, to 0, 10, 50, or 250 mg/m^3 (84% respirable; <13 μm MMAD); 80 rats were exposed for two years, and all surviving rats were killed at the end of exposure. No increase in lung tumors was observed at 10 or 50 mg/m^3. At 250 mg/m^3, bronchioalveolar adenomas were observed in 12/77 male rats and 13/74 female rats. In addition, squamous cell carcinomas were reported in 1 male and 13 females at 250 mg/m^3. These squamous cell carcinomas were later reclassified as proliferative keratin cysts (Carlton 1994), or as a range of responses from pulmonary keratinizing cysts through pulmonary keratinizing eptheliomas to frank pulmonary squamous carcinomas (Boorman et al., 1996). A recent reanalysis of the 16 tumors originally classified as cystic keratinizing squamous cell carcinomas in Lee et al. (1985) had a similar interpretation: two were re-classified as squamous metaplasia, one as a poorly keratinizing squamous cell carcinoma, and 13 as nonneoplastic pulmonary keratin cysts (Warheit and Frame 2006).

Heinrich et al. (1995)

Female Wistar rats were exposed to ultrafine TiO_2 (80% anatase/20% rutile; 15-40 nm primary particle size; 0.8 μm MMAD; 48 ($\pm$ 2.0) m^2/g specific surface area) at an average concentration of 10 mg/m^3, 18 h/d, 5d/wk, for up to 24 months (actual concentrations were 7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months, and 9.4 mg/m^3 for 16 months). After the 2-year exposure, the rats were kept in clean air for an additional 6 months. After 24 months of exposure, four of the nine rats examined had developed tumors (including a total of 2 squamous cell carcinomas, 1 adenocarcinoma, and 2 benign squamous cell tumors). At 30 months (6 months after the end of exposure), a statistically significant increase in adenocarcinomas was observed (13 adenocarcinomas, in addition to 3 squamous cell carcinomas and 4 adenomas, in 100 rats). In addition, 20 rats had benign keratinizing cystic squamous-cell tumors. Only 1 adenocarcinoma, and no other lung tumors, was observed in 217 nonexposed control rats.

NMRI mice were also exposed to ultrafine TiO_2 in Heinrich et al. (1995). The lifespan of NMRI mice was significantly decreased by inhaling approximately 10 mg/m^3 ultrafine TiO_2, 18 hr/day for 13.5 months (Heinrich et al., 1995). This exposure did not produce an elevated tumor response in the NMRI mice, but the 30% lung tumor prevalence in controls may have decreased the sensitivity for detecting carcinogenic effects in this assay.

Recent studies: No subsequent carcinogenicity studies of TiO_2 in animals were found in the literature since those evaluated in Monograph 93 (in press).

Mechanisms of carcinogenicity

Titanium dioxide is poorly soluble low toxicity (PSLT) particles, which can elicit overloading of lung clearance, chronic inflammation, and lung tumors in rats following prolonged exposure at sufficiently high concentrations of particles (Monograph 93 (in press); Baan 2007). Overloading of lung clearance occurs at much lower mass concentrations of ultrafine TiO_2 (10 mg/m^3) than fine TiO_2 (50 or 250 mg/m^3) (Bermudez et al., 2002, 2004). Lung tumors also develop a lower mass concentration of ultrafine TiO_2 (~10 mg/m^3) (Heinrich et al., 1995) compared to fine TiO_2 (250 mg/m^3) (Lee et al., 1985) following chronic inhalation in rats. Particle surface area dose was found to be most predictive of the pulmonary

inflammation and tumor responses in rats when the dose-response relationships are compared for various types and sizes of PSLT including TiO_2 (Driscoll 1995; Dankovic et al., 2007).

Most evidence suggests that TiO_2 and other PSLT-elicited lung tumors develop via a secondary genotoxic mechanism involving chronic inflammation, cell proliferation, and oxidative stress (Schins and Knaapen 2007). Overloading of lung clearance is accompanied by pulmonary inflammation, production of reactive oxygen and nitrogen species, depletion of antioxidants and/or impairment of other defense mechanisms, cell injury, cell proliferation, fibrosis, and as observed in rats, induction of mutations and eventually cancer (Monograph 93 (in press); Baan 2007).

Rats were more sensitive to the adverse effects of inhaling either fine or ultrafine TiO_2 than either mice or hamsters (Bermudez et al., 2002, 2004). Both mice and rats developed overloading of lung clearance (at 50 mg/m^3 of fine TiO_2 and 10 mg/m^3 of ultrafine TiO_2), although rats developed more persistent neutrophilic inflammation, cell proliferation, and fibrotic responses than did mice at 52 weeks after a 13-week inhalation exposure to 250 mg/m^3 fine TiO_2 (Bermudez et al., 2002). Rats were also more sensitive than mice or hamsters to adverse lung effects of inhaled carbon black (Elder et al., 2005). In each of these studies, hamsters had rapid lung clearance and thus low retained dose and response.

Although studies in humans have not shown a direct link between inhaled PSLT and lung cancer, many of the steps in the mechanism observed in rats have also been observed in humans who work in dusty jobs, including increased particle lung retention and pulmonary inflammation in workers exposed to coal dust or crystalline silica (Castranova 2000; Kuempel et al., 2001; Lapp and Castranova 1993); and elevated lung cancer has been observed in some studies of workers exposed to carbon black (Sorahan and Harrington 2007), crystalline silica (Rice et al., 2001; Attfield and Costello 2004), and diesel exhaust particles (Stayner et al., 1998).

An alternative genotoxic mechanism for nanoscale particles may involve direct interaction with DNA (Schins and Knaapen 2007). Nano-TiO_2 particles have been observed inside lung epithelial cells and cell organelles, including the nucleus, of rats 24-hours after a 1-hr inhalation exposure to 0.1 mg/m^3 nanoscale TiO_2 (4 nm primary particle diameter; 22 nm count median diameter; 330 m^2/g specific surface area) (Geiser et al., 2005). Nano-TiO_2 particles were ineffectively cleared by alveolar macrophages and were also observed in all major lung tissue compartments and within capillaries (Geiser et al., 2008).

Recent studies

Several *in vitro* studies have shown that TiO_2 produced reactive oxygen species (ROS) and induced oxidative DNA damage (Gurr et al., 2005; Türkez and Geyikoğlu 2007; Wang et al., 2007). Sayes et al. (2006) reported that nano-anatase produced more ROS and was more cytotoxic than nano-rutile, but only after UV irradiation. Fenoglio et al. (2009) observed that oxygen and carbon-centered free radical generation was associated with the surface area of micro- or nano-sized anatase TiO_2, and that while superoxide production was related to exposure to sunlight, other free radical species were generated in the dark. TiO_2 nanoparticles did not induce DNA breakage (measured by Comet assay) in human lung fibroblast or

bronchial epithelial cell cultures, but did induce a high level of oxidative DNA adduct formation (8-hydroxyl-2-deoxyguanosine or 8-OHdG) (Bhattacharya et al., 2009).

Inflammation in bronchoalveolar lavage (BAL) fluid and in whole blood was examined 24 hours after a single intratracheal instillation (IT) dose of TiO_2 rutile nanorods (1 or 5 mg/kg) in Wistar rats (Nemmar et al., 2008). At both doses, the neutrophilic inflammation in BAL fluid was significantly elevated compared to vehicle controls. The number of monocytes and granulocytes in blood was dose-dependently elevated, while the platelets were significantly reduced at the higher dose, indicating platelet aggregation.

Nanoscale TiO_2 elicited a significantly greater increase in chemokines (associated with pulmonary emphysema and alveolar epithelial cell apoptosis) than did the microscale TiO_2 one week after a single IT dose in a study of adult male ICR mice. Animals were treated by IT administration of a single dose of 0.1 or 0.5 mg per mouse of either nanoscale TiO_2 (rutile, 21 nm average particle size; specific surface area of 50 m^2/g) or microscale TiO_2 (180-250 nm diameter; specific surface area 6.5 m^2/g) (Chen et al., 2006).

Three recent studies compared the pulmonary responses to various types of nanoscale or microscale TiO_2. A similar experimental design was used in each study, including IT dosing of male Crl:CD(SC):IGS BR rats, to a particle dose of either 1 or 5 mg/kg. BAL was performed at 24 hours, 1 week, 1 month, and 3 months after instillation (Warheit et al., 2006a,b; 2007):

In Warheit et al. (2006a), rats were administered IT doses of either 1 or 5 mg/kg of "R-100" or "Pigment A" (two types of hydrophilic TiO_2), carbonyl iron, or Min-U-Sil quartz. Primary average particle sizes were 300 nm, 290 nm, ~1.2 µm, or ~1.5 µm, respectively (Warheit et al., 2006a). Significantly elevated polymorphonuclear leukocytes (PMNs) in BAL fluid were observed for the two types of TiO_2 or carbonyl iron at 24 hours post-exposure, but not at the later time points.

Warheit et al. (2006b) compared nanoscale TiO_2 rods (anatase, 92-233 nm length, 20-35 nm width; 26.5 m^2/g specific surface area), nanoscale TiO_2 dots (anatase, 5.8-6.1 nm spheres; 169 m^2/g specific surface area), and microscale rutile TiO_2 (300 nm primary particle diameter; 6 m^2/g specific surface area). A statistically significant increase in the percentage of PMNs in BAL fluid was seen at the 5 mg/kg dose for all three TiO_2 materials tested (which was higher in the rats administered the nanoscale TiO_2) but returned to control levels at the 1-week time point. There were no statistically significant lung responses (inflammation or histopathology) to either the nanoscale or microscale TiO_2 at either dose (1 or 5 mg/kg) compared to controls at the 1-week to 3-month time points. Because of the low response in rats to either nanoscale or microscale TiO_2 at the doses used in this study, there were insufficient data to compare the dose-response relationships of TiO_2 by particle size.

In Warheit et al. (2007), the lung inflammation, cytotoxic, cell proliferation, and histopathological responses of two types of ultrafine rutile TiO_2, fine rutile TiO_2, and ultrafine 80/20% anatase/rutile TiO_2, and quartz particles were compared. Although the specific surface area of these particles varied from 5.8 to 53 m^2/g, the median particle sizes in the

phosphate buffered saline (PBS) instillation vehicle were similar (2.1-2.7 μm), suggesting that particle agglomeration had occurred reducing the effective surface area. The pulmonary responses (percent PMNs or percent proliferating tracheobronchial epithelial cells) in rats exposed to either type of ultrafine rutile TiO_2 or to fine rutile TiO_2 did not differ significantly from controls at either dose or any time point. The rats exposed to anatase/rutile TiO_2 had significantly greater responses (percent PMNs and percent of proliferating tracheobronchial epithelial cells) at the 5 mg/kg dose than the PBS controls 24-hr and 1-wk after IT, but not at 1 or 3 months. The two ultrafine rutile TiO_2 preparations had been passivated with amorphous silica and alumina coatings to reduce their chemical and photo-reactivity to a low level similar to that of the fine rutile TiO_2, while the ultrafine anatase/rutile TiO_2 was not passivated and was more chemically reactive based on a Vitamin C assay measuring oxidation potential.

Grassian et al. (2007) investigated lung responses in male C57Bl/6 mice exposed to nano-TiO_2 (2-5 nm diameter; 210 m^2/g specific surface area) by whole-body inhalation for either 4 hr (acute) or 4 hr/d for 10 d (subacute). The airborne exposure concentrations were 0.77 or 7.22 mg/m^3 (acute) or 8.88 mg/m^3 (subacute). The TiO_2 primary particle size was 2-5 nm, and the specific surface area was 210 m^2/g. No adverse effects were observed after the 4 hour exposure. Mice in the subacute study were necropsied at the end of the exposure period and at 1, 2, and 3 weeks post-exposure. A "significant but modest" inflammatory response was observed in the mice at 0, 1, or 2 weeks after the subacute exposures, with recovery at the 3rd week post-exposure.

Grassian et al. (2007a) compared the pulmonary toxicity of two sizes of TiO_2 nanoparticles (~5 nm and ~21 nm primary particle diameter, and BET surface area of 219 and 41 m^2/g, respectively) by inhalation and intra-nasal instillation in mice. The crystal structure of these two particles also varied (5 nm was anatase and 21 nm was anatase/rutile, and TEM images showed that the 5 nm particles formed closely compacted agglomerates whereas the 21 nm particles were more loosely agglomerated. The aerosol sizes were 120-123 GM (GSD 1.56) and 139-153 (GSD 1.4) nm. Instillation doses for the 5 nm TiO_2 particles were 5, 20, and 30 μg/mouse, and for the 21 nm TiO_2 particles were 25, 100, and 150 μg/mouse. Inhalation exposures to each particle size were approximately 0.8 and 7 mg/m^3 for 4 hours. Lung responses were examined by BAL and histopathology at 24 hours post-exposure (also at 4 hr for inhalation). By instillation, the pulmonary neutrophilic inflammation was somewhat greater on a mass basis for the 5 nm particles, but was less by BET-surface area. By inhalation, inflammation was similar at 24 hours post-exposure for equivalent mass concentrations of the 5 and 21 nm particles. These findings reinforce previous studies showing that the physical and chemical properties of particles influence toxicity, and that the contribution of a given factor cannot be determined unless the other factors are controlled. In this study, the agglomeration state varied between the two particle samples such that the measured particle sizes and surface areas may not have been a good measure of those to which the lung cells were exposed.

Sager et al. (2008) and Sager and Castranova (2009) investigated the role of particle surface area on pulmonary inflammation in male Fischer 344 rats treated with either fine or ultrafine TiO_2 by intratracheal instillation. The mass doses of ultrafine TiO_2 (primary particle size 21

nm; specific surface area 48 m^2/g; 0.26, 0.52, and 1.04 mg/rat) and fine TiO_2 (primary particle size 1,000 nm; specific surface area 2.3 m^2/g; 5.35, 10.7, and 21.4 mg/rat) corresponded to an equivalent surface area dose (0.031, 0.062, 0.12 cm^2 particles / cm^2 alveolar epithelial cell surface of the lungs). At each post-exposure time point (1, 7, or 42 days) and mass dose, the ultrafine TiO_2 was least 41 times more potent than fine TiO_2 in eliciting pulmonary inflammation (as measured by neutrophil cell count in BAL fluid, relative to control (saline only) rats). When dose was expressed as particle surface area (measured by BET gas absorption), the ultrafine TiO_2 as less than two times more potent than fine TiO_2, and this difference was not statistically significant. This study also showed that ultrafine TiO_2 translocated from the lungs to the lung-associated lymph nodes to a greater extent than fine TiO_2.

Overall, these recent studies are consistent with roles for both particle surface area and particle surface reactivity in the pulmonary responses to TiO_2 and other inhaled particles. Particle surface area and reactivity have been shown to influence the pulmonary inflammation response to various types of inhaled particles including PSLT and crystalline silica (Duffin et al., 2007; Dankovic et al., 2007).

In study in mice of TiO_2 (by oral gavage), Wang et al. (2007) observed that nanoscale and fine TiO_2 translocated to the liver, kidney spleen, and lungs, and that liver damage occurred in mice administered nanoscale TiO_2 (at the high dose of 5 g/kg). This study suggests the need to investigate possible adverse effects or carcinogenicity in other organs and by other routes of exposure, especially to nanoscale TiO_2.

Biomarkers of exposure

No biomarkers of exposure were identified.

Biomarkers of effect

A number of recent studies have identified markers of inflammation, including granulocyte macrophage colony stimulating factor (GM-CSF) mRNA expression and secretion in a human bronchial epithelial cell line (16HBE14o-) (Hussain et al., 2009) and interleukin (IL)-8 production in a human alveolar epithelial type II cell line (A549), which is a pro-inflammatory cytokine also produced *in vivo* (Duffin et al., 2007). MicroRNA signatures may provide a marker linking inflammation, immune response, and cancer (Hussain and Harris 2007), although this has not been examined specifically in conjunction with particle-elicited inflammation. Markers of oxidative DNA damage (e.g., 8-OHdG) may provide an indication of the particle-elicited oxidative stress. However, none of these markers is specific to TiO_2, nor was it determined how feasible these biomarkers would be for testing in exposed populations.

Research needs and recommendations

Possible cohort for future epidemiologic studies

Epidemiological studies with well-characterized exposures and adequate follow-up are needed, especially for workers producing or using nanoscale TiO_2. Exposure data should include information on particle size, crystal structure, and surface properties. A possible cohort for

epidemiologic studies would include workers in industries using TiO_2, particularly the ultrafine (nanoscale) TiO_2 now used extensively in the cosmetics industry. Workers handling or mixing TiO_2 powders with other ingredients would probably be at the greatest exposure. NIOSH is currently conducting exposure studies of TiO_2 users and identifying possible cohorts.

Toxicology studies:

Experimental studies are needed to elucidate the biological mechanisms between particle-induced inflammation and lung cancer. A study examining the relationship between TiO_2 exposure in workers and validated markers of oxidative stress, with quantitative comparison in rodent studies, could provide data on interpretation of the animal studies for predicting lung cancer risk in humans.

Studies are needed that provide mechanistic linkages between the biological responses observed in short-term or subchronic studies and the adverse health effects observed with chronic exposure. The same species, strain, and gender should be used. For example, different mouse strains were used in Heinrich et al. (1995) and in Bermudez et al. (2004), making it difficult to determine whether the subchronic inflammation results are relevant to the chronic lung responses observed in another mouse strain.

The observation of inhaled discrete nanoscale TiO_2 particles inside rat alveolar epithelial cell organelles including the nucleus (Geiser et al., 2005) suggests that possible direct genotoxic mechanisms for lung cancer should be examined.

Given the increasing applications of nano-TiO_2 in consumer products (e.g., food or food packaging and skin care products), there is a need to develop better techniques to detect TiO_2 in tissues and to examine possible carcinogenicity of nano-TiO_2 by other routes of exposure (oral, dermal). A chronic feeding study of nanoscale TiO_2 may be appropriate.

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Welding fumes

by Kirsti Husgafvel-Pursiainen PhD and Jack Siemiatycki PhD

Citation for most recent IARC review

IARC Monographs 49, 1990

Current evaluation

Conclusion from the previous Monograph
Current classification Group 2B

> There is *limited evidence* in humans for the carcinogenicity of welding fumes and gases. There is *inadequate evidence* in experimental animals for the carcinogenicity of welding fumes.

Exposure and biomonitoring

The number of workers worldwide whose work involves some welding is estimated to be about three million. Most welding is performed using electric arc processes - manual metal arc, metal inert gas and tungsten inert gas welding – and most welding is on mild steel.

Welders are exposed to a range of fumes and gases (evaporated metal, metal oxides, hydrocarbons, nanoparticles, ozone, oxides of nitrogen (NOx)) depending on the electrodes, filler wire and flux materials used in the process, but also physical exposures such as electric and magnetic fields (EMF) and ultraviolet (UV) radiation. Fume particles contain a wide variety of oxides and salts of metals and other compounds, which are produced mainly from electrodes, filler wire and flux materials. Fumes from the welding of stainless-steel and other alloys contain nickel compounds and chromium[VI] and [III]. Ozone is formed during most electric arc welding, and exposures can be high in comparison to the exposure limit, particularly during metal inert gas welding of aluminium. Oxides of nitrogen are found during manual metal arc welding and particularly during gas welding. Welders who weld painted mild steel can also be exposed to a range of organic compounds produced by pyrolysis. Welders, especially in shipyards, may also be exposed to asbestos dust. Physical exposures such as electric and magnetic fields (EMF) and ultraviolet (UV) radiation are also common.

Biomarkers of exposure or effect

There are several biomarkers of exposure and effect for various exposures in welding: saliva Mn, serum Mn, urine Mn, Centromere-positive micronuclei in periphery blood lymphocytes, DNA protein-crosslinks in peripheral white blood cells, oxidative stress as urinary 8OHdG, and long-term oxidative damage measured as erythrocytic superoxide dismutase (SOD) activity (measure of systemic oxidative stress) and serum malondialdehyde (MDA) (product of lipid peroxidation and reflect tissue injuries).

Occupational exposure

Different welding environments may present different and complex profiles of exposures. In a Swedish study to characterize welding fume aerosol (Isaxon et al., 2008), nanoparticles in mild steel metal active gas welding showed a mass median diameter (MMMD) of 200-300 nm.

Particle elemental composition was mainly iron and manganese. Ni and Cr exposures were very low in the vicinity of mild steel welders, but much higher in the background in the workshop where there presumably was some SS welding.

In Canadian welders, personal exposures to manganese ranged from 0.01–4.93 mg/m^3 and to iron ranged from 0.04–16.29 mg/m^3 in eight Canadian welding companies in Manitoba. Types of welding identified were mostly (90%) MIG mild steel, MIG stainless steel, and TIG aluminum. Carbon monoxide levels were less than 5.0 ppm (at source) and ozone levels varied from 0.4–0.6 ppm (at source). Ventilation was poor in many companies, and only 7 percent of the welders wore respiratory protection. These Canadian Mn and Fe air concentrations are 300-2600 times *lower* than what was measured in Russian welders: 97 mg/m3 (range 3–4620 mg/m^3; n = 188) and 894 mg/m3 (range 106–20 300 mg/m^3; n = 188), respectively (Ellingsen et al., 2006). No substantial difference was observed in the air Mn concentrations when welding mild steel as compared to welding stainless steel. Three welding methods were included in this study: Shielded-metal arc welding (manual welding), gas metal-arc welding shields (semi-automatic), and fluxed-core arc welding (automated). Welding in a confined space, as expected, created higher ambient Mn breathing zone levels of 1.5 - 0.7 mg/m^3, and lowest in the workshop 0.2 - 0.05 mg/m3 (Lu et al., 2005).

Biological monitoring was also performed in this Russian study, where Mn in whole blood was 25% higher in welders compared to controls. A significant difference was not found comparing urinary Mn. A significant correlation (0.31, p= 0.01) was found between blood Mn and Mn in the workroom air that was collected the day before blood sampling. Former welders had blood Mn concentration higher than controls of similar age (8.7 mg/l vs. 7.0 mg/l), while their urinary concentrations of cobalt, iron and Mn were all statistically significantly lower.

Cancer in humans

(*limited evidence*, vol. 49, 1990)
There has been considerable evidence over the past 3 decades regarding cancer risks in relation to welding activities. Some of the research has focused on the job title of "welders" or the exposure of "welding fumes" as the only descriptor of exposure, while some of the research has focused on more finely discriminated subsets defined by such characteristics as the industry in which the welding occurred (e.g., shipyards), the material welded (e.g., stainless steel, mild steel), or specific chemicals released by the welding activities (e.g., chromium VI, nickel compounds). In 1990 an IARC Monograph Working Group considered that there was limited epidemiologic evidence carcinogenicity of welding fumes, where the main concern was with lung cancer as the outcome. Since then, a large number of additional studies have been published. Some of the accumulated evidence has been used by IARC Monograph Working Groups in assessing the evidence regarding some agents which can be found in welding fumes, notably chromium VI and nickel compounds. But there has not been a formal reevaluation of welding since 1990. The only exception to this statement is a recent evaluation of overexposure of the eyes to UVR, common among electric arc welders. Several case-control studies reported excess risks of ocular melanoma in welders (Guenel et al., 2001; Lutz et al., 2005, Shah et al., 2005). This association may be due to the presence in some

welding environments of fumes of thorium-232, which is used in tungsten welding rods (NCRP, 1988 ; Nuclear Regulatory commission, 2001).

While there has not been such a formal reevaluation recently regarding risks of lung cancer, a widespread consensus seems to have formed to the effect that some welding environments, notably in stainless steel welding, do carry risks of lung cancer. This widespread consensus is in part based on empirical evidence regarding risks among stainless steel welders and in part on the fact that stainless steel welding entails moderately high exposure to nickel and chromium VI compounds, which are recognized lung carcinogens. In this line of reasoning, the presence of nickel and chromium VI compounds would explain the excess lung cancer risk among stainless-steel welders. The corollary is that welding without the presence of nickel and chromium VI compounds, namely mild-steel welding, should not carry risk. But it appears that this line of reasoning in not supported by the accumulated body of epidemiologic evidence.

As reviewed by Ambroise et al. (2006), there have been around 60 studies published that are informative about lung cancer risks in welders. While there remained some uncertainty about possible confounding by smoking and by asbestos, and some possible publication bias, the overwhelming evidence is that there has been an excess risk of lung cancer among welders as a whole in the order of 20%-40%. Further, there was no evidence from the 11 studies of stainless steel welders, the 8 studies of mild steel welders, the 16 studies of shipyard welders, and the 24 studies of unspecified welders that the risks differed by type of welder. Indeed, the meta-estimates of relative risk were very similar among these four categories of welders. In the absence of reliable data on levels of average levels of exposure in these groups it is difficult to conclude that the risks were actually similar, but it can be claimed that there is no evidence that the risks were different. This finding was not only true overall, but also in the largest and arguably best conducted study, the IARC-coordinated multi-center European cohort study (Simonato et al., 1991), and in two large and recently published Danish (Sorensen et al., 2007) and Finnish (Siew et al., 2008) studies.

Cancer in experimental animals

(inadequate evidence, vol 49, 1990)

Recently, lung tumorigenicity of welding fumes was investigated in lung tumour susceptible (A/J) strain of mice (Zeidler-Erdely et al., 2008). Male mice were exposed by pharyngeal aspiration four times (once every 3 days) to 85 μg of gas metal arc-mild steel (GMA-MS), GMA-SS, or manual metal arc-SS (MMA-SS) fume. At 48 weeks post-exposure, GMA-SS caused the greatest increase in tumour multiplicity and incidence, but did not differ from sham exposure. Tumour incidence in the GMA-SS group versus sham control was close to significance at 78 weeks post exposure ($p = 0.057$) (Zeidler-Erdely et al., 2008). Histopathological analysis of the lungs of these mice showed the GMA-SS group having an increase in preneoplasia/tumour multiplicity and incidence compared to the GMA-MS and sham groups at 48 weeks. The increase in incidence in the GMA-SS exposed mice was significant compared to the GMA-MS group but not to the sham-exposed animals, and the difference in incidence between the GMA-SS and MMA-SS groups was of border-line significance ($p = 0.06$). At 78 weeks post-exposure, no statistically significant differences

were observed between the groups. The study concluded that the data are supportive but not conclusive for tumorigenicity of GMA-SS welding fumes in the lung tumor susceptible A/J mouse. The *in vivo* data obtained in the study did not, however, suggest tumorigenicity of MMA-SS or GMA-MS fumes (Zeidler-Erdely et al., 2008).

Mechanisms of carcinogenicity

Genotoxicity in experimental systems in vitro and in vivo

Oxidative DNA damage to lung tissue assessed by immunohistochemical staining of 8-OH-dG was shown in rats exposed to MMA-SS welding fumes at low-dose (~ 65 mg/m^3) and high-dose (~ 116 mg/m^3) concentrations in whole body exposure. Higher 8-OH-dG levels were detected after high-dose exposure (Yu et al., 2004). Genotoxicity in individual lung cells determined by alkaline Comet assay was increased in a dose-dependent manner. It was concluded that a variety of major welding fume components were associated with ROS production, including Fe, Mn, Cr, Cr^{VI}, gaseous ozone, NO_2, nitrous fumes and SO_2 (Yu et al., 2004).

MMA-SS welding fumes induced DNA damage, likely via generation of reactive oxygen species, in an *in vitro* DNA strand breakage assay (Antonini et al., 2005). The study concluded, as many other studies by the same research group, that the DNA damage was attributable to the soluble components present in MMA-SS welding fumes, suggested to be predominantly chromium well known for its capacity to generate radical oxygen species and to induce diverse forms of genotoxicity (Antonini et al., 2003b; Antonini et al., 2005; Salnikow and Zhitkovich 2008).

Toxic effects in lungs in vivo and in lung cells in vitro

In all, the *in vivo* studies suggest that different welding fumes cause varied responses in rat lungs *in vivo*, and the toxic effects typically correlate with the metal composition of the fumes and their ability to produce free radicals. In many studies both soluble and insoluble fractions of the stainless steel welding fumes were required to produce most types of effects, indicating that the responses are not dependent exclusively on the soluble metals (Antonini 2003, Antonini et al., 2003a, b; Antonini et al., 2004).

In human A549 lung cells *in vitro*, three types of welding fumes (NIMROD 182, NIMROD c276, COBSTEL 6), all high in Cr content, and their soluble fractions induced cytotoxicity, generation of ROS, and significant increase in pro-inflammatory cytokine expression (McNeilly et al., 2004). The two nickel-based fumes were found to significantly increase intracellular ROS production, but not the cobalt-based fume. These pro-inflammatory responses were proposed to be attributable to soluble transition metal components and ultrafine particulate composition (mean diameter <0.1 um) of welding fumes, likely effective via oxidative stress mechanisms (McNeilly et al., 2004).

Histological analysis of nasal respiratory mucosa in rats exposed to MMA-SS welding fumes indicated various types of histopathological changes, suggesting diminished protection and defence mechanisms in nasal respiratory (Jeong et al., 2006).

Genotoxicity in humans

The latest IARC evaluation recognised only one study out of three indicating increased chromosome aberrations and sister chromatid exchanges in stainless steel welders (IARC 1990). Since then, a number of studies have investigated a variety of genotoxicity endpoints in subjects exposed to welding fumes, as summarised briefly in the following.

Urinary 8-OH-dG, DNA-protein crosslinks, and single strand DNA breaks in welders

Statistically significant pre- to post-shift changes in urinary 8-OH-dG of boilermakers have been found (Nuernberg et al., 2008). Significant increases in DNA-protein crosslinks have been observed in welders in several studies (Costa et al., 1993; Zhitkovich et al., 1998). Levels of Cr and Ni in blood of MMA welders in India correlated positively with the DNA damage using the Comet assay. Smoking did not to have a significant effect on DNA damage in these welders (Danadevi et al., 2004). A statistically significant association between polymorphism for *XRCC*, one of the DNA repair genes, and DNA damage (Iarmarcovai et al., 2005) have also been found. These two studies agree with earlier studies showing significantly increased DNA single strand breaks in welders (Werfel et al., 1998).

Cytogenetic effects in welders

Some cytogenetic studies have shown significant increases in chromosomal aberrations (Elias et al., 1989, Knudsen et al., 1992, Jelmert et al., 1994), while others did not (Jelmert et. al., 1995, Halasova et al., 2008). A recent study reported some suggestive influence of *XRCC1* polymorphism on the total chromosome aberration frequency (Halasova et al., 2008).

SCE frequency studies also show inconsistencies (Werfel et al., 1998, Knudsen et al., 1992).

Micronuclei from welders

A significantly higher frequency of micronuclei in peripheral blood lymphocytes (binucleated cell assay) and higher mean levels of both centromere-positive and centromere-negative micronuclei was observed in welders (n=27) who worked without protective device compared to controls (n=30).The rate of micronucleated cells did not correlate with the duration of exposure (Iarmarcovai et al., 2005, Iarmarcovai et al., 2006). Polymorphisms, smoking, or drinking did not affect the outcome.

Induction of micronuclei in buccal epithelial cells were found, and the frequency was associated with increasing duration of welding work as well as blood chromium levels; however increase in micronuclei showed age dependence (Danadevi et al., 2004).

Research needs and recommendations

Research needs based on current epidemiological data

Thus it seems that the reasons for excess risk among welders, whether stainless steel or mild steel, remains unknown. It may still be the case that excess risks are due to nickel and

chromium VI compounds among stainless steel welders and other factors among mild steel welders, but this seems unlikely. The most parsimonious explanation is that there is an as-yet unexplained common reason for excess lung cancer risks that applies to all types of welders. Siew et al. (2008) have proposed that iron fumes may play such a role, and their Finnish data appear to support this hypothesis, though not conclusively. This hypothesis would also imply that excess lung cancer risks among welders are not unique to welders, but rather may be shared among many types of metal working occupations.

Further evidence needs to be systematically assembled to compare and synthesize lung cancer risks among metal working occupations, including welders. Further, to the extent possible, existing cohorts of welders should be re-examined to try to describe risks according to various dimensions of exposure, including the type of welding process, the type of metal being welded, the types of rods and fluxes used, and other characteristics of the welding environment such as abrasives, cleaners and degreasers used. It would be justified to initiate new studies (industry-based cohorts or community-based case-control) if information on these various dimensions could reliably be collected. If biomarkers of exposure to Mn or Fe could be integrated into such studies, that could be helpful. But this is hampered by the difficulty of obtaining biospecimens in retrospective cohort studies, and the questionable historic relevance of biospecimens collected at time of disease diagnosis in case-control studies.

Research needs based on current experimental and mechanistic data

Welding fumes are complex particulate mixtures containing multiple hazardous metals, metal oxides and gases; also radiation hazards and heat are involved in welding. Given such huge variation, just a fraction of the types of fumes and processes of welding has been studied. Research needs include experimental animal studies using inhalation as the route of exposure. Employment of transgenic mouse models, e.g. the heterozygous p53 deficient ($p53^{+/-}$) mouse assay, may offer one option for future studies. It would also be useful if experimental carcinogenicity assays were carried out on different components of welding fumes.

Studies to characterize the generation of radical oxygen species and oxidative DNA damage in human and animals for each of the numerous types and processes of welding would be informative. Some of the genotoxicity studies suggest somewhat differential effects of welding fumes containing Cr or Ni as the predominant metal component; such questions may deserve more attention.

Large proportion of ultrafine or nanosized welding fume particles (mean diameter of ~ 0.1 μm and range of 0.01 - < 1.0 μm) (McNeilly et al., 2004; Ayers et al., 2008), together with the high content of metals and metal oxides, further points to a efficient capacity of welding fumes to generate oxidative stress, both direct generation of reactive oxygen species and indirectly through inflammatory responses in the lungs. Inhaled ultrafine welding particles easily reach the lower respiratory tract, including bronchioles, alveolar ducts, alveolar sacs, and alveoli (Zeidler-Erdely et al., 2008) and may also be translocated in a facilitated way elsewhere in the body. Studies on welding fumes in this area of particle research may aid in understanding cellular and molecular mechanisms involved in welding-related lung carcinogenesis.

Finally, research using the current powerful technologies to study epigenetic mechanisms, such as DNA methylation and histone modification, gene expression pathways, and functional level changes following welding fume exposure may provide further insight into molecular mechanisms of welding fume carcinogenesis.

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Refractory ceramic fibres (RCF)

by Hartwig Muhle PhD and Kyle Steenland PhD

Citation for most recent IARC review

IARC Monographs 81, 2002

Current evaluation

Conclusion from the previous Monograph:
Refractory ceramic fibres are possibly carcinogenic to humans (Group 2 B).

Exposure and biomonitoring

RCFs are produced by melting a combination of predominantly alumina (Al_2O_3) and silica (SiO_2).

Exposure intensity in the small U.S. cohort (n=942), the only existing cohort with epidemiologic data, was relatively low (<10 fb/ml maximum in 1950s, dropping to <0.6 fb/ml by the late 1980s) (LeMasters et al., 2003), especially in comparison for example to historical asbestos exposures which resulted in excess lung cancer (intensity 10-100 fb/ml) (Hodgson and Darnton, 2000, Berman and Crump, 2008). Cumulative exposures in this cohort were low (average approximately 40 fb/ml months, by the end of 2000).

NIOSH has estimated that there were about 30,000 U.S. workers exposed to ceramic fibers in the 1980s.

Maxim et al. (2008) and Rice et al. (2004) have summarized exposures in the United States since the late 1980s. The current recommended level (ACGIH) is 0.2 fb/ml. Finnish data indicates most current levels in production are low (0.01-0.29)(Linnainmaa et al., 2007), although peaks occurred in maintenance work (0.01-14.2 fb.ml. Ontario construction workers in the early 2000s generally had low RCF exposures (Verma et al., 2004) although some exceeded the ACGIH recommended level.

Cancer in humans

(*inadequate***,** Vol 81, 2002)

There are no new data on cancer in humans since the 2002 Monograph 81, although it appears that the Working Group at that time had a preliminary draft of a manuscript which published a year later (LeMasters et al., 2003). Results in the published manuscript differ only slightly from the Monograph results; lung cancer shows no excess (SMR 0.78) but the cohort (n=942) and the numbers of lung cancer deaths (n=9) are small, hence few firm conclusions can be drawn. A review of all death certificates and medical records for 35% of the deceased found no mention of mesotheliomas. There was a significant (at the $p<0.05$ level) excess of bladder cancer death based on only 3 deaths (SMR=5.0). This may have been a chance finding given the lack of a priori evidence. This cohort also shows an exposure-related excess of pleural plaques, with adjustment for asbestos exposure (asbestos exposure was apparently common, often far in the past, but prevalence data are not presented) (Lentz et al., 2003). ORs for plaque among 652 current and former workers by quartile of cumulative fiber-months/cm^3 were 1.0, 1.0, 7.7 (0.9-67), 22 (2.9-180), by quartile of exposure (0-2, 3-16, 16-51, >51 fiber-months/cm^3) (observed plaques 0,1, 6, 14 by quartile). Results using estimated cumulative dose to the lung were similar. Plaques are biomarkers of fiber (usually asbestos) exposure, but here may reflect RCF exposure. Their presence in this cohort may suggest increased subsequent risk of lung cancer of mesothelioma. There was also a dose-related increased in damage to lung function, for either FEV or FVC (Lockey et al., 1998)

There is a small European convenience cohort of ceramic fiber production workers (n=774), but there are no cancer data on this cohort. There was some evidence of lung function damage in relation to increased ceramic fiber exposure (Cowie et al., 2001). There was a relation between pleural plaques and time since last exposure to ceramic fibers.

Cancer in experimental animals

(*sufficient***,** Vol 81, 2002)

No new carcinogenicity assays have been performed since the last evaluation since 2002.

Mechanisms of carcinogenicity

Respirability (the fraction of inhaled fibres reaching the alveolar region) is an important aspect of fiber pathogenicity (ILSI Working Group, 2005). Surface charge and hydrophilicity, as well as adsorbed finishes and other physical and chemical factors, determine whether fibres can be easily dispersed or will agglomerate into larger, non respirable masses.

For respirable fibres tested in rodent bioassays, the dose, dimensions, durability in the lung, and in some cases surface reactivity of the fibres have been identified as critical parameters

related to adverse health effects. Fiber length is hypothesized to be a major determinant of pathogenicity: fibers that are not efficiently cleared or altered by physicochemical processes (e.g. breaking, or leaching) are termed biopersistent. Fibres that are too long to be completely phagocytized by macrophages are cleared less efficiently if the fiber type shows a high biopersistence. Refractory ceramic fibres have the potential to interact with target cells in the lungs or to be translocated to the interstitium or the pleura where they may cause disease. Chronic inhalation assays used in man-made vitreous fibres in rodents have correlated fiber length and biopersistence with persistent inflammation, fibrosis, lung cancer, and malignant mesothelioma (ILSI Working Group, 2005).

Direct genotoxicity

Mineral fibres may directly induce genotoxicity by catalyzing generation of reactive oxygen species (ROS) resulting in oxidized DNA bases and DNA strand breaks that can produce gene mutations if not adequately repaired (reviewed in Institute of Medicine, 2006). In addition to direct clastogenic and aneuploidogenic activities that may be induced to target cells in lungs, persistent inflammation and macrophage activation can secondarily generate additional ROS and reactive nitrogen species (RNS) that can indirectly induce genotoxicity in addition to intracellular signaling pathways, stimulation of cell proliferation. There are indications that RCFs acting by a similar mechanism. Somatic gene alterations were induced by refractory ceramic fibres in mesothelial cells (Andujar et al., 2007).

Combination effects

Granular breakdown products of refractory ceramic fibres which are produced at the preparation of a rat respirable fraction of RCF1 show a higher deposition and retention in lungs of rats compared to fibres. The granular particles may partly act by the same adverse effects in lungs like chronic inflammation and induction of fibrosis (Davis, 1996; Bellmann et al., 2001). A mixed fibrous and non-fibrous dust exposure may have led to combination effects in the chronic rat inhalation experiments published by Mast et al., 1995.

Research needs and recommendations:

Combination effects of RCF and granular, low biosoluble particles should be investigated. The presence of granular dust retained in lungs could significantly aggravate effects of inhaled fibres (Davis, 1996)

The impact of fiber length on carcinogenicity should be investigated. Fibres longer than 20 µm are supposed to be more carcinogenic than fibres in the range between 5 and 10 µm.

The validity of dose response data in rats after inhalational exposure is potentially questionable as there are indications that the sensitivity of this assay is relatively low. This can be concluded from inhalation experiments with asbestos in rats compared to results in the asbestos epidemiology (Muhle and Pott, 2000, Wardenbach et al., 2005). More sensitive models for investigating carcinogenicity of man-made fibres should be developed

Further follow-up of the United States cohort is recommended, and mortality follow-up is currently planned. Incidence follow-up would be useful. However it is unlikely that this

small cohort will yield important results until many more years of follow-up. Mortality to date is 13% for this relatively young cohort. Follow up for cancer mortality or incidence in the European cohort would also be useful.

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Diesel Exhaust

by Joellen Lewtas PhD and Debra T. Silverman ScD

Citation for most recent IARC review

IARC Monographs 46, 1989

Current evaluation

Conclusion from the previous Monograph:
DE is *probably carcinogenic to humans (Group 2A)* because of limited evidence of carcinogenicity in humans coupled with sufficient evidence of the carcinogenicity of whole engine exhaust in experimental animals.

Exposure and biomonitoring

Environmental exposure to DE is ubiquitous in urban areas, with substantial DE exposure to those who commute on highways for years. Occupational exposure to DE is widespread, affecting 1.4 million workers in the United States (Steenland et al., 1996) and 3 million workers in the European Union (Kauppinen et al., 2000).

Occupational exposure

Because of the ubiquitous nature of environmental exposure to DE, it is difficult to measure environmental DE exposure for risk estimation in epidemiologic studies. In contrast, DE exposure is potentially quantifiable among the some of the following DE-exposed occupational groups: miners, professional drivers (truck drivers, bus drivers, taxicab drivers), railroad workers, vehicle mechanics, heavy equipment operators, dockworkers, tunnel workers, firefighters, farmers and shipping engineers. Since the last Monograph, industrial hygiene (IH) surveys of DE exposure have been conducted in truck drivers and underground miners.

For truck drivers, two IH surveys have been carried out. In the late 1980s, NIOSH (Zaebst et al., 1991; Steenland et al., 1992) measured elemental carbon, the primary surrogate for DE, in the cabs of long haul truck drivers and pick-up & delivery (P&D) drivers. The investigators reported the following elemental carbon levels: 5.1 μ/m^3 for in-cab long haul truckers; 5.4 μ/m^3 for in-cab local drivers; 3.4 μ/m^3 for roadway background; and 1.4 μ/m^3 for residential background in urban areas. In 2001-05, a second IH survey was conducted to determine elemental carbon levels in the trucking industry. Davis et al. (2007) reported lower levels of EC exposure for in-cab long haul truckers (1.1 μ/m^3) and in-cab P&D drivers (1.2 μ/m^3) than those reported by Zaebst et al. (1991). They attribute the recent lower levels to a downward trend in ambient particulate levels and tighter regulations on fuel composition and emissions over time. However, missing data on a number of factors, including trucker driving patterns, are needed in order to build a comprehensive model of driver exposure for epidemiologic analysis. Thus, results to date of both these IH surveys have not provided the data needed to estimate quantitative DE exposure among truck drivers in epidemiologic studies.

For underground miners, two IH surveys have been completed. In 1992-94, NIOSH and NCI conducted an IH survey of a New Mexico potash mine (Stanevich et al., 1997) to determine the feasibility of conducting retrospective exposure assessment for DE in a cohort and nested case-control study of lung cancer in DE-exposed underground nonmetal miners. The survey results indicated that DE exposure is higher in the mining industry than in other industries. Underground worker average EC levels ranged from 53-345 μ/m^3 and surface worker EC levels ranged from 12-31 μ/m^3. IH surveys in the six other US nonmetal mines in the epidemiologic study have been completed and results are expected to be published soon. In 1992, an industrial hygiene survey of German potash miners in underground jobs was conducted (Neumeyer-Gromen et al., 2009). Total carbon (TC) was used as the surrogate for DE because more data were available for TC than for EC. (Typically, EC is roughly 40 - 60% of TC.) The reported average TC exposure was 0.120-0.244 mg/m^3, which is similar to underground levels reported for the New Mexico potash miners. The German investigators assumed that the 1992 TC estimates were representative of DE exposure in the period from 1970 (when diesel equipment began to be introduced in the study mines) through 1992 because diesel technology had not changed during the 20-year timeframe. Thus, factors that affect level of DE, such as changes in number of pieces of equipment in operation, maintenance of diesel equipment, ventilation, and fuel composition, were not taken into account. In contrast, the retrospective exposure assessment in the U.S. study has included an intensive effort to incorporate all factors affecting DE exposure over time in the study mines.

In addition to IH surveys in truck drivers and miners, retrospective assessments of DE exposure have been conducted for a number of other occupational groups, including railroad workers (Laden et al., 2006), Swedish bus garage workers (Gustavsson et al., 1990) and Swedish dockworkers (Emmelin et al., 1993), since the last Monograph. However, these exposure assessments were based on virtually no measurement data, and thus, it is difficult to compare DE exposure levels in these occupational groups to levels reported for truck drivers and miners.

Cancer in humans

(*limited,* Vol 46, 1989)

Lung cancer

Much relevant research on the relation between DE exposure and risk of dying from lung cancer has been published since the last Monograph. Two meta-analyses have estimated the summary risk to range from 1.33 (95%CI = 1.24-1.44)(Bhatia et al., 1998) to 1.47 (95%CI=1.29-1.67)(Lipsett and Campleman, 1999). Although each meta-analysis was based on about 30 studies, most of the studies inferred DE exposure based on job title rather than from data on individual exposure, which may have led to misclassification of exposure and estimates of risk biased towards the null.

A small number of studies in the past 20 years have included a retrospective assessment of DE exposure. In the German study of potash miners (Neumeyer-Gromen et al., 2009), 5,862 potash miners were followed from 1970 to 2001, yielding 61 lung cancer deaths. A non-significant, positive trend with increasing cumulative TC exposure was observed after adjustment for age and smoking. Relative risks (RR) were 1.13(0.46-2.75), 2.47(1.02-6.02), 1.50(0.56-4.04) and 2.28(0.87-5.97) for cumulative TC up to 2.04, 2.73, 3.90 and >3.90 (mg/m^3)*years, respectively. This study was based on only 61 lung cancer deaths and none of the reported trends achieved statistical significance. Other limitations include possible residual confounding by smoking and an inability to adjust for other confounders, such as employment in other high-risk occupations for lung cancer; and a relatively short latent period since DE exposure was not introduced in the study mines until 1970 or later.

Two epidemiologic studies of DE exposure and lung cancer risk have been conducted among truck drivers. A nested case-control study of 996 cases and 1,085 controls in a Teamsters Union cohort (Steenland et al., 1990; Steenland et al., 1992) reported a significant trend in risk with increasing duration of employment (based on union records) as a long-haul truck driver after 1959 (the year when many trucking companies had completed dieselization of their fleets)(p=0.04), with the odds ratio (OR) peaking at 1.55 for 18 or more years duration of employment after adjustment for age, smoking and asbestos exposure. Next-of-kin interviews also indicated that drivers of primarily diesel trucks for 35 years or more had increased risk (OR of 1.89 (95%CI=1.04-3.42)). In a cohort study of 31,135 male truck drivers followed from 1985 to 2000 (Garshick et al., 2008), long-haul truckers with 20 years of employment had an hazard ratio (HR) of 1.40 (95%CI=0.88-2.24) and P&D drivers had an HR of 2.21 (95%CI=1.38-2.52) for 20 years of employment after adjustment for age in 1985,

decade of hire, calendar time, race, census region, the healthy worker-survivor effect, and indirect adjustment for smoking. The reason for the higher risk among P&D drivers compared to long haul truck drivers is unclear since the IH survey indicated that the in-cab EC exposure levels measured for the two groups were virtually the same. The investigators indicate that their findings suggest that driver exposure comes predominantly from surrounding vehicles and from background air pollution, as well as from the driver's own vehicle. Both studies of truck drivers are limited because the IH surveys of DE exposure were conducted at the same time as the epidemiologic studies, and no historical measurement data were available to quantify individual truck driver DE exposures in either study.

For railroad workers, Garshick et al. (2004) extended follow up through 1996 on the original 54,973 US railroad workers in the cohort included in the last Monograph, identifying 4,351 lung cancer deaths. They reported a RR of 1.4 (95%CI=1.30-1.51) for workers in jobs associated with operating trains, but risk did not increase with increasing years employed at these jobs. Lung cancer mortality was elevated in selected DE-exposed jobs such as conductor and engineer, but risk did not increase with increasing years of employment in these DE-exposed jobs. Although historical measurement data on DE exposure in this cohort were unavailable, Laden et al. (2006) obtained extensive historical information on diesel locomotive use by railroad. They found that workers hired after 1945 (when diesel locomotives began to be introduced) had a RR of 1.77 (95%CI=1.50-2.09), with increasing risk with increasing duration of employment in a DE-exposed job. In contrast, railroad workers hired before 1945 had an RR of 1.30 (95%CI=1.19-1.43) for any diesel exposure and no evidence of a trend in risk with duration of employment. However, no trend in risk with increasing cumulative DE exposure was apparent. Findings reported by Laden et al. (2006) were not adjusted for smoking despite the fact that indirect adjustment for smoking in the earlier analysis reported by Garshick et al. (2004) did attenuate estimates of risk.

Studies of Swedish bus garage workers (Gustavsson et al., 1990) and Swedish dock workers (Emmelin et al., 1993), based on retrospective exposure assessments in the absence of historical measurement data on DE levels, yielded elevated ORs for heavily exposed bus garage workers (2.4, 95%CI=1.3- 4.5) and for heavily exposed dock workers (2.9, 95%CI=0.8-10.7). However, both studies were small, with only 20 and 50 lung cancer deaths among the bus garage workers and the dock workers, respectively. In addition, smoking was not taken into account in the bus garage workers study.

Other cancers

Epidemiologic studies of the carcinogenicity of DE have focused primarily on lung cancer. In addition, DE exposure has been linked to a number of other neoplasms including cancers of the bladder, kidney, pancreas, colon and rectum, prostate and multiple myeloma and leukemia (IARC, 1989; Boffetta and Silverman, 2001; Seidler et al., 1998; Lee et al., 2003). Following lung cancer, the strongest evidence of increased risk associated with DE is apparent for bladder cancer. A meta-analysis (Boffetta and Silverman, 2001) yielded a summary of RR of 1.44 (95%CI=1.18-1.76) for high DE exposure. However, some evidence of publication bias was apparent, with a paucity of small studies with null or negative results.

Cancer in experimental animals

(*sufficient,* Vol 46, 1989)

Diesel emissions research was initiated in 1977 by scientists at the US EPA to evaluate the health impact of increasing diesel vehicle emissions (US EPA/625/9-79-004), 1979. The first diesel studies designed to characterize the chemical and bioassay characterization led to the discovery that diesel particles contained a relatively large quantity of mutagenic organic compounds (Lewtas et al., 1979). Animal studies were initiated to characterize the tumor potency of different diesel emissions and determine which diesel extracts, fractions, and specific chemicals were carcinogenic (Nesnow et al., 1982). Mutagenesis and carcinogenesis studies of a range of diesel particles was published in the early 1980s (Lewtas, 1982, Nesnow et al, 1982) as well as inhalation (Pepelko, 1982) (Pepelko et al., 1983) and toxicology studies (Lewtas, 1982). Comparative cancer potency studies of diesel and gasoline particle extracts were compared to a series of organic extracts from known human carcinogens (coal tar, a coke oven, tobacco smoke) with respect to chemical composition (Williams et al., 1986), mutagenicity (Lewtas, 1983), and animal tumor potency (Nesnow,1982). The tumor initiation potency of the three known human carcinogens compared to the tumor potency of a series of diesel combustion emissions and one gasoline combustion emission sample was used to estimate the range of relative cancer unit risks from diesel emissions (Albert et al., 1983, Lewtas et al., 1983). Lung implantation studies reported that PAH and nitro-derivatives contributed to the carcinogenic impact of diesel exhaust condensates evaluated by implantation into the lungs of rats (Grimmer et al., 1987).

Biodiesel fuel derived from soybean oil was used in two 1998 Cummins diesel engines operated by the US EPA heavy-duty engine dynamometer schedule. This biodiesel exposure to F344 rats (30/exposure group) was to sub-chronic inhalation exposure levels of 0.04, 0.2, and 0.5 mg particles/m^3. Significant exposure-related effects were limited to the lung and were greater in female rats than in males and the effects were primarily found in the highest exposure (0.5 mg particles/m^3equal to 50 ppm). Among the high-level (50 ppm) females, the lung weight/body weight ratio was increased and multifocal bronchiolar metaplasia of alveolar ducts was reported in 4 of 30 rats. There were no other significant exposure-related effects on survival, clinical signs, or toxicology evaluations (Finch et al., 2002).

Mechanisms of carcinogenicity

Since the 1960s, evidence has increasingly supported the theory that chemical carcinogens (e.g., polycyclic aromatic hydrocarbons (PAH) and nitro- polycyclic aromatic hydrocarbons (nitro-PAH) are metabolized via oxidative pathways to produce electrophilic reactive products (e.g., epoxides) that react covalently with DNA and possibly with other nucleophiles (e.g., diesel particles and other combustion particles). Bioassay-directed chemical analysis of the constituents of complex combustion emissions has facilitated the identification of mutagenic and carcinogenic constituents of these complex mixtures (Schuetzle and Lewtas, 1986) (Lewtas, 1988). In 1986, el-Bayoumy and Hecht reported the mutagenicity of several K-region lactone derivatives of 1-nitropyrene that were highly mutagenic and ten years later 3-nitrobenzanthrone was isolated from both diesel and air particles (Enya et al., 1997). Diesel and other soot particles have also been reported to generate free radicals that lead to biologically damaging hydroxyl radicals. The formation of DNA adducts by nitro-PAH and PAH in animal and cellular studies of diesel particles is very well documented in a series of

studies reviewed in Mutation Research Reviews (Lewtas, 2007). This review also compares a wide range of combustion emissions, including diesel emissions and the causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects that have been reported (Lewtas, 2007).

Research needs and recommendations

Possible future epidemiologic studies:

Epidemiologic evidence to date suggests that the relation between DE exposure and lung cancer risk may be causal. To establish causality will require well-designed epidemiologic studies of large cohorts of DE-exposed workers with (a) quantitative estimates of DE exposure for study subjects, (b) with adequate latent period for the development of lung cancer, and (c) with information on smoking and other potential confounders (e.g., employment in other high-risk occupations for lung cancer). Both heavily exposed workers (i.e., nonmetal miners) and light-to-moderately exposed workers (i.e., truck drivers) are currently being studied to estimate risk for a wide range of DE exposure. The study of US nonmetal miners will be particularly informative because of several unique features. First, based on a cohort of 12,400 workers who have exposures many times higher than that observed in other DE-exposed occupations, adequate information is available to quantify historical exposures to DE for all cohort members. The historical assessment is strengthened by the availability of industrial hygiene data from NIOSH surveys carried out at study mines in 1976 and again in the late 1990s. Second, the follow-up period for the cohort ranges up to 50 years, which should provide a sufficient latent period to detect an elevation in lung cancer mortality. Lastly, information on cigarette smoking and other potential confounders was obtained from interviews with next of kin. This study is close to completion and results are expected to be published soon. In ongoing and future studies, it will also be important to evaluate any potential interaction between cigarette smoking and DE exposure. If ongoing epidemiologic studies of nonmetal miners and/or truck drivers yield consistent significant, positive exposure-response relationships, it will be important to conduct research into the underlying mechanisms of DE-induced carcinogenesis. Cross-sectional molecular epidemiological studies in DE-exposed human populations will be needed to evaluate the relationship between DE exposure and biomarkers of inflammation, genotoxicity, and other relevant early biological effects, and to study potential sources of genetic susceptibility. In addition, such studies may help us identify the components of DE that are most biologically active in humans, to the extent that these components are not highly correlated. In the long-term, the design and implementation of technologies for population surveillance of DE exposure coupled with biomarkers of effect merit consideration.

Impact of biodiesel fuels on the emissions, mutagenicity, and carcinogenicity of diesel emissions:

Biodiesel fuels (e.g., rapeseed oil, rapeseed oil methyl ester, soybean oil methyl ester) and blends of biodiesel fuel with petroleum diesel fuel (e.g., a biodiesel blend with petroleum diesel designated as B35 is 35% biodiesel and 65% petroleum diesel) are now being investigated as well as natural gas-derived synthetic fuels (gas-to-liquid). Vegetable oils have been used as fuels for diesel engines as early as the late 1800's by Rudolf Diesel, who used peanut oil in a diesel engine, and recent studies are investigating renewable biological sources for fuels such as vegetable oils or animal fats. Heavy duty diesel trucks performed well with

35% biodiesel and 65% petroleum diesel blend designated B35 and B20 (20% biodiesel) (Wang et al., 2000). A series of publications by Bunger et al., 2006 and 2007 report on the influence of fuel properties, nitrogen oxides (NOx), and exhaust treatment by oxidation catalytic converters that increased the mutagenicity of the diesel engine emissions. The fuels included common fossil diesel fuel (DF), low-sulfur DF (LSDF), rapeseed oil (RSO), rapeseed oil methyl ester (RME) biodiesel, and soybean oil methyl ester (SME). The strong increase in mutagenicity (~10 to 60 fold) when using RSO or RME as diesel fuels needs to be considered before the rapeseed-based fuels are used to replace established diesel fuels (Bunger et al., 2007).

With the increased use of biodiesel in recent years, the potential carcinogenicity of biodiesel warrants future evaluation. Although several biodiesel fuels derived from rapeseed oil or rapeseed methyl ester have been found to be highly mutagenic (Bunger et al., 2007), the soy-oil-based biodiesel emissions are less mutagenic. It is premature to conduct epidemiologic studies of biodiesel because the latent period for the development of solid tumors is currently inadequate. However, experimental laboratory studies of biodiesel should be a priority in view of the increasing prevalence of use of biodiesel in the US and European populations.

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Carbon Black

by Eileen D. Kuempel PhD and Tom Sorahan PhD

Citation for most recent IARC review

IARC Monographs Volume 93 (in press)

Current evaluation

Conclusion from the previous Monograph: Carbon black is *possibly carcinogenic to humans (Group 2B)* based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.

Exposure and biomonitoring

Carbon black is a powdered form of elemental carbon manufactured by the controlled vapor-phase hydrolysis of hydrocarbons. Different types of carbon black have a wide range of particle sizes, surface areas per unit mass, and contents of toluene-extractable materials. Carbon black is variously known as acetylene black, channel black, furnace black, lampblack or thermal black depending on the specific process by which it is manufactured.

Occupational exposure

Some 8,000 inhalable dust measurements and 7,400 respirable dust measurements were taken in the period 1987-92 at 19 carbon black production plants as part of a European respiratory morbidity study (Gardiner et al., 1996). Arithmetic mean respirable dust exposures were shown for various job titles including process operator (0.41 mg.m.-3) and warehouse packer (0.78 mg.m-3). Estimates of occupational exposure to carbon black have been incorporated into the UK and German cohort mortality studies of carbon black production workers but not in the United States study of carbon black production workers. It seems likely that carbon black exposures in these cohorts are much higher than those found in user industries such as rubber manufacture and newspaper printing.

Environmental exposures

No data were found on environmental exposures to carbon black. The ambient fine mode particulate matter <2.5 μm in diameter (PM2.5) consists mainly of combustion-derived carbonaceous particles (fine and ultrafine sizes) with organic compounds and transition metals adsorbed on the surfaces (Stoeger et al., 2006).

Cancer in humans

(*inadequate*, Vol 93, in prep)

A Working Group from the International Agency for Research on Cancer (IARC) met in 2006 to review the available literature on cancer risks from exposure to carbon black. Three cohort studies of carbon black production workers were available, relating to plants in the UK (Sorahan et al., 2001), Germany (Wellman et al., 2006; Morfeld et al., 2006), and the USA (Dell et al., 2006). The focus of these three studies was on possible excess risks of lung cancer. Cohort studies of rubber workers exposed to carbon black were also available (e.g. Straif et al., 2000), but these studies are not considered here because of the difficulties of controlling for all potential lung carcinogens in the rubber industry.

UK carbon black production workers

The cohort study of UK carbon black production workers included follow-up details of 1,147 male manual workers employed for at least twelve months at one of the five main UK plants (Sorahan et al., 2001). The definition of the factory sub-cohorts differed but all workers were first employed before 1975; follow-up was analyzed for the period 1951-96 and expectations were based on mortality rates for the general population of England and Wales. Mortality from lung cancer was elevated (Observed 61, SMR 173, 95% CI 132 to 222), whereas mortality from all other causes combined was not (Observed 311, SMR 106, 95% CI 95 to 118). Exposures to carbon black were considerable. For example, it was estimated that process workers in the 1960s had typical exposures of 4.0-7.0 $mg.m^{-3}$ and bag packers in the 1960s had typical exposures of 15.0-20.0 $mg.m^{-3}$. Poisson regression analyses found no significant positive trends of lung cancer risks with estimated cumulative exposure (lagged or unlagged) to carbon black.

German carbon black production workers

The cohort study of German carbon black production workers included follow-up details of 1,535 male manual workers employed for at least twelve months in the period 1960-98 at a single German plant (Wellmann et al., 2006). All study subjects had to be alive on 1st January,

1976, and follow-up was analyzed for the period 1976-98. Expectations were based on mortality rates for the general population of Germany. Lung cancer mortality was significantly elevated (Observed 50, SMR 218, 95% CI 161 to 287), in contrast with mortality from all other causes (Observed 282, SMR 111, 95% CI 99 to 125). A Cox regression analysis of these data (Morfeld et al., 2006) did not find cumulative exposure (lagged or unlagged) to be positive predictors of lung cancer risk.

U.S. carbon black production workers

The cohort study of U.S. carbon black production workers included follow-up details of 5,011 workers with potential exposure to carbon black, whilst employed for at least twelve months at one of the eighteen U.S. plants (Dell et al., 2006). The definition of the factory sub-cohorts differed but all workers were first employed between the mid-1930's and the end of 2003; follow-up was analyzed for the same period. Expectations were based on mortality rates for the general U.S. population, specified by age, sex, calendar year, and race. Mortality from lung cancer was not elevated (Observed 138, SMR 97, 95% CI 82 to 115). This observed figure included 11 deaths of unknown cause (15% of the 76 deaths with unascertained cause of death). Mortality from all other causes was significantly depressed (Observed 1,188, SMR 72, 95% CI 68 to 76), suggesting a possible failure to trace all deaths, or incorrect allocation of person-years-at-risk. Work history details were not collected and, consequently, no analyses of lung cancer risks in relation to quantitative estimates of carbon black exposure have been attempted.

Recent Studies

Sorahan and Harrington (2007) updated the UK mortality study to the end of 2004. Based on serial rates for the general population of England and Wales, significantly elevated mortality was observed for lung cancer (Observed 67, SMR 146, 95% CI 113 to 185) but not for all other causes combined (Observed 426, SMR 106, 95% CI 97 to 117). There was highly elevated lung cancer mortality at two of the plants but no excess mortality at the other three plants combined. Analyses by period since leaving employment indicated elevated lung cancer risks were limited to those workers with some employment in the most recent 15 years. SMR analyses found an overall positive significant trend between lung cancer risks and cumulative carbon black exposure received in the most recent 15 years (so-called 'lugged' analysis). Poisson regression analyses provided different results depending on which variables were adjusted for. The authors concluded that carbon black, or chemicals associated with the production of carbon black, may have had an effect on late stages of lung cancer carcinogenesis at two of the plants but that no such effect was found at the other plants.

Morfeld and McCunney (2007) re-analyzed data from the German cohort but found no evidence of lung cancer SMRs declining with period since leaving employment. The same authors have now carried out further tests of the hypothesis that carbon black is a late stage human lung carcinogen, but find no suggestion of "lugged" exposures (cumulative exposures in recent years) being related to lung cancer risks (Morfeld and McCunney, 2009).

A summary of findings from the three available cohorts of carbon black production workers is shown in Table 1.

Table 1. Summary of lung cancer findings from cohort studies of carbon black production workers.

					Relationship between lung cancer risks and estimated cumulative exposure to carbon black		
Country	Obs	Exp	SMR	(95% CI)	Lifetime[a]	Lagged[b]	Lugged[c]
UK	67	45.9	146	(113 to 185)	No	no	Yes
Germany	50	23.0	218	(161 to 287)	No	no	No
USA	138	142.1	97	(82 to 115)	n/k	n/k	n/k
Total	255	211.0	121	(107 to 137)			

n/k not known
a. total
b. distant exposure.
c. recent exposure.

Cancer in experimental animals

(*sufficient*, Vol 93, in prep)

Previous Studies

Two chronic inhalation studies and two intratracheal instillation studies found significantly elevated lung cancer in rats exposed to carbon black.

Heinrich et al. (1995)
Rats (female Wistar Crl:(WI)BR) were exposed by whole-body inhalation, 18 hr/d, 5 d/wk for 24 months, to furnace black (Printex 90, primary particle size 14 nm; specific surface area 227 $\pm$ 18.8 m^2/g) at an average airborne concentration of 11.5 mg/m^3, then kept in clean air for another 6 months. At 30 months, 39/100 exposed rats had lung tumors (11 of which had benign cystic keratinizing squamous cell tumors only), whereas 1 of the 217 control (unexposed) rats had a lung tumor (adenocarcinoma).

Mice (female Crl:NMRI BR) were exposed to furnace black (Printex 90, primary particle size 14 nm; specific surface area 227 m^2/g) for 18 h/day, 5 d/wk, 13.5 months, to an average airborne concentration of 11.5 mg/m^3 (whole-body inhalation) then kept in clean air for another 9.5 months. The lung tumor incidence in the exposed mice was not significantly elevated compared to that in control (unexposed) mice, which had a high background tumor incidence (not related to carbon black exposure).

Nikula et al. (1995)

Rats (male and female Fischer 344/N) were exposed for 16 hr/day, 5 d/wk, for up to 24 months to 2.5 or 6.5 mg/m^3 furnace black (Elftex-12; particle size distribution: 1.95 µm (67%) and 0.1 µm (33%)). The tumor response was 8/213 and 25/211, respectively, in rats exposed to 2.5 or 6.5 mg/m^3, compared to 2/214 in control (unexposed) rats.

Intratracheal Instillation
Rats (female Wistar) were exposed by intratracheal instillation, once per week for 15 weeks, to furnace black Printex 90 (specific surface area 270 m^2/g; 3 mg/rat). Control rats were treated with the vehicle control (0.4 ml of 0.9% saline). At 131 weeks, 24 (65%) of the treated rats had benign and malignant lung tumors, while no tumors were observed in the control rats (Pott and Roller 1994; Pott et al., 1994).

Rats (female Wistar Crl:(WI)BR) received intratracheal instillation, once per week (approximately 1 mg/rat) for 16-17 weeks, to furnace black Printex 90 (specific surface area 270 m^2/g; primary particle size 14 nm) or Lampblack 101 (specific surface area 22 m^2/g; primary particle size 95 nm). Control rats were treated with the vehicle control (0.9% sodium chloride, 0.25% Tween 80 solution). At 27 months, the tumor incidence was 10/48 in rats treated with Printex 90 (benign and malignant), and 4/48 rats treated with lampblack 101 had benign squamous cell tumors. No lung tumors were observed in the control rats (Heinrich et al., 1994; Dasenbrock et al., 1996).

Other routes of exposure
In a study of C57B1 mice exposed by subcutaneous injection of 300 mg of furnace black containing benzo[a]pyrene, 18/46 mice developed subcutaneous sarcoma (Steiner 1954). In Wistar rats exposed by intraperitoneal injection (once per week for 4 weeks) with 20 mg of furnace black, 1 of the 35 treated rats developed an abdominal sarcoma (Pott et al., 1991).

Recent Studies

No carcinogenicity studies of carbon black in animals were found in the literature except those already reported in Monograph 93 (in press), discussed above.

Mechanisms of carcinogenicity

Carbon black appears to act like other poorly soluble low toxicity (PSLT) particles, which can elicit lung tumors in rats following prolonged exposure to sufficiently high concentrations of particles (Monograph 93 (in press); Baan 2007). Particle surface area dose was found to be most predictive of pulmonary inflammation and tumor response in rats when comparing the dose-response relationships for various types and sizes of PSLT including carbon black (Driscoll 1995; Elder et al., 2005). Compared to fine PSLT, much lower concentrations of ultrafine PSLT (e.g. 2.5, 6.5 or 11.5 mg/m^3 carbon black and ~10 mg/m^3 ultrafine titanium dioxide) were associated with impaired clearance, persistent inflammation, and malignant lung tumors in chronic inhalation studies in rats (Heinrich et al., 1995; Nikula et al., 1995). The retained particle volume, grouped by particle size, was also shown to describe the rat lung tumor responses to various PSLT administered by intratracheal instillation (Roller and Pott 2006).

Most evidence suggests that carbon black and other PSLT-elicited lung tumors occurs through a secondary genotoxic mechanism, involving chronic inflammation and oxidative stress (Knaapen et al., 2003). Experimental studies have shown that when the particle lung dose reaches a sufficiently high concentration (e.g., mass dose of ~0.5 mg fine-sized PSLT/g lung in rats), the alveolar macrophage-medicated clearance process begins to be impaired (complete impairment occurs at ~10 mg/g lung (Muhle et al., 1990)). Overloading of lung clearance is accompanied by pulmonary inflammation, leading to increased production of reactive oxygen and nitrogen species, depletion of antioxidants and/or impairment of other defense mechanisms, cell injury, cell proliferation, fibrosis, and as seen in rats, induction of mutations and eventually cancer.

Rats appear to be more sensitive to carbon black and other PSLT than other rodent species that have been tested. Fisher-344 rats had more severe and persistent lung responses (inflammation and histological changes) than those in B6C3F1 mice or F1B Syrian golden hamsters at equivalent exposure concentrations (females of each species) (Elder et al., 2005). Rats also had a greater proinflammatory response and a lower antioxidant response than mice or hamsters (Carter et al., 2006), indicating a greater oxidative stress response in rat lungs. Gallagher et al. (2003) observed dose-related progressive oxidative DNA damage in the lungs of rats exposed to carbon black.

Although studies in humans have not shown a direct link between inhaled PSLT and lung cancer, many of the steps in the mechanism observed in rats have also been observed in humans who work in dusty jobs, including increased particle lung retention and pulmonary inflammation in workers exposed to coal dust or crystalline silica (Castranova 2000; Kuempel et al., 2001; Lapp and Castranova 1993); and elevated lung cancer has been observed in some studies of workers exposed to carbon black (Sorahan and Harrington 2007), crystalline silica (Rice et al., 2001; Attfield and Costello 2004), and diesel exhaust particles (Stayner et al., 1998).

Recent studies

Recent findings have strengthened the association between inflammation and cancer (Hussain and Harris 2007; Calin and Croce 2006), and between the particle surface area dose of carbon black and other PSLT particles and the pulmonary inflammation response in mice (Stoeger et al., 2006) and rats (Duffin et al., 2007; Sager and Castranova 2009), and the proinflammatory effects in lung cells *in vitro* (Duffin et al., 2007; Hussain et al., 2009). Recent evidence suggests that in addition to a cancer mechanism involving indirect genotoxicity through inflammation and oxidative stress, nanoparticles may act as direct carcinogens (Mroz et al., 2008; Schins and Knaapen 2007), although additional research is needed in this area.

Recent finding concerning microRNA-mediated gene regulation in immune response during inflammation provide additional mechanistic information for an association between chronic inflammation and cancer (Hussain and Harris 2007). MicroRNAs, which are small gene-silencing RNAs that cause cleavage and degradation of target RNA, are involved in the innate immune response during inflammation and have been linked to the initiation and progression

of human cancer (Calin and Croce 2006). Studies to date have not investigated the role of microRNA in particle-elicited inflammation and cancer.

In an study of six different ultrafine carbon particles administered to mice (female BALB/cJ) by intratracheal instillation, the particle surface area dose correlated best with the inflammatory response (Stoeger et al., 2006). A no observed adverse effect level (NOAEL) of 20 cm^2 per mouse was observed for acute inflammation in healthy, in-bred mice. By comparison, mortality studies in humans in association with ambient fine particle air pollution have not shown any evidence of a threshold dose-response relationship (Schwartz et al., 2002), which may reflect a greater susceptibility to the effects of particulate air pollution in sensitive individuals such elderly individuals with preexisting cardiopulmonary diseases.

Duffin et al. (2007) investigated the role of particle surface area on acute pulmonary inflammation in a male Wistar rats, which were instilled with 125 μg of fine carbon black (surface area 9.9 cm^2) or nanoparticle carbon black (surface area 317 cm^2), in addition to titanium dioxide and polystyrene of different sizes (0.5 ml saline control). Rats were killed 18-24 hr later, and the number and type of cells in the lung was determine by bronchoalveolar lavage (BAL). A linear relationship between particle surface area and neutrophil cell count in BAL fluid was observed for all particle types and sizes (R^2=0.99), while no clear dose-response relationship was observed with the particle mass dose metric. *In vitro*, a linear relationship was also observed between the surface area dose of these PSLT particles and the induction of the proinflammatory cytokine IL-8.

Sager and Castranova (2009) investigated the role of particle surface area on pulmonary inflammation in male Fischer 344 rats treated with either fine or ultrafine carbon black by intratracheal instillation. The mass doses of ultrafine carbon black (primary particle diameter 14 nm; specific surface area 269 m^2/g; 0.047, 0.094, and 0.19 mg/rat) and fine carbon black (primary particle diameter 260 nm; specific surface area 8 m^2/g; 1.53, 3.06, and 6.12 mg/rat) corresponded to an equivalent surface area dose (0.031, 0.062, 0.12 cm^2 particles / cm^2 alveolar epithelial cell surface of the lungs). At each post-exposure time point (1, 7, or 42 days) and mass dose, the ultrafine carbon black was at least 65 times more potent than fine carbon black in eliciting pulmonary inflammation (as measured by neutrophil cell count in BAL fluid, relative to control (saline only) rats). When dose was expressed as particle surface area (measured by BET gas absorption technique (Brunauer et al., 1938)), the ultrafine carbon black was less than two times more potent than fine carbon black, and this difference was not statistically significant.

In an *in vitro* study of carbon black and titanium dioxide in a human bronchial epithelial cell line (16HBE14o-), Hussain et al. (2009) found that oxidative stress (production of reactive oxygen species) correlated with the BET-measured particle surface area of carbon black or titanium dioxide and with the internalized amount of nanoparticles (confirmed for titanium dioxide only by energy dispersion spectroscopy). The primary particle size of the carbon black nanoparticles was 13, 21, and 95 nm, and doses ranged from 5 to 160 $\mu g/cm^2$ (24-760 μg/ml); cells were treated for 24 hours.

In an *in vitro* study in a human epithelial cell line (A549), Mroz et al. (2008) were treated with several types and sizes of particles, including fine carbon black (primary particle diameter 260 nm) and nanoparticle carbon black (Printex 90, primary particle diameter 14 nm), at doses of 100 μg/ml. Nanoparticle carbon black and other nanoparticles tested caused single-strand DNA breaks, disruption of cell cycle kinetics, and induction of genes involved in cell signaling pathways, while fine carbon black did not. Urban dust caused both single and double-strand DNA breaks. The nanoparticles elicited reactive oxygen species, DNA damage, and activation of p53 and proteins related to DNA repair, which reportedly mimics the processes induced in irradiation carcinogenesis pathways.

Ultrafine carbon black (Printex 90) was selected as being representative of the carbon core of combustion particles in an *in vitro* study of components of particulate air pollution using two human epithelial cell lines (alveolar type II-derived A549 cells and bronchial-derived BEAS-2B cells) (Ovrevik et al., 2009). Despite different physical and chemical properties, the compounds tested showed similar responses to the positive control crystalline silica, suggesting that the generation of reactive oxygen species may be a common mechanism for all the particles tested. The predominant response was increased gene expression of the neutrophil-recruiting CXC-chemokines.

Translocation of ultrafine black (Printex 90, 14 nm diameter) from the lungs in mice following intratracheal instillation was observed by electron microscopy (Shimada et al., 2006). Particles were seen in gaps between lung epithelial cells, inside the capillary lumen in the lungs, and attached to red blood cells. This study suggests possible pathways for the distribution of ultrafine carbon black from the lungs, which may influence biological responses including in the immune system.

Biomarkers of exposure

No biomarkers of exposure were found that would be observed at lower doses than markers of early effect. For example, chest x-ray or computerized tomography may show deposits of particles in the lungs, but by the time these opacities are visible, adverse lung effects are also likely to have occurred. Markers of early effect may be more sensitive.

Biomarkers of effect

The genes involved in the early response to inhaled ultrafine carbon particles were elucidated in mice (female BALB/cJ) exposed by inhalation to ultrafine carbon particles (produced by electric spark generator from ultrapure graphite electrodes in an argon atmosphere) at an average mass concentration of 380 $\mu g/m^3$ for 4 or 24 hours (Andre et al., 2006). Upregulation of mRNA expression for immune-modulatory genes (e.g., heat shock protein) after 4 h of exposure, followed by several genes regulated by the NF-kB signaling pathway involved in oxidative stress and antioxidant response. Osteopontin, aglectin-3, and lipocalin-2 are secreted proteins observed in mice after acute inhalation to ultrafine carbon particles, which have also been observed in humans. These proteins may be useful markers for particle-induced pulmonary inflammation, although validation studies are needed.

Lung proteins involved in lung injury from ultrafine carbon black were identified in a study of ICR male mice administered 200 μg of ultrafine carbon black (Printex 90; 14 nm diameter,

surface area 254 m^2/g) and killed 24 hours later (Chang et al., 2007). Leukemia inhibitory factor receptor (LIFR) and epidermal growth factor receptor (EGFR) were associated with epithelial shedding and with vascular endothelial growth factor (EGRF) in bronchoalveolar lavage fluid.
The antioxidant cerulopasmin (Cp) was produced by lung epithelial cells.

Standard tests of oxygen diffusion capacity may provide an early marker of alveolar changes that affect gas exchange.

Research needs and recommendations

Epidemiology

Currently there is a single study of UK carbon black production workers providing strong (though novel) evidence that carbon black is a late stage human lung carcinogen. This hypothesis has been given no support whatsoever from the German study of carbon black production workers, although it would be worthwhile for an IARC Working Group to evaluate these recent studies. Ideally, at least one further test of the hypothesis that carbon black is a late stage lung carcinogen is also required. The most obvious candidate is the United States study of carbon black production workers. Attempts should be made to a) locate the 76 unascertained death certificates for this study, b) review plant-specific frequency distributions by year of hire and year of leaving employment to make sure that expected numbers of deaths are not being increased artifactually by including periods of follow-up when no deaths could have occurred, c) collect work history details and derive estimates of cumulative exposure, and d) carry out analyses of lung cancer risks in relation to "lugged" (recent) exposures. Examination of 1) possible relationships between lung cancer risks and other exposure metrics and 2) possible effects of age at exposure need to be carried out for all three published cohorts of carbon black production workers.

The cancer experience of workers at many other carbon black production factories remains unexamined, including workers at Columbian (Hannover, Germany), Hanan (Germany), Columbian (Trecate, Italy), Cabot (Ravenna, Italy), Degussa (Ravenna, Italy), Cabot (France), Cabot (Berre L'etang, France), Carbon Black Nederland (Botlek, Netherlands), Cabot (Rozenburg, Netherlands), Carbesa (Cadiz, Spain), Columbian (Santander, Spain), Cabot (Santurce, Spain), and Nordisk (Malmo, Sweden). The outcome variable could be cancer incidence, mortality, but preference would be to use an effect biomarker such as 8-OHdG with the exposure biomarkers.

Toxicology

Experimental studies are needed that improve our understanding of the mechanisms of particle-elicited lung cancer. A study examining the relationship between occupational exposure to carbon black and validated biomarkers of oxidative stress may provide information on the early biological responses relevant to particle-induced lung cancer mechanisms. These exposure-response relationships should be quantitatively compared in humans and rodents, and the role of particle size should also be examined.

Given the recent findings of an association between the production of microRNA and immune and inflammation processes (Hussain and Harris 2007), it may be useful to investigate the production of microRNA *in vitro* and *in vivo* in experimental systems with conditions equivalent to those in which inflammation and tumors were observed in rats (e.g., Elder et al., 2005; Heinrich et al., 1995; Nikula et al., 1995). Investigation of microRNA in other rodent species (mice and hamsters) could provide data on whether the microRNA responses are associated with the chronic inflammation and lung tumor responses. However, investigating a role of microRNA in particle-induced lung responses in humans may not be feasible without the availability of noninvasive or minimally invasive procedures.

Studies elucidating the role of particle size in the biological mechanism of particle-elicited carcinogenesis would be useful. Currently, there is uncertainty of whether nanoparticles can interact directly with DNA, in addition to the secondary genotoxic mechanism involving inflammation, oxidative stress, and oxidative DNA damage (Schins and Knaapen 2007).

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Styrene-7,8-oxide and Styrene

by Paolo Vineis PhD and Lauren Zeise PhD

Citation for most recent IARC review
IARC Monograph 82, 2002

Current evaluation:

Styrene is *possibly carcinogenic to humans (Group 2B)*. The Working Group found *limited evidence* in humans and *limited evidence* in experimental animals for the carcinogenicity. Evidence from mechanistic studies did not contribute in making the overall classification decision.

Styrene-7,8-oxide is *probably carcinogenic to humans* (*Group 2A*). The Working Group found inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity. In making the overall evaluation, the Working Group took into consideration supporting evidence that styrene-7 ,8-oxide: (i) forms covalent adducts with DNA in humans, rats and mice; (ii) induces gene mutation in bacteria and rodent cells in vitro; (iii) induces chromosomal aberrations, micronuclei and sister chromatid exchange in human cells in vitro; and (iv) induces chromosomal aberrations and sister chromatid exchange in mice in vivo.

Recent Authoritative Review

The National Toxicology Program recently finalized a review of styrene (NTP, 2008). The review covered use, exposure, and evidence for carcinogenicity from epidemiology, animal, mechanistic and other relevant studies. Except where citations are given, the discussion below relies on the NTP review. The NTP review was finalized after peer review by the "Report on Carcinogens Expert Panel for Styrene" The Panel made conclusions about the evidence for the carcinogenicity of styrene. The Panel found limited evidence of carcinogenicity in humans and sufficient evidence in animals. Major considerations in the Panel's recommendation included the established animal carcinogenicity and genotoxicity of the metabolite styrene-7,8-oxide, and the evidence for styrene-related DNA adducts and cytogenetic effects in styrene-exposed workers. In writing this summary we relied on existing authoritative reviews such as the NTP document, which has been subjected to careful peer review, exhaustive fact

checking and public comment. Except where citations are given, the discussion relies on the NTP review.

Exposure and biomonitoring:

Exposure to styrene is common in the general population and in occupational settings. Since the release of the Monograph, a number of publications report on environmental levels of styrene.

There are a number of emission sources of styrene, which on average occurs in the United States in sub-ppb levels in outdoor and indoor air, with indoor concentrations generally higher than outdoor. Styrene occurs in tobacco and marijuana smoke (Moir et al., 2007); the smoker is exposed as well as those exposed to his or her smoke. Styrene is released in wood burning, fuel combustion, and a number of industrial sources. Styrene is used in the manufacture of polystyrene and numerous copolymers and resins, and emissions result from manufacture and fabrication. In the United States, styrene ranked fifth in fugitive air emissions in 2005. Besides smoke sources, indoor sources include consumer product off-gassing of residual styrene monomer in polymer and copolymer materials. This offgassing results in higher levels indoors than outdoors. Higher levels have recently been found inside stores in some metropolitan US cities. In personal monitoring studies, indoor sources produced greater exposures than outdoor. In a recent study in Leipzig, indoor sources dominated as well, with greater contributions to personal exposure from home than from office buildings (Gokhale et al., 2008).

Styrene exposures via tap water are currently low in the United States.

Styrene migrates into food packaging into food. There are few new references on food migration since the publication of the IARC Monograph. The United States Food and Drug Administration includes styrene as a chemical it monitors in its Total Diet Study, and there are recent data. Levels of styrene of 10 ppb and above are common in a variety of food products. There also can be non-residue sources of food exposure. One recent paper also demonstrated that certain food molds produced styrene in foods.

For the general population, aside from tobacco smoke, food appears to be the largest source of exposure, followed by indoor air. For smokers, the largest source is cigarette smoke. Styrene was detected by the United States Centers for Disease Control and Prevention (US CDC) in 87.5% of blood samples from a group 624 individuals aged 20-59 year, sampled in the late 1980's and early 1990's (http://ntp.niehs.nih.gov/files/Styrene_Background_Document_(9-29-08)F%5B1%5D.pdf, p.42). The highest value measured was 4 µg/L and the median was 0.04 µg/L. The caveat for this study was that: "It is important to note that because this study was conduced with a nonstatistical subsample of NHANES III participants, statistical weights cannot be assigned, and estimates for the total U.S. population therefore cannot be calculated (NCHS 2000).", making it non-representative for the general population. More recently styrene was biomonitored over a 2 year period in 150 disadvantaged minority children in Minneapolis Minnesota. The mean concentration was 0.12 µg/L and upper 95th percentile was 0.5 µg/L. In a follow-up study of 43 children aged 3 to 6 years and also from a disadvantaged neighborhood levels were lower and similar to those in the non-represented sample by the United States CDC.

Occupational exposure data collected in epidemiological studies are often of poor quality. Occupational exposure to styrene-7,8-oxide, for which there is sufficient evidence of carcinogenicity in animals, can occur directly, e.g. in "lamination". The extent of such exposure needs to be established.

Air concentrations measured in occupational settings where styrene is used in fabrication or manufacturing are orders of magnitude higher than environmental concentrations. This includes measurements reported in recent studies published since the IARC Monograph. In addition to characterizing exposures, biomonitoring studies continue to evaluate possible markers of styrene exposure. For example, Fustinoni et al. (2008) investigated "urinary analytes and haemoglobin and albumin adducts as biomarkers of exposure to airborne styrene (Sty) and styrene-(7,8)-oxide (StyOX) and to evaluate the influence of smoking habit and genetic polymorphism of metabolic enzymes GSTM1 and GSTT1 on these biomarkers." While protein adducts were not associated with styrene or styrene oxide exposures urinary metabolites were. Saturating metabolism was observed for all metabolites but for the mercapturic acids. The extent to which metabolic saturation in humans should be addressed in characterizing exposures in epidemiologic studies deserves greater attention.

Cancer in humans:

Styrene: limited, Vol 82; styrene oxide: inadequate, Vol. 60

Since the Monograph, two epidemiology studies were published (Ruder et al., 2004, Delzell et al., 2006 and Sathiakumar et al., 2009; Delzell et al., more completely reports on same cohort as Sathiakumar et al., 2005). The questions that remained open from the 2002 evaluation are:

- why was cancer risk concentrated in cohorts less exposed to styrene (in particular not in the reinforced plastics, where exposure levels are higher)
- the effect of potentially confounding exposures, in particular 1,3-butadiene
- why different types of hematolymphopoietic cancers are found in different cohorts
- whether cancers other than hematolymphopoietic (e.g. pancreatic) are increased in exposed workers.

The recent studies do not help address these questions in a conclusive manner. The study by Ruder is small and finds no excess of hematolymphopoietic cancers, while it finds a strong excess of kidney and bladder cancers. Like many cohort studies with information only from personnel records, it could not address properly the issue of multiple confounders. Sathiakumar et al. (update of Delzell) find a slight (16%) excess of leukemias in styrene-butadiene workers and again cannot rule out confounders. As noted in the NTP report, significant increases in leukemia were reported for this cohort with longer employment and latency, with highest cumulative styrene exposure. Also, relative risk increased with increasing cumulative styrene exposure, but was attenuated when controlled for butadiene, and disappeared when controlling for dimethyldithiocarbamate. Risk did increase with increasing exposure to styrene peaks, which persisted after controlling for butadiene and dimethyldithiocarbamate.

Cancer in experimental animals

Styrene: limited, Vol 82; styrene oxide: sufficient, Vol. 60

No new bioassays on styrene or styrene oxide have been published since the release of the IARC Monograph. In considering the need for further experimental evidence for evaluating animal carcinogenicity of styrene, three issues are noteworthy.

1) IARC updated the Preamble; the guidance for evaluating the evidence for carcinogenicity, after the 2002 styrene review. IARC updated the guidance on use of historical control data in evaluating tumor increases, e.g.,:

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

IARC also has updated the approach for evaluating a robust study conducted in both sexes:

> "An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*. "

2) Further statistical analyses of the available bioassay data have been conducted and are included in the report released by the NTP in 2008. The statistical significance of a variety of findings in the experimental animal studies is now clarified.

The 2008 expert NTP panel concluded that there was sufficient evidence of carcinogenic activity from multiple studies in mice by multiple routes.

A consistent finding across studies is the occurrence of lung neoplasia in mice. These are seen in a robust inhalation study in male and female CD-1 mice (Cruzan et al., 2001), and in male B6C3F1 mice exposed by oral intubation (NCI, 1979). The data in the intubation study was questioned because the incidence of lung tumors in the control males was thought to be low. The NTP reviewed the appropriate historical control data (animals from the same source, same study protocol and same chronological window, with similar duration) and found that the increases were significant. Furthermore, they were significant when compared with the concurrent controls. Early onset of lung tumors and increased incidence of lung tumors was also seen in male and female mice O_{20} mice receiving styrene by gavage, although the study had notable design limitations. It is also noteworthy that in the study of a mixture of styrene and beta-nitrostyrene lung tumors in mid-dose male mice were substantially higher than in controls (mortality in the top dose group was high, limiting the power to detect findings in this group).

Given the consistent finding of lung tumors and little else in the mouse, further long-term bioassays of traditional design in this species does not appear justified, even given IARC's label of limited evidence for animals. As discussed below, at the time of the IARC monograph and to present time, mechanisms by which styrene induces lung tumors in the mouse has been an active area of research.

3) The modulation of mammary tumors in a few rat studies (increased in three studies, and decreased in one study (Cruzan,) add justification for conducting research on potential carcinogenesis of styrene at tissues in addition to the lung, and in the mammary gland in particular, given the further discussion below. The lack of finding of mammary gland in another robust study in the same animal strain limited the weight given to the mammary tumor findings.

Styrene oxide consistently caused benign and malignant tumors of the forestomach, but not at other sites, following oral administration. Thus, tumorigenic effects have only been seen local to the site of administration, and not at distal sites. The localized impact of styrene oxide on cells, like the Clara cell, with have activities for metabolic transformation is the subject of research. Pharmacokinetics may in part explain the apparently different sensitivities and site specificities among rats, mice and humans but not necessarily on the cellular or microcompartment level. This deserves further study.

Mechanism of carcinogenicity

IARC did not upgrade or downgrade styrene, but as described above did upgrade styrene oxide, based on mechanistic data.

Styrene itself (unmetabolized) is a weak genotoxic agent. Studies in humans on chromosome aberrations are small and most are limited in design. However it is interesting to note that (a) styrene adducts have been found in lymphocytes of workers; (b) styrene is metabolized mainly to styrene-7,8,-oxide, which is genotoxic and is carcinogenic to animals with sufficient evidence; (c) the same DNA adducts have been found in humans and animals.

There are at least 70 research publications released since the styrene monograph that address various facets of the possible mechanisms of carcinogenic action in humans and rodents. Most of these have been reviewed in the NTP (2008) report and many by Vodicka et al. (2006). Briefly:

- A large number of studies address different aspects of human variability in enzymes involved in the metabolic activation and detoxification of styrene, some studying the impact on cytogenetic markers, DNA strand breaks and repair.
- Dose response relationships between DNA damage and styrene or styrene oxide exposure have been further explored.
- In addition to styrene-7,8-oxide, there has been some study of the possible role of other metabolites (e.g., styrene-3,4-epoxide and styrene-2,3-oxide), sometimes using 2E1 knockout mice.
- Related to the potential importance of extrahepatic metabolism, studies have recently been published that identify and quantify CYPs in a variety of human tissues, in addition to compartments of human lung.

- Induction of prolactin has been recently observed in humans, adding to the concern about possible mammary cancer risk. The vast majority of subjects in worker studies are men; women have not been studied to any significant degree.
- Some studies have explored the role of oxidative stress.
- There has been recent compartmental modeling of the pharmacokinetics in the form of physiologically based pharmacokinetic models, but validation of these models is in large part lacking.

The 2008 NTP review noted that at least two mechanisms of carcinogenesis were supported by the literature. First, it noted that the compound's genotoxicity to human lymphocytes involves sister chromatid exchange and chromosomal aberrations and that further that it involves the 7,8-oxide. It noted that CYP2F2 in mouse cells can efficiently metabolize the compound to this oxide, and that this enzyme and CYP2A13 and CYP2S1 that also have activity for styrene are expressed in various extrahepatic human organs and thus may bioactivate styrene. The panel also noted that the same adducts formed in vitro by incubating styrene oxide in culture also form in workers exposed to styrene.

The second mechanism noted is one discussed at length in the IARC review – the activation of the compound to an epoxide that is toxic to mouse lung cells by way of the formation of 4-vinylphenol and its further oxidation. The NTP panel noted that this may be operative in the human lung as well.

Priorities involve a further understanding of the detail of the chemicals pharmacokinetics. including the development and validation of PBPK models, and an expansion of studies on DNA adducts in humans and animals.

Research needs and recommendations

Research recommendations are:

1. to perform a pooled analysis of human studies on chromosome aberrations and other genotoxic effects (building on Bonassi et al. (1996))
2. to explore further extrahepatic metabolism of styrene by mammary, hematolymphopoietic, human lung and other tissue/cells
3. to study the formation of other possible genotoxic metabolites such as the 2,3- and 3,4- oxides, and their genotoxicity.
4. to compare DNA adduct formation in humans and animals
5. to refine estimates of the extent of internal exposure to styrene-7,8-oxide, and explore the possible magnitude of exposure to the cell by considering microcompartments or quantifying possible exposure at the cellular level.
6. to further explore and quantify the distribution of susceptibility to styrene based on interindividual differences in activation, detoxification enzymes and repair capacities.
7. to further examine the extent of human exposure to styrene-7,8-oxide, which has sufficient evidence of carcinogenicity in animals, e.g. in "lamination"

8. to update existing studies in humans, in particular those of reinforced plastics workers, addressing the open questions above and clarifying the issue of hematolymphopoietic cancer classification; also, a pooled analysis of these studies would be helpful
9. to recruit new cohorts
10. to promote nested case-control studies in the largest cohorts, particularly on hematolymphopoietic, bladder, kidney and pancreatic cancers, to look at: (a) levels of exposure and dose-response relationship; (b) potential confounders, e.g. 1,3-butadiene; (c) measures of biomarkers of long-term exposure, such as DNA or hemoglobin adducts.
11. to further explore the relationship between styrene exposure, potential increases in prolactin, and potential for mammary carcinogenesis by styrene since there are limited data on the elevation of prolactin in exposed workers, and since some of the animal studies produced suggestive evidence for this site.

Styrene is currently classified as Group 2B. The large body of evidence that has been generated since the last monograph together with the evidence in the monograph may support a re-evaluation of carcinogenicity. Some of the research recommendations above are to analyze and synthesize existing data. This could be useful in a reevaluation of the compound.

Selected relevant publications since IARC review

Fustinoni S, Campo L, Manini P, et al. An integrated approach to biomonitoring exposure to styrene and styrene-(7,8)-oxide using a repeated measurements sampling design. *Biomarkers* 2008; 13: 560-78.

Gokhale S, Kohajda T, Schlink U. Source apportionment of human personal exposure to volatile organic compounds in homes, offices and outdoors by chemical mass balance and genti algorithm receptor models. *Sci Tot Environ* 2008; 407: 122-138.

National Toxicology Program (NTP, 2008), Report on Carcinogens Background Document for Styrene, U.S. Department of Health and Human Services, NTP, Research Triangle Park, North Carolina, September, 29, 2008.

Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. *Am J Ind Med* 2004; 45: 165-176.

Sathiakumar N, Graff J, Macaluso M, et al. An updated study of mortality among North American synthetic rubber industry workers. *Occup Environ Med* 2005; 62: 822-829.

Vodicka P, Koskinen M, Naccarati A et al. Styrene metabolism, genotoxicity, and potential carcinogenicity. *Drug Metab Rev* 2006; 38: 805-853.

Propylene oxide (PO)

by Paul A. Schulte PhD, Kari Hemminki PhD, and Nancy B. Hopf PhD

Citation for most recent IARC review

IARC Monographs 60, 1994

Current evaluation:

Conclusion from the previous Monograph

Propylene oxide is *possibly carcinogenic to humans (Group 2B)*.

There is *inadequate* evidence in humans for the carcinogenicity of propylene oxide.
There is *sufficient* evidence in experimental animals for the carcinogenicity of propylene oxide.

Exposure and biomonitoring

Biomarkers of exposure or effect

There are three biomarkers of effect currently being applied to measure PO exposure in humans: DNA adducts, hemoglobin adducts, and sister chromatid exchanges (SCE). These effect biomarkers integrate exposure for different time intervals, and correlation between all three end points is not expected.

DNA adducts of propylene oxide are formed in various organs of mice, rats and dogs. PO binding in mouse liver DNA was about one-twentieth that of ethylene oxide (EO). The major adduct formed in DNA after *in vitro* reaction of PO is 7-HP-guanine, followed by the adducts 3-HP-, 1-HP-adenine, and 3-HP-cytosine. These adducts are chemically unstable: 7-HP-guanine and 3-HP-adenine will depurinate, forming apurinic sites, and 1-HP-adenine and 3-HPcytosine will spontaneously convert to *N*6-HP-adenine and 3-HP-uracil, respectively. The level of 1-HP-adenine corresponded to 2% of 7-HP-guanine. 1-HP-adenine is chemically more stable ($t_{1/2}$ = 9.2 days for rearrangement to an *N*6 adduct compared with $t_{1/2}$ = 5 days for depurination of 7-HP-guanine). Therefore, this minor adduct could be an alternative for monitoring PO exposures (Czene et al., 2002 and references therein). DNA adducts are subject to DNA repair and necrosis of the cell, which will alter the cumulative dose estimate depending if the exposure is intermittent (allowing for DNA repair time) or constant (no DNA repair time).

PO forms adducts with albumin and hemoglobin (Hb) in man, dog, rat and mouse. Adducts to Hb are especially suitable because small molecules such as PO form chemically stable adducts with the N-terminal valine of Hb, N-(3-hydroxypropyl)valine (HOPrVal). Concentration of HOPrVal (Hb) is linearly related to air concentrations of PO (see below Boogaard et al., 1999). These adducts do not appear to affect the average life span of the erythrocytes. The exposure dose as measured by determination of Hb-adduct concentrations is integrated over the average life span of human erythrocytes, which is approximately 120 days.

Hb-adducts are not subject to repairs and therefore a cumulative dose over the past 120 days is achieved.

PO may cause SCE, which is a nonmutational genotoxic event (do not alter genetic information); however, when they arise in vivo, they do indicate that a putative mutagen/carcinogen has reached the critical cellular target for direct genotoxicity. SCE is not specific to PO exposures, and may be caused by other chemicals.

For dose estimates, monitoring Hb adducts is preferred to DNA adducts for several reasons: availability of large amounts (erythrocytes in whole blood), availability of methods for chemical identification (specific and sensitive), and the well-defined life span due to absence of DNA repair (Ogawa et al., 2006). Also, the hemoglobin adduct is strongly correlated with measured concentrations of PO (8h TWA) (Boogaard et al., 1999). Limitation of Hb adducts is that they cannot be used for monitoring of acute or short term exposures.

Polymorphisms:
Hypoxanthine phosphoribosyl transferase (*HPRT*) gene has been used for studies of the mutagenic effect of EO but not for PO. High exposures to EO in workers showed a significant increase in *HPRT* mutant frequency compared to controls, while at low exposures no such association was found.

Occupational exposure

Occupational exposure occurs during the production of propylene oxide and its derivatives and during production of hydroxypropyl starch ethers. (IARC, 1994). Since the IARC review, three new occupational biomonitoring studies have been conducted: one in a chemical plant manufacturing glycols and glycol ethers from PO and two in PO producing plants. Included also is an exposure to polyethylene (PE) study because PE metabolizes to PO.

An occupational exposure study was performed to establish a relationship between ambient exposure to EO and PO in air and the formation of their respective adducts to Hb (Boogaard et al., 1999*). The personal air monitoring (PAM) data were measured by gas-diffusion monitors in the breathing zone in three groups of workers: (1) In 1990: male operators (N=20) and controls (N=36 operators from a different division of the same plant with no exposure to EO or PO) in a chemical plant manufacturing glycols and glycol ethers from EO and PO; (2) In 1997 follow-up: male operators (N=18) from the same plant maintaining and inspecting a shutdown of the plant; (3) In 1997: male workers (N=28) maintaining a styrene-PO plant during shutdown. No correlations between random PAMs and Hb-adducts were found in the 1990 survey of male operators and controls, but correlations were found between PO air concentrations spanning four months (the life span of the human erythrocyte) and the Hb-adducts of male operators in the 1997 follow-up. However, in the follow-up study, 89 of 112 PAMs (79.5%) were below the limit of detection (0.2 mg m−3 = 0.08 ppm 8-h TWA), but did allow calculations of increments for the 4-mo interval from which daily (8-h) increments and steady-state concentrations were derived. Background levels of HOPrVal were found in some controls; however, the publication does not reveal how many nor what level of HOPrVal

levels were found in controls and exposed operators. In the 1997 study of maintenance workers the median HOPrVal concentrations increased 21.3 pmol/g globin, a 2-fold increase from pre-maintenance activities (24.4 pmol/g globin) to end of shutdown period (45.7 pmol/g globin). A PO air concentration of 10 mg m-3 is equal to 5.3 nmol/g globin, and the operators' blood levels were measured to 0.005 to 0.161 nmol/g globin in 1997.

Hemoglobin adducts were assessed as a measure of PO exposure in a chemical plant using PO, EO, acrylamide and acrylonitrile in the production of surfactants for the textile industry in exposed (N=62) and controls selected from the laboratory (N=10) (Schettgen 2002). In addition to PO globin adduct (HOPrVal), other globin adducts (N-2-carbamoylethylvaline, N-2-cyanoethylvaline, HOEtVal) were also measured for workers at this plant. HOPrVal were not found in either exposed workers or controls. The authors contribute this fact to either the limit of the analytical detection (LOD, 80 pmol/g globin), or due to the low adduct formation of PO, which is five times lower than for EO. This study does not report PO air concentrations or describe what type of jobs the workers were performing.

A small study in China was conducted to determine DNA adducts (1-hydroxypropyl-adenine or 1HPAdenine), hemoglobin adducts (HOPrVal), and SCE in blood of workers (N=8) occupationally exposed to PO at a PO-producing plant and control subjects (N=8) at an institute of occupational health (Czene et al., 2002). PO concentrations were determined based on area air samples collected in areas workers occupied the day before blood samples were drawn. PO concentrations ranged from 0.9 to 6.9 ppm, and workers were reported to be present in the areas of highest PO concentration for 1 to 1.5 h per day. HOPrVal adduct concentrations in the exposed workers ranged from 0.13 to 4.92 pmol mg−1 globin for a mean of 2.69 ± 1.52 pmol mg−1 globin, which is in the same range as found in the glycol and glycol ether from PO producing plants (Boogard et al., 1999). For comparison, values for controls ranged from 0.005 to 0.008 pmol mg−1 globin. The mean difference between exposed and controls was highly significant. In this study, Hb- and DNA-adducts were measured in the same worker (Czene et al., 2002). DNA adducts were detected in 7 of the 8 exposed workers with a mean value of 0.66 ± 0.34 mol per 109 mol normal nucleotides. DNA adducts were not detected in controls. The LOD was 0.1 mol per 109 mol normal nucleotides. 1HPAadenine rather than the N7HPG adducts were measured in this study because they are more stable. The group mean difference for DNA adduct concentrations between exposed workers and controls was highly significant, as was the correlation between the individual OHPrVal hemoglobin and 1HPAdenine DNA adduct concentrations when all subjects were included in the analysis (including the controls with no detectable adducts) ($r = 0.887$, $P < 0.0001$). However, limiting the correlation to only the exposed workers resulted in a correlation with marginal statistical significance ($r = 0.713$, $p = .047$). For the third biomarker, the difference in mean SCE frequency was significant ($p \geq 0.011$) with mean SCE frequency was 3.7 ± 2.11% for the exposed workers and 2.0 ±0.52% for the control group (Czene et al., 2002). Strong and significant correlations were found between levels of N7HPG and SCE frequencies ($r = 0.792$; $p = 0.00026$) and between OHPrVal and SCEs ($r = 0.766$; $p = 0.0014$). Excluding smokers gave significant associations between SCE frequency and 1HPAdenine adducts $r = 0.851$ ($p = .00044$), and between SCE and OHPrVal $r = 0.757$ ($p = 0.011$).

Exposures to PO at three manufacturing sites in France and the Netherlands were assessed by determining Hb-adducts (n>800 samples) over a 2-year period from operators, maintenance fitters and office staff (Jones et al., 2005***). The geometric means range from the three plants was 2.9-12.6 pmol g-1 globin, which is four to 16 times lower than the other two plants mentioned previously (Boogard et al., 1999, Czene et al., 2002). To evaluate smoking as a confounder, workers at one of the plants were separated into smokers (N=51) and non-smokers (N=177) and their GMs were 3.8 and 3.0 pmol g-1 globin, respectively. Smoking does not seem to be a major confounder when measuring Hb-adduct in blood of very low exposed workers.

Polyethylene (PE) is metabolized to PO. PO concentrations in blood from healthy male non-smoking volunteers (N=4) resulting from inhalation of PE (mean concentrations of 9.82 and 23.4 ppm for 180 min) was measured to 0.44 and 0.92 nmol/l for the two PE air levels (Filser et al., 2008**). The authors found a distinct species difference between rats and human in the activities of PO metabolizing enzymes of liver and lung cell fractions. Human microsomal epoxide hydrolase activities toward PO 2-4x (liver) and 6-8x (lung) higher in humans than those of rats.

Environmental exposures
Household and industrial detergents, paints, adhesives, textiles, defoamers, oil field chemicals, cosmetics, functional fluids and lubricants, heat transfer fluids, and automotive brake fluids contain PO. PO is also an additive in food. Levels of PO exposures from using these articles have not been measured (or reported).

PO is also found in tobacco smoke and is an environmental pollutant.

In a 2-year cancer bio-assay study in rats, the authors compared the results obtained from rats being exposed to a constant PO concentration with an unexposed human. The unexposed human level of PO Hb-adduct level was 0.006 pmol/mg globin (compared to Boogaard et al., 1999 for occupational exposure of 5.32 pmol/mg globin) (Rios-Blanco et al., 2002*****).

Human carcinogenicity:
From the last Monograph (vol. 60, 1994): One case-control study provides information about cancer risk in relation to exposure to propylene oxide specifically but does not allow any firm conclusion regarding carcinogenicity. Since then only one epidemiological study has been conducted.

Mortality study of workers formerly employed in a ethylene chlorohydrin and propylene chlorohydrin process plant (Olsen et al., 1997****). The objective was to compare the SMRs at this plant with previous excess mortality from pancreatic cancer and lymphopoietic and hematopoietic cancer found among workers in another chlorohydrin unit. All male workers (N=1361) who had worked in the ethylene or propylene production area for a month and worked at either manufacturing site for a year were included in the study. These workers were identified using work histories. Vital status was determined from 1940 to 1992. SMR was non-significantly elevated for lymphopoietic and hematopoietic cancers (SMR=129; 95%CI 62-238, observed 10 cases, expected 7.7), and not elevated for malignant neoplasms

(SMR=94) and pancreatic cancer (SMR=25),. Including a latency of 25 years gave increased the SMR to 144 for lymphopoietic and hematopoietic cancers, but did not reach statistical significance (95%CI 52-312). Comparing the SMRs across plants gave similar results except for lymphopoietic and hematopoietic cancer deaths at one plant SMR=181 (95%CI 66-393, observed 6 cases and expected 3.3). Comparing SMRs across process gave similar non-significant results; however, including a latency of 25 years gave a SMR= 194 (95%CI 71-423, 6 observed, 3.1 expected,) for lymphopoietic and hematopoietic cancer among those employees with exposure only to the ethylene chlorohydrin process. At 10-20 years of employment in the chlorohydrin plants, there was a significant Mantel-Haenszel RR (RR 3.56, 95% CI 1.23-10.29) for lymphopoietic and hematopoietic cancer based on three observed deaths. This effect disappeared with different categories were used in a Poisson regression. Limitation of this study is that following exposures are not considered: cigarette smoking, the ratio of exposure to chlorohydrin versus oxide, exposure in enclosed versus outdoor production facility, earlier versus later exposure periods (usually industrial hygiene improves over the years). The mean follow-up time was 25 years and additional follow-up time might change the outcome.

Animal cancer data (excluding mechanisms):

From the last Monograph (vol. 60, 1994): Propylene oxide was tested by oral gavage in one study in rats, by inhalation in one study in mice and in three adequate studies in rats and by subcutaneous administration in one study in mice and in one study in rats.

Propylene oxide administered by oral gavage to rats produced tumours of the forestomach, which were mainly squamous-cell carcinomas. In mice exposed by inhalation, propylene oxide produced hemangiomas and hemangiosarcomas of the nasal cavity and a few malignant nasal epithelial tumours. In a study in rats of each sex exposed by inhalation, papillary adenomas of the nasal cavity were observed in males and females and thyroid adenomas and carcinomas were found in females; in the second study, in males, papillary adenomas of the nasal cavity and an increased incidence of adrenal phaeochromocytomas were observed; in the third study, in females, increased incidences of mammary fibroadenomas and adenocarcinomas were observed. Subcutaneous administration of propylene oxide to mice produced local sarcomas; the study in rats was inadequate for evaluation.

Male Fischer 344/N rats in closed exposure chambers were exposed to constant PE concentrations, between 20.1 and 3000 ppm (7 h at least) and the PO concentrations were measured in the blood of these rats (Filser et al., 2008**). The PO blood concentrations ranged from 53 nmol/l at 20.1 ppm PE to 1750 nmol/l at 3000 ppm PE. The PO blood concentrations measured in this study are too low for resulting in PO treatment–related tumors seen in a long-term study (Renne et al., 1986; U.S. National Toxicology Program, 1985b).

Mechanisms of carcinogenicity:

From the last Monograph (vol 60, 1994): In rats exposed by inhalation, there is strong uptake of propylene oxide, which is then metabolized extensively and eliminated rapidly. Metabolism occurs predominantly by conjugation with glutathione. Propylene oxide can also

be hydrolyzed by epoxide hydrolase to 1,2-propanediol, which is subsequently metabolized to lactic and pyruvic acids.

Dominant lethal mutations were not induced in rats or mice, and sperm abnormalities were not observed in mice exposed to propylene oxide in vivo. Micronuclei and, in single studies, chromosomal aberrations and sister chromatid exchange were induced in mouse bone marrow after intraperitoneal injection of propylene oxide. Neither sister chromatid exchange nor chromosomal aberrations were induced in monkeys exposed by inhalation to 300 ppm. Propylene oxide induced chromosomal aberrations and sister chromatid exchange in human lymphocytes and DNA damage, gene mutation, chromosomal aberrations and sister chromatid exchange in mammalian cells in vitro. It caused dominant lethal mutation in Drosophila and was mutagenic to yeast, fungi and bacteria.

PO binds covalently to the cyclic ring nitrogens in DNA producing hydroxypropyl (HP) adducts. HP adducts can be non-promutagenic lesions being eliminated spontaneously, leaving behind the depurinated sites that can result in mutations only if not repaired before DNA replication occurs, or the adduct may be at the coding site resulting in potentially promutagenic lesions (Albertini et al., 2007). These adducts may cause mutation by transversions. PO can also react with the phosphate groups in the DNA backbone.

PO is a less potent mutagen than the week EO mutagen.

In a 2-year cancer bioassay with rats exposed to constant PO concentration the pattern for DNA and Hb-adduct accumulation did not correlate with the incidence curve for nasal tumors (Rios-Blanco et al., 2002*****). Tumor formation was only seen at >300 ppm exposure (highest levels tested 300 and 500 ppm). Neither adduct accumulation in tissues can explain the threshold in nasal tumor formation. The authors suggest an increased cell proliferation in the nose occurs at high PO concentration to be a critical factor for tumorigenesis in this tissue.

Research needs and recommendations:

The animal data consist of oral, inhalation and subcutaneous studies in three strains of rats and two strains of mice. Propylene oxide caused tumors at or near the site of administration in rodents, causing nasal tumors after inhalation exposure (NTP, 1985).

One of the major limitations in cancer epidemiological studies is that records of exposure are often incomplete or lacking (Kolman et al., 2002). Measuring hemoglobin adducts, an extremely sensitive effect biomarker, which does not undergo repair as DNA-adducts, may overcome this problem. In addition, low background level of OHPrVal shows that contributions from non-occupational sources are minor. The levels of OHPrVal of the smokers were similar to those of the nonsmokers, indicating that tobacco smoking is not the major contributing factor to the found background levels. Therefore, a future prospective study exploring cytogenetic effect would be feasible even though PO exposures are low because:

1) the great difference in means between exposed and controls seen in the pilot study will require few participants in the two groups (exposed and unexposed workers) and

2) the OHPrVal is a sensitive and specific biomarker to PO exposures (even detected in controls with unknown exposures).

The OHPrVal adduct represents cumulative exposures over a 3-month time period. The number of workers recruited to such a study would only be about 11 assuming equal variances in the two groups; however, other possible confounders should be included in the study such as gender, age, ethnicity, years of education, smoking, and possibly polymorphisms in the GST metabolizing pathway of PO. In addition, loss of follow-up and errors in power calculations would benefit from a larger study (~ 100 exposed and 100 controls in a balanced design). Various exposure-selective cross sectional epidemiological studies that look at OHPrVal adducts and cytogenetic effects would be useful.

Possible sources of future cohorts for such a study might be the already established cohorts in PO manufacturing workers (USA) (Olsen et al., 1997), which did not show an increased mortality rate due to cancer by duration with or without latency or cancer risk by process (PO versus EO); or the manufacturing cohorts in France and the Netherlands (Jones et al., 2005), and China (Czene et al., 2008). PO production is being expanded, and workers at these new PO manufacturing sites should be recruited for a future study. Other possible cohorts for propylene oxide biomarker epidemiological studies are: processing workers where PO is used as a starting material in polyurethane polyols (NTP 11th RoC), surfactants for textiles (Schettgen et al., 2002) and glycol/glycol ether manufacturing (Boogaard et al., 1999), and manufacturing of polyethylene (PE), which metabolizes to PO. Women should be included in the study as PO might be mammary carcinogen (Rudel et al., 2007). Workers' exposures to PO in paint and automotive fluids have not been adequately characterized. If these workers have exposures to PO then these could potentially be included in a future biomarker epidemiological study. Therefore recruiting sufficient workers to a future prospective study should be feasible.

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** Study paid for by CEFIC – an organization representing the European chemical industry
*** Authors from: Lyondell Chemical Europe – a chemical manufacturing company
**** Authors from: Dow Chemical Company, Health and Environmental Sciences
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Formaldehyde

by Bernard D. Goldstein PhD and Martyn T. Smith PhD

Citation for most recent IARC review

IARC Monograph 88, 2006

Current evaluation

Conclusion from the previous Monograph:

Formaldehyde is *carcinogenic to humans (Group 1)*. There is *sufficient evidence* in humans for the carcinogenicity of formaldehyde. There is *sufficient evidence* in experimental animals for the carcinogenicity of formaldehyde.

However, on p.276 of IARC Monograph 88 with regard to the ability of formaldehyde to cause leukemia it states: "In summary, there is strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde. Increased risk for leukemia has consistently been observed in studies of professional workers and in two of three of the most relevant studies of industrial workers. These findings fall slightly short of being fully persuasive because of some limitations in the findings from the cohorts of industrial and garment workers in the USA and because they conflict with the non-positive findings from the British cohort of industrial workers."

The Working group concluded that the epidemiological findings provided 'strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde'. However, after reviewing the toxicological and mechanistic data available, the Group concluded that 'Based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukemia in humans'. Several possible mechanisms were considered for the induction of human leukemia, such as clastogenic damage to circulatory stem cells. The Working Group was not aware of any good rodent models that simulate the occurrence of acute myeloid leukemia in humans. Therefore, on the basis of the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukemia in humans.

Exposure and biomonitoring

A number of new papers regarding exposure levels in several countries have been published since the monograph in 2006. A detailed review of exposure levels in China and elsewhere has recently been published (Tang et al., 2009). A summary of outdoor ambient concentrations worldwide was provided in Table 2 of a recent review and meta-analysis (Zhang et al., 2009).

Cancer in humans

(*adequate***,** Vol 88, 2006)

Formaldehyde has been evaluated by IARC to be a known cause of human nasal cancer, based on epidemiological and toxicological evidence. There is also strong support for a mechanism of action for nasopharyngeal carcinogenesis in which inhaled formaldehyde causes DNA-protein crosslinks in nasopharyngeal tissue.

Only one new report from an original epidemiology study in relation to leukemia induction by formaldehyde has been published since the last review. The NCI group has published a recent update of one of their studies, with an additional 10 years of follow-up, and it continues to suggest a possible link between formaldehyde exposure and mortality due to lymphohematopoietic malignancies, particularly myeloid leukemia (Beane Freeman, Blair et al., 2009).

Details are as follows: In the NCI's formaldehyde cohort, previously followed through December 31, 1979, and updated through December 31, 1994, formaldehyde exposure was associated with an increased risk for leukemia, particularly myeloid leukemia, that increased with peak and average intensity of exposure. Beane-Freeman et al. extended follow-up an additional 10 years through December 31, 2004 (median follow-up = 42 years), for 25 619 workers employed at one of 10 formaldehyde-using or formaldehyde-producing plants before 1966 (Beane Freeman et al., 2009). There were statistically significant increased risks for the highest vs. lowest peak formaldehyde exposure category ($\geq$ 4 parts per million (ppm) vs. >0 to <2.0 ppm) and all lymphohematopoietic malignancies (RR = 1.37; 95% CI = 1.03 to 1.81, *P* trend = .02) and Hodgkin lymphoma (RR = 3.96; 95% CI = 1.31 to 12.02, *P* trend = .01). Statistically nonsignificant associations were observed for multiple myeloma (RR = 2.04; 95% CI = 1.01 to 4.12, *P* trend > .50), all leukemia (RR = 1.42; 95% CI = 0.92 to 2.18, *P* trend = .12), and myeloid leukemia (RR = 1.78; 95% CI = 0.87 to 3.64, *P* trend = .13). There was little evidence of association for any lymphohematopoietic malignancy with average intensity or cumulative exposure at the end of follow-up in 2004. However, disease associations varied over time. For peak exposure, the highest formaldehyde-related risks for myeloid leukemia occurred before 1980, but trend tests attained statistical significance in 1990 only. After the mid-1990s, the formaldehyde-related risk of myeloid leukemia declined. The authors concluded that evaluation of risks over time suggests a possible link between formaldehyde exposure and lymphohematopoietic malignancies, particularly myeloid leukemia but also perhaps Hodgkin lymphoma and multiple myeloma. Observed patterns could be due to chance but are also consistent with a causal association within the relatively short induction – incubation periods characteristic of leukemogenesis.

Epidemiological issues worthy of further research include the appropriateness of the dose metric used in the NCI studies in which a significant association with hematological neoplasms was observed primarily with highest peak exposure rather than with average intensity or cumulative dose of formaldehyde. The implication of the association with highest peak exposure to both toxicological mechanisms of action as well as to the dose-response pattern appropriate to formaldehyde risk analysis would also be pertinent subjects for further research.

It should be noted that Marsh and Youk (2004) criticized the highest peak exposure metric used in the NCI studies, including pointing out that the peak exposures used in the metric were not based on actual measurements but estimated from estimated average time-weighted exposures (Marsh and Youk 2004). In their reanalysis, no association was observed between duration of time at the highest peak or the time since the first highest peak and leukemia mortality. They also suggest that some of the key methods of analysis used to evaluate the highest peak exposure metric are flawed or deficient). However, they did find that standardized mortality ratios for peak exposure categories and all leukemia and myeloid leukemia increased from deficits in the lowest exposed (e.g., 0.4 – 0.5) to excesses in the highest exposed (e.g., 1.2 – 1.4) categories (Marsh and Youk 2004).

A recent meta-analysis also used a "highest exposure" category to evaluate leukemia risk from formaldehyde exposure (Zhang et al., 2009). Using data from 19 studies, the summary relative risk (RR) for all types of lymphohematopoietic cancer combined was 1.25 (95% CI,

1.09–1.43, Shore adjusted). The summary relative risk was elevated in the 15 studies reporting data on all leukemia (RR = 1.54; 95% CI, 1.18–2.00, p < 0.001, Shore adjusted) with the highest summary relative risk seen in the six studies of myeloid leukemia (RR = 1.90; 95% CI, 1.31–2.76, p = 0.001, Shore adjusted). All six studies of myeloid leukemia had relative risks of 1.4 or higher. This new meta-analysis provides additional evidence of an association between formaldehyde exposure and human leukemia, especially for myeloid leukemia.

Based on the original data (observed deaths) in the above six studies, Zhang, Steinmaus et al. 2009 (Table 3) showed for the first time that myeloid leukemia (51%) is the primary type of leukemia observed with 19% being lymphocytic leukemia, while the remainder are unspecified. Furthermore, AML (64%, acute myeloid leukemia) is the major subtype of myeloid leukemia among leukemia deaths reported in formaldehyde-exposed individuals.

Bosetti et al. also published a quantitative analysis of pooled results from the published cohort studies through February 2007 (Bosetti, McLaughlin et al., 2008). They concluded that brain cancer and lymphohematopoietic neoplasms were modestly elevated in risk in professionals, but not in industry workers. They did not specifically examine myeloid leukemia.

Cancer in experimental animals

(*sufficient***,** Vol 88, 2006)

Mechanisms of carcinogenicity

Although the updated meta-analysis of Zhang et al. adds weight to the association between formaldehyde exposure and myeloid leukemia (Zhang, Steinmaus et al., 2009), several impediments remain to current full acceptance of formaldehyde as a cause of human myeloleukemogenesis. These include difficulty in understanding the pathway for this highly reactive inhaled agent to reach the human bone marrow; the high background levels of exposure to formaldehyde; and the recognition that it appears to have distinguishing features from other known human myeloleukemogens, including the current absence of evidence of pancytopenia in the published English literature and a relatively long latency period for AML.

It is not uncommon that epidemiological associations which are initially met with skepticism because of the absence of a perceived biological causal linkage spur the performance of basic mechanistic studies and animal toxicology that then provide the basis for confirming the causal validity of the epidemiological association. When the putative cause is an agent of major industrial importance to which exposure is common, such as formaldehyde, and for which there is an extensive toxicological data base, there is ample incentive to carefully and comprehensively investigate the potential biological linkage, as well as to replicate the epidemiological findings.

Described below are key issues related to the current issue of whether formaldehyde is a cause of human hematological cancers, and research that might help resolve the issue or at least narrow the extent of the current uncertainty. One of us co-authoring this document (MS) has been senior author of a paper containing a meta-analysis of the epidemiological literature

supporting a causal relation between formaldehyde and AML, as well as proposing biological mechanisms by which formaldehyde could cause AML. The other co-author (BG) has had a recent manuscript accepted for publication which concludes that current toxicological and hematological evidence does not support formaldehyde as a human myeloid leukemogen (Goldstein 2009).

New research related to mechanisms of formaldehyde penetrance to hematopoietic stem cells

Given the fact that formaldehyde is a highly reactive gas, the question arises as to how it reaches the blood and bone marrow to elicit toxic effects and produce leukemia. Several studies have reported increased chromosomal damage in the form of aberrations and micronuclei in circulating peripheral blood lymphocytes of workers exposed to formaldehyde (Suruda et al., 1993; Kitaeva et al., 1996; Ye et al., 2005; Yu et al., 2005; Orsiere et al., 2006). Increased levels of cytogenetic damage have also been reported in the bone marrow of exposed mice and rats, suggesting that it reaches the bone marrow in experimental animals (Kitaeva et al., 1990; Tao et al., 2004).

In aqueous solution, formaldehyde is converted mostly to oligomers of its diol form methanediol (formaldehyde hydrate, $CH_2(OH)_2$, or methylene glycol) and a dynamic equilibrium with formaldehyde is formed. The concentration of the diol oligomers versus that of formaldehyde depends on the precise conditions (temperature, pH, formaldehyde concentration) under which the reaction occurs (Walker 1964). Thus, methanediol, with a molecular weight of only 48, which can readily penetrate into tissues, may travel to the marrow through the blood where it is in equilibrium with reactive formaldehyde. The formaldehyde, once regenerated, can react with cellular macromolecules producing toxic injury (Fox et al., 1985). Further research into the generation of methanediol and its persistence in the circulation would be of value.

It is also possible that formaldehyde promotes leukemogenesis through direct induction of DNA damage and chromosome aneuploidy in hematopoietic stem or early progenitor cells in the nasal circulation or the nose. This hypothesis clearly requires additional testing and there at least two alternate mechanisms. As suggested by Zhang et al. formaldehyde may induce leukemia by damaging hematopoietic stem/progenitor cells circulating in the peripheral blood; or, by damaging the primitive pluripotent stem cells present within the nasal turbinates and/or olfactory mucosa. In these two alternate models, damaged stem/progenitor cells would then travel to the bone marrow and become initiated leukemic stem cells (Zhang et al., 2009).

New research related to the possible relation of formaldehyde leukemogenesis to known human leukemogens

Known human myeloid leukemogens are ionizing radiation, benzene and various systemic cancer chemotherapeutic agents. While the specific physicochemical processes vary greatly, common to all are mechanisms which result in the disruption of bone marrow DNA. Some act by direct alkylation of DNA; some through the action of free radicals or active states of oxygen; some through intercalating metals within the DNA structure; and some by inhibiting enzymes involved in cell division. Yet all of these agents produce pancytopenia at high doses,

which has not been described in the English literature with formaldehyde. Further, the epidemiological literature on AML associated with formaldehyde suggests a much longer latency period than usually observed with other known human myeloleukemogens (Goldstein 2009).

In their recent review Zhang et al. concluded that the published data on formaldehyde hematotoxicity are limited and inconsistent (Zhang et al., 2009). Several previous studies showed that formaldehyde altered the counts of different types of blood cells. One study reported that exposure to formaldehyde in humans reduced white blood cell counts (Kuo et al., 1997). Another recent study concluded that formaldehyde increased B cells, but decreased total T cells (CD3) and T-helper cells (CD8) in the blood of exposed workers, while T-suppressor (CD4) cells remained unchanged (Ye et al., 2005). In male rats exposed to a high dose of formaldehyde, increased monocytes, red blood cells and hemoglobin were detected, but lymphocyte counts were decreased (Vargova et al., 1993). Pancytopenia has not been a feature of classic long term safety assessment studies in which laboratory animals are exposed to the maximum tolerated dose of formaldehyde (Rusch et al., 1983; Maronpot et al., 1986; Appelman et al., 1988; Monticello et al., 1996). However, as the hematological system had not been perceived as a primary target of formaldehyde, it is perhaps possible that subtle effects were overlooked that may be observed with further study. The inconsistencies and limitations in the published studies suggest that more comprehensive studies of the hematological effects of formaldehyde in exposed populations and in laboratory animals are needed.

Recently, Tang et al. have evaluated the Chinese literature and describe eight studies conducted in China on hematological parameters in formaldehyde-exposed humans (Tang et al., 2009). These are published mainly in Chinese journals. The majority of these studies show that long-term exposure can decrease the number of white blood cells and possibly lower platelet and hemoglobin counts (see Table 9 in (Tang et al., 2009)). In a detailed study of occupationally exposed nurses, personal and area exposure data, as well as complete blood cell counts, were collected. This data was reported as a correlation matrix for complete blood count, formaldehyde concentration and work duration. The study concluded that the correlation between the decrease in WBCs and increase in formaldehyde concentration is the best indicator of exposure among the other outcomes (Kuo et al., 1997). One study of only 10 exposed subjects showed a non-significant decrease in WBC counts compared to the 10 controls (Tang et al., 2009), likely due to the small sample size. Another study reported no significant differences in WBC and Hb in individuals occupationally exposed to formaldehyde (Tang et al., 2009). Further evaluation of these Chinese studies is warranted.

Implications of the possibility that the nose is the site of formaldehyde leukemogenesis

Two inferences can be drawn from the proposal that formaldehyde may be leukemogenic through virtue of its reaction with hematopoietic stem cell precursors within the nose which would allow indirect assessment of this possibility.

A. Analysis of other nasal carcinogens and leukemia incidence in relation to formaldehyde

One inference is that other known nasal carcinogens might then be expected to be leukemogens. For chromium, a meta-analysis of 49 epidemiologic studies evaluating cancer mortality found an SMR for leukemia of only 88 (Cole and Rodu 2005). However, the major study of workers in a sulfur mustard factory showed an increase in leukemia risk (13 deaths observed; 8.51 expected) which was not statistically significant (Easton et al., 1988). This may reflect the fact that sulfur mustard is a known cause of human and laboratory animal pancytopenia – it was its pancytopenic effect observed in World War I mustard gas casualties that led to mustard derivatives being evaluated as a chemotherapeutic agent. Nickel, another known human nasal carcinogen, is not listed as causing leukemia; nor is arsenic, which may be a nasal carcinogen (Hayes 1997; Navarro Silvera and Rohan 2007). Both nickel and chromium are thought to act as carcinogens through DNA-protein cross-linking, the proposed mechanism of action of formaldehyde. Research into understanding the pathways of DNA damage of formaldehyde in relation to other know nasal carcinogens, as well as clarification of the risk of leukemia in cohorts exposed to other nasal carcinogens, would be helpful in interpreting the potential for formaldehyde leukemogenesis.

B. Analysis of the implications of the apparent rarity of chloroma formation within the nose

Another implication of the possibility that nasal tissue contains myelopoietic precursor cells is that nasal tissue would also be a location for isolated accumulations of myeloid tumor cells known as chloromas. Chloromas classically are collections of extramedullary malignant hematopoietic precursor cells that are sometimes observed prior to the development of frank AML.

Chloromas have been reported in virtually every tissue. However, if they occur at all in the nasal cavity they are relatively rare. For example, Yamauchi and Yasuda describe the site location of 102 tumor nodules in 74 patients with non-leukemic granulocytic sarcoma, i.e., chloromas that are diagnosed prior to systemic evidence of leukemia (Yamauchi and Yasuda 2002). The 23 tissues listed are skin, adipose tissue, bone, mediastinum, lymph nodes, tonsil, spleen, uterus, ovary, vagina, breast, testis, stomach, small intestine, liver, pancreas, epidural spine, meninges, brain, orbit, heart, lungs and urinary bladder – but not nasal tissue. Underdiagnosis of nasal tissue chloromas is unlikely.

Chloromas do occur in paranasal sinuses (O'Brien et al., 2008). However, based on data in the rhesus monkey penetration of formaldehyde into sinuses is restricted (Monticello et al., 1989; Kepler et al., 1998). Similarly the IARC monograph (2006), points out that the usual practice of combining cancers of the nose and nasal sinuses might dilute a true effect of formaldehyde on nasal cancers, presumably because nasal sinuses would not be an expected location for formaldehyde exposure. More research is needed, including a thorough review of the literature, to both substantiate the apparent lack of chloromas in nasal tissue, and to further understand its implications. These include the finding that chloromas preferentially have the [t(8:21)] chromosomal translocation (Byrd et al., 1997).

Pyatt et al.(Pyatt et al., 2008) have also made a series of theoretical arguments against the nose as the site of formaldehyde leukemogenesis in response to the United States Environmental Protection Agency (EPA) recently proposed mode of action (MOA) to explain

how inhaled formaldehyde (FA) might induce leukemia, lymphoma and a variety of other lymphohematopoietic (LHP) malignancies in occupationally exposed workers. As discussed above the hypothesis requires that B lymphocytes or hematopoietic progenitor cells (HPC) present at the "portal of entry (POE)" undergo sustained mutagenic change as a result of direct FA exposure. These modified cells would then migrate back to the bone marrow or primary lymphatic tissue and subsequently develop into specific LHP disease states. Chemical interaction at the POE is an absolute requirement for the hypothesized MOA as, according to Pyatt et al., and they claim there is no convincing evidence that inhaled FA causes distant site (e.g., bone marrow) toxicity. The authors further claim the available data does not support the proposed concept of "peripheral transformation" at the chemical entry site and that the existing science does not support the proposed MOA as a logical explanation for proposing that FA is a realistic etiological factor for any LHP malignancy (Pyatt et al., 2008).

Implications of the possible epidemiological association of lymphoproliferative tumors to considerations of formaldehyde as a leukemogen

Modern molecular biological tools have amply demonstrated the ability of early hematopoietic precursor cells to differentiate broadly in both myeloproliferative and lymphoproliferative directions. The recent update by the NCI (Beane Freeman et al., 2009) suggests that there may also be an increase in lymphoid tumors in this cohort. This suggests that further study of the molecular mechanisms by which formaldehyde might cause AML consider the broader implications of an effect on an early hematopoietic precursor cell capable of differentiating to lymphoid and myeloid cell types.

Research on implications of epidemiological and mechanistic findings to risk assessment

The potential for leukemogenesis is of particular importance to the quantitative assessment of formaldehyde risks and its regulation as a risk to workers and the general population. Classic risk assessment dose response models for carcinogenesis tend to use linear “one-hit” models. The findings in the NCI cohort of a relationship of leukemia with highest peak exposure rather than standard dose measures potentially has implications for the dose extrapolation model. Similarly, the implications of a mechanism model which first leads to a malignant transformation of nasal pluripotential cells, and then subsequently to dislodge these cells, also needs to be explored.

Molecular events involved in formaldehyde carcinogenesis

Ridpath et al. (Ridpath et al., 2007) reported that cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde. They assessed the DNA damage response to plasma levels of formaldehyde (13 to 97 micromol/L) using chicken DT40 cells with targeted mutations in various DNA repair genes. Hypersensitivity to formaldehyde was detected in DT40 mutants deficient in the BRCA/FANC pathway, homologous recombination, or translesion DNA synthesis. Human cells deficient in FANCC and FANCG were also hypersensitive to plasma levels of formaldehyde. These results indicate that the BRCA/FANC pathway is essential to counteract DNA-protein crosslinks caused by formaldehyde. Based on the results obtained in their study, the authors proposed that endogenous formaldehyde might have an effect on highly proliferating cells, such as bone marrow cells. Further, homologous recombination induced by formaldehyde in DNA-deficient cells may lead to leukemia-inducing translocations, a possibility that should be investigated.

Recently, Swenberg and colleagues (Lu et al., 2009) have produced findings which support the idea that formaldehyde may cause cancer by altering epigenetic regulation. Using mass spectrometry, the N-terminus of histone and lysine residues located in both the histone N-terminal tail and the globular fold domain were identified as binding sites for formaldehyde. The observation that only lysine residues without post-translational modification (PTM) can be attacked by formaldehyde indicates that PTM blocks the reaction between lysine and formaldehyde. Additionally, Lu et al. found that formaldehyde-induced Schiff bases on lysine residues could inhibit the formation of PTM on histones that may affect their function in gene regulation (Lu et al., 2009).

Research needs and recommendations

More molecular epidemiological studies examining the genotoxic effects of formaldehyde are needed (Zhang et al., 2009). For example, the only human studies performed to date showing elevated DPCs in the peripheral mononuclear cells of formaldehyde-exposed workers (Shaham et al., 1996; Shaham et al., 2003), need to be replicated due to the excessively high levels of DPCs reported in the controls.

Studies showing increased CA in humans have a number of methodological weaknesses, including poor exposure assessment, non-current measurement of exposure and outcome, small sample size, etc, necessitating replication of the findings in better-designed studies (Bauchinger and Schmid 1985; Chebotarev et al., 1986; Vozenilkova et al., 1991; Kitaeva et al., 1996; He, Jin et al., 1998; Lazutka et al., 1999). Despite these limitations, many studies report positive results indicating that formaldehyde is able to cause a range of genotoxic effects in the DNA and chromosomes of lymphocytes, and possibly other bone marrow-derived cells. Recent studies have investigated the potential mechanisms underlying DNA damage (Wang et al., 2007) and the DNA repair pathways (Ridpath et al., 2007) induced by formaldehyde.

It is hypothesized that the induction of DPCs by endogenous formaldehyde plays a critical role in the initiation of progressive bone marrow failure or predisposition to malignant tumors in Fanconi anemia patients. Exposure to exogenous sources of formaldehyde could push susceptible individuals into a dangerous zone in which genotoxic levels of DPC are induced. One of the big limitations to this hypothesis is the uncertainty over whether exogenous formaldehyde can reach the bone marrow. A mouse model, such as a Fanconi anemia deficient mouse could be a useful tool to better understand whether formaldehyde causes bone morrow toxicity by inhalation. Such a model would also allow us to investigate the potential role of endogenous formaldehyde on the etiology of acute myeloid leukemia in Fanconi anemia patients.

Although leukemia arises from damaged blood stem cells, little is known about the sensitivity of blood stem cells to formaldehyde and whether formaldehyde produces mutations related to leukemia in these cells. As discussed above, some studies report that formaldehyde produces chromosome damage in circulating blood cells of exposed humans, but it is not known if it

also does so in blood progenitor/stem cells or how consistent its effects are. Studies of $CD34^+$ cells exposed to formaldehyde in culture were suggested to address these issues.

Molecular epidemiology/biomarker studies of occupationally-exposed populations should be designed to address whether formaldehyde causes hematotoxicity, as it has not been definitively shown. A biomarker discovery approach should be applied in these studies using toxicogenomic, proteomic, and metabolomic tools. Leukemia-specific markers, such as chromosome translocations, should be examined in peripheral blood leukocytes and progenitor cells. Together, the study of leukemia-specific chromosome damage in cultured $CD34^+$ cells and of hematotoxicity in human populations will strengthen the biological plausibility and help to elucidate a mode of action.

Further studies in transgenic mice with DNA repair deficiencies is one possible future research direction. The determination of whether adducts are formed in the bone marrow of mice treated with formaldehyde-generating chemicals and whether the FANC/BRCA pathway is involved in the response to such damage in the bone marrow could help to determine if exogenous formaldehyde reaches the bone marrow. The potential application of the *Pig-A* mutation assay and/or a knock-out mouse model to clarify the mechanisms of formaldehyde-induced leukemogenesis is also proposed. These various research approaches will provide lines of evidence that can be used to ascertain causality.

Finally, because few tools are available to measure formaldehyde exposure internally, chemical-specific methodologies to specifically detect adducts of formaldehyde to DNA and proteins in blood, bone marrow and other target tissues are urgently needed. The recently developed assay for the formaldehyde-DNA adduct N^6-HOMe-dAdo in leukocytes is one example. The ability to accurately measure formaldehyde exposure, particularly if the marker was linked to a potential pathway of formaldehyde carcinogenesis, would address one of the key aspects of causality judgment in risk assessment, that of biologic gradient or exposure-response relationship. According to this relationship, increasing effects associated with greater exposure strongly suggest cause and effect. Swenberg and colleagues recently demonstrated that formaldehyde can cross-link GSH with DNA by forming *S*-[1-(N^2-deoxyguanosinyl)methyl]glutathione in the test tube, and proposed utilizing this adduct as a biomarker of formaldehyde exposure and toxicity (Lu et al., 2009). Further, the authors proposed that this adduct, coupled with isotope-labeled formaldehyde, could differentiate between endogenous and exogenous origin of formaldehyde-derived adducts.

In conclusion, much of the uncertainty in the risk assessment of formaldehyde and leukemia could be limited through a concerted effort among all associated disciplines in the design of future studies. Risk assessment does not weigh one type of evidence against another, but rather weighs all of the evidence taken together. Research that strengthens the consistency, strength, specificity, exposure-response relationship, or biological plausibility of an observed association, or that provides experimental evidence in human populations, will aid in making supportable causality judgments.

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Acetaldehyde

by Tania Carreón-Valencia PhD

Citation for most recent IARC review:

IARC Monograph 71, 1999

Current evaluation

Conclusion from the previous Monograph:

Acetaldehyde is *possibly carcinogenic to humans (Group 2B)* because there is *inadequate evidence* in humans for the carcinogenicity of acetaldehyde, and there is *sufficient evidence* in experimental animals for the carcinogenicity of acetaldehyde.

Exposure and biomonitoring

Acetaldehyde is primarily used as an intermediate in the manufacturing of acetic acid, flavorings, aniline dyes, plastics and synthetic rubber, in some fuel compounds and in the manufacture of numerous other products (Muttray et al., 2009). Acetaldehyde is also a ubiquitous indoor and outdoor air pollutant. Sources of acetaldehyde are industrial burning processes, traffic emissions or emissions emerging from the combustion of wood. It is also a component of tobacco smoke. Acetaldehyde is also an endogenous metabolite produced from ethanol. During alcohol consumption, acetaldehyde is formed in the digestive system by microbes in normal gut and flora. Ethanol oxidation also occurs, to a limited extent, in nearby tissues. As ethanol is distributed to the aqueous phase of the human body, it is metabolized continuously to acetaldehyde as long as it remains in the blood and saliva, leading to its accumulation in the saliva and intestinal contents during and after the consumption of alcohol (Lachenmeier et al., 2009a).

Lachenmeier and Sohnius (2008) analyzed and evaluated a large sample of different alcoholic beverages. Beer (9 ± 7 mg/l, range 0–63 mg/l) had significantly lower acetaldehyde contents than wine (34 ± 34 mg/l, range 0–211 mg/l), or spirits (66 ± 101 mg/l, range 0–1159 mg/l). The highest acetaldehyde concentrations were generally found in fortified wines (118 ± 120 mg/l, range 12–800 mg/l). Foods and beverages produced or preserved by fermentation may contain small amounts of ethanol and mutagenic (>100 μM) concentrations of acetaldehyde. These include dairy products (i.e. yogurts), fermented soy products (e.g., soy sauces), tofu products, fermented vegetables (e.g., Chinese pickles and kimchi), vinegar and homemade beers. Many fruits, such as apples, may have their own metabolic pathways for acetaldehyde production. In addition, acetaldehyde is used widely as a food additive and aroma agent (Salaspuro 2009a).

Occupational exposure

Occupational exposure to acetaldehyde may occur by inhalation and skin exposure at workplaces where this compound is produced or used. Acetaldehyde has also been detected in cutting fluids. It is also one of the major aldehyde components in tobacco smoke (Muttray et al., 2009). The OSHA Permissible Exposure Limit

In an investigation to evaluate environmental tobacco smoke exposure among casino dealers in three U.S. casinos, NIOSH (2009a) found that the levels of acetaldehyde in full-shift personal breathing zone ranged from 4.8 to 17.0 $\mu g/m^3$. For all three casinos combined, the geometric mean for personal breathing zone was 10.2 $\mu g/m^3$. The area air samples ranged in concentration from below the minimum detectable concentration to 20 $\mu g/m^3$. For all three casinos, the geometric mean for area acetaldehyde was 11.0 $\mu g/m^3$.

Three recent studies have reported acetaldehyde air concentrations in facilities that produce and use flavorings. In a facility that manufactures flavorings, modified dairy products and bacterial additives, NIOSH (2007) reported mean, full-shift time-weighted average (TWA) acetaldehyde air concentrations of 0.14 ppm in the powder production room, 0.07 ppm in the liquid production room, and 0.07 ppm in the pre-production corridor. A task-based acetaldehyde air concentration of 0.19 ppm was measured during pouring and mixing of ingredients for a fruit flavor in the liquid production room. In a follow-up visit, mean full-shift TWA acetaldehyde air concentrations were 0.44 ppm in the spray-drying room, 0.343 ppm in the powder production room, 0.273 ppm in the liquid production room, and 0.029 ppm in the pre-production corridor. The highest task-based acetaldehyde air concentration (4.02 ppm) was measured during packaging of a powdered dairy-flavored product in the powder production room.

In a small popcorn popping plant, NIOSH (2009b) reported that acetaldehyde concentrations in air were less than the detectable (0.09 ppm) or quantifiable (0.15 ppm) concentrations.

In a flavoring manufacturing plant in The Netherlands, control measures taken to enclose the process, led to a reduction in air concentrations from 7.6 to 0.7 mg/m^3 (geometric mean). Personal task-based sampling among process operators ranged from 0.2 to 14 mg/m^3 acetaldehyde (van Rooy et al., 2007).

Environmental exposures

Lachenmeier et al. (2009a) estimated exposure to acetaldehyde due to alcoholic beverage consumption, based on products from the EU. According to these estimates, a 60-kg person with mean alcoholic beverage consumption in Europe and a mean content of acetaldehyde would be exposed to 0.112 mg/kg body weight/day of acetaldehyde. A heavy drinker (99^{th} percentile) exposed to a mean content of acetaldehyde would be exposed to 0.305 mg/kg/day. An average drinker consuming beverages with high content of acetaldehyde (99^{th} percentile) would be exposed to 0.56 mg/kg/day. Lastly, a heavy drinker of beverages with high acetaldehyde content would be exposed to 1.639 mg/kg/day of acetaldehyde.

Furthermore, Lachenmeier et al. (2009b) have estimated that twice-daily use of alcohol-containing mouthwashes leads to a systemic acetaldehyde exposure of 0.26 µg/kg/day on average.

Acetaldehyde levels in drinking water were measured through a U.S. EPA Information Collection Rule (ICR) effort to gather water quality and treatment information in 500 treatment plants over an 18 month period. Acetaldehyde was observed at sub- to low-µg/L levels; the maximum level of 11 mg/L was measured in ozonated drinking water, but levels were generally below the detection limit (<5 mg/L) in chlorine dioxide-treated waters (Richardson, 2007).

In a recently published study, McCarthy et al. (2009) compiled 3-year averages for ambient measurement of air toxics collected at monitoring locations in the United States from 2003 through 2005. They used national distributions of risk-weighted concentrations to identify the air toxics of most concern. The authors found that concentrations of acetaldehyde were above the 10^{-6} cancer risk at 99% of 163 sites nationally with a high degree of confidence.

A recent investigation of indoor airborne aldehyde levels in the bedrooms of 196 French infants, showed the presence of acetaldehyde in most dwellings, with geometric mean levels (geometric standard deviation) of 8.9 (1.8) µg/m^3 (Dassonville et al., 2009).

Cancer in humans: (*inadequate***,** Vol 71, 1999)

IARC Monograph 71 (1999) included a case series of nine cancers (five bronchial tumors and two carcinomas of the oral cavity) among workers in an acetaldehyde dimerization plant in the German Democratic Republic. All cases were smokers. Main exposures included acetaldol (3-hydroxybutanal), acetaldehyde, butyraldehyde, crotonaldehyde, and other aldehydes, as well as traces of acrolein. The relative frequencies of these tumors were reported to be higher than those expected in the GDR, but the Working Group noted the mixed exposures, the small number of cases and the poorly defined exposed population. Other epidemiologic studies of cancer in populations occupationally-exposed to acetaldehyde were not identified.

The most compelling evidence of the carcinogenicity of acetaldehyde is provided by studies of alcohol drinkers. Acetaldehyde is the first metabolite of ethanol oxidation. The conversion from ethanol to acetaldehyde is catalyzed by the enzyme alcohol dehydrogenase (ADH), and the subsequent oxidation from acetaldehyde to acetate is catalyzed by the enzyme aldehyde dehydrogenase (ALDH).

$$\text{Ethanol} \xrightarrow{\text{ADH}} \text{Acetaldehyde} \xrightarrow{\text{ALDH}} \text{Acetate}$$

The ALDH2 gene is polymorphic. The variant allele *ALDH2*2* encodes an enzyme with a deficient ability to detoxify acetaldehyde. After consumption of alcohol, homozygous carriers of the allele (*ALDH2*2-2*) (<5% of Asians) develop a severe flushing reaction, physical discomfort and other toxic responses. Most of them rarely consume alcohol. The heterozygous carriers (*ALDH2*1-2*) have about 10% residual ALDH activity (30-50% of Asians), and therefore less severe adverse effects. They may become heavy drinkers and alcoholics, and studies have shown that they have markedly elevated concentrations of acetaldehyde in their saliva after consumption of ethanol (Yokohama et al., 2008). Several studies conducted in Japan, China and Taiwan have shown increased risk of cancer of the esophagus associated with ALDH2 deficiency and alcohol consumption (Salaspuro 2009b). A study from Taiwan, published after the Monograph 96 meeting that evaluated the carcinogenicity of alcoholic beverages, included 406 cases with esophageal squamous cell carcinoma (ESCC) and 656 matched controls. Compared to non-drinkers, the odds ratio for

*ALDH2*1-2* carriers drinking at a low to moderate rate (0.1-30 g/day) was 14.5 (95% CI 7.1-29.6), and for *ALDH2*2-2* carriers was 17.3 (95% CI 1.4-213.7), whereas the risk for those with the active isoform (*ALDH2*1-1*) was 2.2 (95% CI 1.1-4.5). The risk for the ALDH2 heterozygous drinkers of over 30 g/day was 102.5 (95% CI 38.3-274.8) (Lee et al., 2008).

Increased risks for gastric cancer, alcohol consumption and ALDH2 deficiency have also been reported in Asian populations. The association with colorectal cancer has not been consistently demonstrated (Salaspuro, 2009b).

Another polymorphic ALDH2 variant has been identified in Poland, and the encoded enzyme may be functionally deficient in eliminating acetaldehyde. A case-control study reported a stomach cancer risk of 2.6 (95% CI 1.0-6.9) among heterozygous carriers that drank alcohol daily, and of 3.7 (95% CI 1.2-11.2) among those with 40 or more drink-years (Zhang, 2007).

The 2 ADH enzymes responsible for most of ethanol metabolism are ADH1B and ADH1C. The *ADH1B*2* and the *ADH1C*1* alleles encode enzymes that result in fast metabolism of ethanol. *ADH1B*2* is highly prevalent in Asians. Studies of alcohol drinkers in Japan, China, Thailand and Central Europe have shown that the *ADH1B*1-1* genotype (enzyme with 1/40 activity of the normal) is a strong risk factor for esophageal and oropharyngolaryngeal cancers (Salaspuro, 2009b). It appears that after these individuals consume alcoholic beverages, ethanol remains elevated in blood and saliva for a longer time than in those with the normal enzyme, resulting in a longer exposure.

Among Caucasians, ADH1C is the main enzyme involved in alcohol metabolism. The *ADH1C*1* allele has been shown to increase the risk of esophageal, hepatocellular and head and neck cancers in some studies but not in others (Boffetta and Hashibe, 2006). This lack of consistency has been explained by differences in the geographic distribution of ADH1C genotypes in Europe and by the fact that negative studies have generally included controls and patients with little or moderate alcohol consumption (Homann et al., 2006).

Several studies have shown that the risk for upper digestive tract cancer is highest among ALDH2-deficient Asian drinkers who simultaneously have the low-activity *ADHB*1-1* genotype (Salaspuro, 2009b). In a recent case-control study by Lee et al. (2008) in Taiwan, moderate alcohol users with the *ADH1B*1-1* genotype and the *ALDH2*2* allele had an increased risk of esophageal cancer (OR 37.5, 95% CI 10.4-134.7), and the risk was stronger for those drinking >30g/day (OR 382.3, 95% CI 47.4-3084.9). Furthermore, smoking had an independent and interactive effect on esophageal cancer risk among *ADH1B*1* and *ALDH2*2* carriers.

The evidence suggests that an increased risk for upper digestive tract cancer is associated with both a deficient ability to detoxify acetaldehyde and an enhanced or even deficient ability to produce it.

Cancer in experimental animals: (*sufficient***,** Vol 71, 1999)

Oral administration of acetaldehyde has resulted in the development of tumors in experimental animals. After Monograph 71, a lifetime study was conducted in female and male rats given drinking water containing acetaldehyde at concentrations of 0, 50, 250, 500, 15000 or 2500 mg/L. The study showed an increase in total malignant tumors and specific carcinogenic effects on various organs and tissues (Soffritti et al., 2002).

Acetaldehyde, when inhaled, causes nasopharyngeal and laryngeal carcinoma in rats and hamsters (Woutersen et al., 1984; 1986).

Mechanisms of carcinogenicity:

Acetaldehyde interferes with DNA synthesis and repair, and *in vitro* studies have shown that acetaldehyde causes cytogenetic abnormalities in eukaryotic cells. Acetaldehyde causes point mutations in the hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) locus in human lymphocytes, and induces sister chromatid exchanges and gross chromosomal aberrations. Acetaldehyde also binds to proteins, resulting in structural and functional alterations, such as enzymes involved in DNA repair (O6 methyl guanine methyltransferase) and DNA cytosine methylation, as well as glutathione, an important anti-oxidative peptide (Seitz and Stickel 2007).

Acetaldehyde binds to DNA, forming stable DNA adducts, and acetaldehyde DNA adducts have been found in alcohol consumers. The steady state level of DNA adducts, which can also be produced by reactive oxygen species (ROS), is influenced by various factors, including the activity of the anti-oxidative defense system, glutathione-*S*-transferase, the DNA repair system and apoptosis. Chronic ethanol ingestion may affect all of these mechanisms either directly or indirectly (Seitz and Stickel 2007).

A recent study showed that cells deficient in homologous recombination repair and Fanconi anemia like (KO40) cells were more sensitive to acetaldehyde, suggesting that these pathways are very important for the repair of acetaldehyde-induced lesions, confirming the evidence that this agent may induce DNA crosslinks (Mechilli, 2008).

Biomarkers of exposure:

No biomarkers of occupational exposure to acetaldehyde have been described in the literature.

Acetaldehyde has been measured in human saliva after ethanol consumption to demonstrate endogenous production of acetaldehyde after ingestion of ethanol (Homann, 1997). Acetaldehyde concentrations of 50–100 μM, which are known to be mutagenic, can be detected following the intake of 0.5 g alcohol per kg of body weight, equaling approximately half a bottle of wine. Salivary acetaldehyde concentrations are decreased after an antiseptic mouthwash by approximately 30–50%, underlining the importance of oral bacteria and poor oral hygiene in acetaldehyde generation (Seitz and Stickel 2007). It has also been used to demonstrate that use of alcohol-containing mouthwash increase salivary acetaldehyde levels to concentrations normally found after alcoholic beverage consumption (Lachenmeier, 2009b). Breath acetaldehyde has been used to investigate the production of acetaldehyde after ethanol ingestion. Additional research is necessary to standardize the technique used for breath sampling and to control the influence of the factors that are known to affect breath acetaldehyde determination (Tardif, 2007). Levels of acetaldehyde have been measured in blood and urine samples of alcohol consumers. The results showed an increase over time of free acetaldehyde, followed by a subsequent decrease. Acetaldehyde bound to biological components increased over time, suggesting that this is the mechanism by which acetaldehyde accumulates in the body as a result of chronic alcohol consumption (Tominaga, 2009).

The most abundant DNA adduct resulting from the reaction of acetaldehyde is N^2-ethylidene-2′-deoxyguanosine (N^2-EtidG). N^2-EtidG needs a reduction step to become a stable adduct,

N^2-ethyl-2′-deoxyguanosine (N^2-EtdG). Fang and Vaca (1995) reported levels of N^2-EtdG in Swedish drinkers and controls, and found higher adduct levels in lymphocytes of alcohol consumers compared with controls. They also found an increase of the same adducts in mice exposed to 10% alcohol in their drinking water.

α-Methyl-γ-OH-propano-deoxyguanosine is another DNA adduct with acetaldehyde that has been identified. As this adduct has been observed previously in DNA treated with crotonaldehyde, it is referred to as Cr-PdG. The formation of Cr-PdG adducts can be facilitated in the presence of basic amino acids, histones or polyamines. Relevant polyamine concentrations are present in tissues with hyper-regeneration. Chronic alcohol consumption results in mucosal hyperproliferation of the upper digestive tract, as well of the large intestine, probably due to the local toxic effect of highly concentrated acetaldehyde. In addition, high acetaldehyde concentrations are found in the saliva and colonic content following moderate alcohol consumption due to the bacterial oxidation of ethanol. As a consequence of high acetaldehyde concentrations in a hyper-regenerative environment, the generation of the highly-mutagenic Cr-PdG may be facilitated in these tissues (Seitz and Stickel 2007). Matsuda et al. (2006) reported that the level of acetaldehyde-derived DNA adducts in Japanese alcoholics with the *ALDH2*1-2* genotype is much higher than that in alcoholics with the *ALDH2*1-1* genotype, indicating that the ALDH2 genotype plays a crucial role in the formation of acetaldehyde DNA adducts.

Biomarkers of effect

Two genetic markers, chromosome aberrations and micronuclei, were used to evaluate genetic damage in peripheral lymphocytes from alcoholics, abstinent alcoholics, and controls. A statistically significant increase was observed in the frequencies of chromosomal aberrations and micronuclei in lymphocytes of alcoholics as compared both with controls and abstinent alcoholics. However, no correlation was found between the length of alcohol abuse and the frequencies of either biomarkers in alcoholics. Chromosomal aberrations and micronuclei frequencies in abstinent alcoholics were similar than those in controls (Maffei et al., 2002). In addition, sister chromatid exchanges and micronuclei were more frequently found in lymphocytes of habitual drinkers with *ALDH2*1-2* than in lymphocytes of drinkers with fully active ALDH2 (Seitz and Stickel 2007).

Research needs and recommendations:

An epidemiologic study that evaluates the association between acetaldehyde exposure and upper digestive tract cancer will require evaluation of all potential sources of exposure to acetaldehyde, to address their contribution to the overall risk.

Different study designs could be proposed for such a study. Prospective studies could be designed to assess all sources of exposure using a combination of questionnaires and environmental and biological monitoring, as well as genotyping to identify individuals with ALDH2, ADH1C, and ADH1B deficiencies. However, given the long induction and latency of most cancers, such a study may not be feasible. Retrospective studies, conversely, have the limitation that exposures have to be evaluated retrospectively, increasing the potential for misclassification. Alternatively, acetaldehyde-derived DNA adducts could be used as biomarkers of exposure to acetaldehyde (Matsuda et al., 2006).

On the other hand, there is substantial evidence that acetaldehyde, the first product of ethanol metabolism, is predominantly responsible for carcinogenesis of alcoholic beverages. Numerous epidemiologic studies in alcohol drinkers with ALDH2 deficiency or low ADH1B activity described above, strongly suggest that acetaldehyde derived from the metabolism of ethanol contributes towards causing upper digestive tract cancers. This notion is also supported by two meta-analyses that used a Mendelian randomization approach (Boccia et al., 2009) and a recent large-scale case-control study that reported a multiplicative combined risk for esophageal cancer among alcohol and tobacco consumers, who were low ADH1B and ALDH2-deficient carriers (Lee et al., 2008).

The IARC Working Group that evaluated the carcinogenicity of alcoholic beverages (2007, Monograph 96) concluded that "acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to causing malignant esophageal tumors" (Baan, 2007). Furthermore, recent risk assessments that consider individual sources of exposure such as acetaldehyde in alcoholic beverages, acetaldehyde in saliva after alcohol drinking and cigarette smoking, acetaldehyde levels in foods and beverages such as yogurt, homemade beer and apples, have concluded that the lifetime cancer risks for many of these sources of exposure greatly exceed the usual limits for cancer risks from the environment ($1:10^4$-$1:10^6$). Acetaldehyde exposure is cumulative and in some cases synergistic (as occurs with alcohol exposure and smoking) (Salaspuro, 2009a). Exposure scenarios that consider multiple sources of exposure and genetic deficiencies in alcohol metabolism convey increased risks. It is thus recommended that the IARC classification of acetaldehyde is reviewed in a Monograph meeting.

Selected relevant publications since IARC review:

Boccia S, Hashibe M, Gallì P, De Feo E, Asakage T, Hashimoto T, et al. Aldehyde dehydrogenase 2 and head and neck cancer: A meta-analysis implementing a mendelian randomization approach. *Cancer Epidemiol Biomark Prev* 2009; 18: 248-254.

Lachenmeier DW, Kanteres F, Rehm J. Carcinogenicity of acetaldehyde in alcoholic beverages: Risk assessment outside ethanol metabolism. *Addiction* 2009; 104: 533-550.

Lee CH, Lee JM, Wu DC, Goan YG, Chou SH, Wu IC, et al. Carcinogenetic impact of adh1b and aldh2 genes on squamous cell carcinoma risk of the esophagus with regard to the consumption of alcohol, tobacco and betel quid. *Int J Cancer* 2008; 122: 1347-1356.

Matsuda T, Yabushita H, Kanaly RA, Shibutani S, Yokoyama A. Increased DNA damage in aldh2-deficient alcoholics. *Chem Res Toxicol* 2006; 19: 1374-1378.

Muttray A, Gosepath J, Brieger J, Faldum A, Pribisz A, Mayer-Popken O, et al. No acute effects of an exposure to 50 ppm acetaldehyde on the upper airways. *Int Arch Occup Environ Health* 2009; 82: 481-488.

Salaspuro M. Acetaldehyde as a common denominator and cumulative carcinogen in digestive tract cancers. *Scand J Gastroenterol* 2009a [Epub ahead of print].

Salaspuro M. Acetaldehyde: A cumulative carcinogen in humans. *Addiction* 2009b; 104: 551-553.

Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007; 7: 599-612.

Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann N Y Acad Sci* 2002; 982: 87-105.

Dichloromethane, methylene chloride (DCM)

by Jane Caldwell PhD and Ruth Lunn DrPH

Citation for most recent IARC review

IARC Monographs 71, 1999

Current evaluation

Conclusion from the previous Monograph: Dichloromethane (DCM) is *possibly carcinogenic to human*s (Group 2B) based on sufficient evidence in experimental animals [lung and liver tumors in mice exposed by inhalation, and mammary tumors in rats (both sexes) exposed by inhalation].

Exposure and biomonitoring

Exposure

DCM is used primarily as a solvent in paint removers, degreasers, aerosol products and the manufacture of foam polymers. Production is estimated to be on the order of 2 X 10^8 kg/year in the United States (Watanabe et al., 2007 reporting from http:// www. atsdr.cdc. gov/tp14-c4.pdf). Exposure occurs during the manufacturing and use of consumer products. Occupation exposure occurs through its use as a degreaser, paint remover, aerosol propellant, blowing agent for polymer foam, and as a solvent in the textile industry, photographic film production (cellulose triacetate). The general public can be exposed from releases of DCM into the ambient air and water. Workers employed in furniture refinishing or furniture stripping are also exposed to DCM. The NIOSHTIC-2 database (NIOSH, 2009) contains multiple entries for reports involving methylene exposure and furniture stripping. Sources of exposures in indoor air come from spray painting paint removal and metal degreasing. DCM has also been found in some foods.

Biomononitoring

Exposure biomarkers

The available data on biomarkers of exposure for DCM are limited, and thus represent a major research gap. Three studies of DCM-exposed workers (ranging from 20 to 96 workers) have reported a positive correlation with urinary DCM (although small amounts) and time-weighted average DCM in the breathing-zone air of the workers (reviewed by Imbriani and Ghittori, 2005). No sex differences were observed. Ukai et al. (1998) stated that urinary DCM assays were sensitive enough to separate workers exposed to 10 ppm from non-exposed workers. Sakai et al. (2002) reported that urinary DCM levels increased with the start of exposure and decreased during lunch and dinner breaks in subjects with multiple samples.

The biological half life was estimated to be 210 to 410 minutes. Urinary levels of DCM did not differ according to *GST-T1* (a glutathione *S*-transferase of the theta class) genotypes, but a lower correlation (not statistically significant) was observed among workers with the cytochrome P450 (*CYP) 2E1 c1* allele than among the c2 allele. DCM is very volatile and thus sampling and storage conditions of biological fluids are important.

Biomarkers of effect

A dose-response relationship was observed between ambient levels of DCM (8-hour TWA) and carboxyhemoglobin concentrations among cellulose triacetate production workers who were non-smokers (Amsel et al., 2001). The formation of carboxyhemoglobin has been proposed to be a potential marker for estimating DCM exposure levels (Shusterman et al., 1990).

Human: Cancer in humans

(inadequate, Volume 71, 1999)

The previous IARC Monograph (71, 1999) reviewed seven cohort studies (photography film industry, textile fiber manufacturing industry and aircraft maintenance workers) and three case-control studies. The studies were limited by small numbers of exposed cases and few studies were able to evaluate exposure-response relationships. Excesses of several cancers were found in one or two cohort studies; pancreatic and breast cancer in two studies, and non-Hodgkin lymphoma, multiple myeloma, and cancer of the brain, cervix, prostate, liver and bile duct in one study. The case-control studies reported associations with astrocytic brain cancer, breast cancer, and rectal cancer; the risk of astrocytic brain cancer increased with increasing probability of exposure, duration of exposure and average intensity, but not cumulative exposure to DCM. The Working Group concluded that no type of cancer was elevated across studies to make a causal interpretation credible.

Since the 1999 IARC review, several more epidemiological studies have been published. These include an update (and/or expansion) of two of the cohort studies (Hearne and Pifer, 1999; Radican et al., 2009), one new cohort study (Goldberg and Thériault, 1994a, 1994b), an update of one of the case-control studies (Dumas et al., 2000), and four new case-control studies: CNS (Cocco et al., 1999), renal cell cancer (Dosemeci et al., 1999), childhood leukemia (Infante-Rivard et al., 2005), and lymphoma (Seidler et al., 2007). ~~The~~ Table 1 summarizes studies evaluating cancer and DCM exposure in tabular format. [This table is an updated version of the supplemental tables to the review by Ruder (2006)]. In addition, Ojajävi et al. (2001) conducted a meta-analysis of exposure to DCM and pancreatic cancer using four of the cohort studies that were part of the 1999 IARC review. The authors reported a meta-relative risk (MRR) of 1.42, 95% CI = 0.80 to 2.53.

Cohort studies

Hearne and Pifer (1999) reported the findings of two overlapping cohorts of photograph film-manufacturing workers at Eastman Kodak. [Cohort I was an update of the cohort described by Hearne et al., (1990)]. Statistically non-significant increased SMRs were reported for several cancers including stomach, brain and CNS, colorectal (only one cohort), pancreatic (only one cohort), leukemia, and Hodgkin lymphoma. Risks of leukemia ($P = 0.01$) (both cohorts) and pancreatic cancer ($P = 0.08$) (Cohort 1) increased with increasing cumulative

exposure to DCM. In an update of aircraft maintenance workers exposed to solvents, increased risks were observed for non-Hodgkin lymphoma and multiple myeloma among men, and breast cancer among women, confirming the findings from the earlier cohort (Radican et al., 2009). The third cohort study found statistically significant increased risks for (1) non-Hodgkin lymphoma among textile workers employed in the extrusion units (acetate, DCM or polypropylene depending on the time period) (Goldberg and Thériault, 1994a), and (2) colon cancer among male workers in the polypropylene and cellulose triacetate extrusion unit (Goldbert and Thériault, 1994b). Risks for both cancers increased with increasing exposure duration (This study did not measure exposure to DCM but the authors stated it was used during extrusion of cellulose triacetate.)

Case-control studies

Increased risks were found for CNS cancer (although risks did not increase with increasing probability or intensity of exposure) (Cocci et al., 1999), rectal cancer (with substantial exposure) (Dumas et al., 2000), and lymphoma (at the highest exposure level) (Seider et al., 2007), but not for renal cell cancer (Dosemeci et al., 1999). Increased risks (although not statistically significant) were also found for childhood leukemia and maternal exposure to DCM in a population based case-control study (Infante-Rivard et al., 2005).

In summary, excesses of cancer (lymphohemaopoietic, brain or CNS, pancreas, and breast) have been found in some studies but the findings for a specific site are not consistent across studies. The studies published since the IARC review provide some additional support for an association between exposure to DCM and lymphohematopoietic cancer. There is some site-concordance with animal studies for brain and breast cancer. The cohort study by Hearne and Pifer (1999) is probably the most informative study because it was able to evaluate exposure-response relationships. Most of the tumor sites of interests are rare or uncommon tumors, and the available cohort studies do not provide the power to detect these tumor. Few studies included adequate numbers of women, and thus there is very limited information for the evaluation of breast cancer. Some of the cohorts are young cohorts. None of the studies used biomarkers to measure exposure.

Animal: Cancer data in experimental animals

(*sufficient***,** Vol 77, 1999)

No chronic studies of DCM for carcinogenicity in rats or mice have been conducted since the 1999 IARC evaluation. In $B6C3F_1$ mice there is evidence of liver and lung tumors with inhalation exposures in both sexes, and liver tumors with drinking water exposures in males. In rats there is evidence of a trend for increased risk of liver tumors in female F344 rats exposed via drinking water or inhalation. Additional tumorigenic potential of DCM is provided by increased benign mammary tumors following inhalation exposure and the presence of the relatively rare astrocytoma or glioma tumors at relatively low exposure concentrations in rats. Although not as strong as mouse data, on the whole the rat data provide supporting evidence of carcinogenicity.

Mechanisms of carcinogenicity

Previously no genetic and related effects data were noted by IARC to be available in humans. DCM is positive for mutagenicity in a number of *Salmonella typhimurium* strains with and

without addition of exogenous metabolic activation. In mammalian systems, studies published before 1985 were primarily negative. In more recent publications, positive results have been reported for assays [DNA damage and HPRT mutations conducted in the mid 1990s by Graves et al., (1995, 1996)]. Since the IARC DCM evaluation, a few more *in vivo* studies have reported positive results (Rodriguez-Arnaiz, 1998; Sasaki et al., 1998). For *in vivo* genotoxicity, many more studies have been conducted in mice for lung and liver than in rat so it is difficult to link key events or results with positive cancer bioassays across rats and mice, which is a limitation of the database.

DCM is thought to be metabolized via two pathways, one involving *CYP2E1* and the other *GST-T1*. There is experimental evidence exists that indicates manipulation of the glutathione (GSH) pathway can alter toxicity (i.e., enhanced GST metabolism in bacteria results in greater genotoxicity. In humans, there is evidence of large individual variability in the rate of metabolism for both pathway; these include lifestyle factor induction of *CYP2E1,* and polymorphisms in *CYP2E1* and *GST-T1 (*e.g., the distribution *of GST-T1* polymorphisms varies among ethnic groups and were estimated to be 32% for wild (+/+), 48% +/- (heterozygote), and 20% -/- (null) among Caucasians) (Haber et al., 2001). Although concentrated in the liver, there is evidence of *CYP2E1* activity in the brain (Nishimura et al., 2003; Miksys and Tyndale, 2004). The variability in metabolism can be key for how epidemiological studies can examine and take into account DCM-induced toxicity or cancer.

Although the previous IARC evaluation placed emphasis on the GST pathway as the probable toxicity induction pathway, Landi et al. (2003) report that in primary cultures of human epithelial cells from 4 healthy human subjects there was DNA damage (comet assay) in 2 of the 4 cultures (2 subjects were *GST* + and 2 were *GST* -) after DCM exposure. However, there was no correlation with DNA damage and GST phenotype; GST activity was low in all cultures. Activity in half of this limited sample is suggestive of GST-independent genotoxicity. This finding, along with the positive genotoxicity assays without additional metabolic activation, raises issues regarding the proposed mode of action (MOA) of DCM. New literature on the metabolism of DCM also raises issues of which pathways, what actions of metabolites, and how manipulations of the GSH pathway are responsible for DCM carcinogenicity (Watanabe et al., 2007; Watanabe and Guengerich, 2006). Watanabe and Guengerich (2006) demonstrated that formyl chloride (a P450 metabolite of DCM) does not react with GSH. Watanabe et al. (2007) examined male and female rats and mice treated with DCM for GSH-linked DNA adducts using a relatively sensitive technique. Despite overcoming difficult technical problems, Watanabe and Guengerich (2007) reported no DCM GSH-adducts were detectable *in vivo*. More research needs to be conducted on these pathways and metabolites. Several PBPK models use assumptions about metabolism and the importance of these pathways in determination of the relevance of human risk. Uncertainties raised by the data gaps for pathway toxicity affect the accuracy of the models and inferences from them.

Research needs and recommendations

Human cancer studies

Identification of possible new cohorts for future epidemiologic studies should be undertaken. Such cohorts may include auto repair technicians (Enander et al., 2004) and furniture-

stripping workers (NIOSH, 2009). Larger or multi-plant cohort studies, that include women, need to be conducted with rigorous exposure assessment that allows for the evaluation of exposure response relationships. The studies should use internal or nested case control analyses to help reduce potential confounding. Case-control studies should be conducted for lymphohematopoietic cancer, and cancers of the breast and brain. The working group is aware of several large brain case-control studies (NCI, NIOSH, Interphone), which will be analyzed in the next year or two for an association between exposure to chlorinated solvents and risk of brain cancer (Inskip et al., 2001; Ruder et al., 2006; Cardis et al., 2007).

Ideally the studies should look at cancer incidence, especially when looking at lymphomas. If possible, medical records, or data on molecular markers of lymphoma should be obtained to provide more information on the diagnosis of lymphohematopoietic cancers (For further discussion, see the TCE review). The validation of urinary DCM as a useful biomarker of exposure needs to be done for its use in epidemiological studies.

A meta-analyses approach is also warranted given the modest relative risk estimates and the relative rarity of the cancers observed, and therefore the limited statistical power of individual studies.

Genetic susceptibility studies

Studies looking at genetic susceptibility are useful for understanding mechanisms and increasing the power to detect an effect. Candidate genes include *GST-T1* and *CYP2E1*. Large studies are needed to be able to detect gene-environment interactions. Other candidate genes for genetic susceptibility includes those involve in regulating immune function. Studies should also be conducted using entire genome scans to identify new susceptibility genes.

Genetic-related genomic damage studies

Studies evaluating genetic damage (such as adducts, mutations, chromosomal aberrations, micronuclei and sister chromatid exchange) are also needed. These studies should also include look at genetic susceptibility such as *GST-T1* and *CYP2E1*. These studies could be conducted among the Eastman Kodak workers in the cohorts established by Hearne and Pifer (1999) since this study had sampling data for DCM and was able to calculate cumulative exposure.

Mechanistic concerns

Research needed includes the (1) identification and role of metabolic pathways involved in carcinogenicity, (2) identification of modes of actions from the numerous toxicologically active metabolites of the solvents, and (3) development and validation of physiologically based pharmacokinetic (PBPK) modeling. Measurements of DCM levels need to be a part of future studies with further development of biomarkers.

Immunologic mechanism may be involved in lymphomagenesis from solvents (Vineis et al., 2007) and this should also be an area of future research. Brain tumor is also a potential target for DCM, and Perc (tetrachloroethylene), and has not been adequately studied for TCE.

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TABLE 1. Cancer Studies Evaluating Occupational Exposure To DMC (adapted from Ruder 2006)

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Bond et al. 1990	Dow Chemical	Case-control nested in cohort	44 cases, 1,888 controls	Liver & biliary tract ca death	All employed in chemical production 1940-82, VS to 1982, controls randomly selected from cohort of 21,437	Work histories to classify by exp potential to 11 substances (any/none)	Carbon tet, chloroform, PCE, 1,1,2-trichloro-ethane, vinyl chloride, PCB, dioxins, TCP, ethylene dibromide & dichloride	Exp: 4.6% cases, 8.9% controls RR 0.8 (0.2-3.6)	Workers could have had multiple exp, exp not quantified or qualified
Cantor et al. 1995	US NCI	Case-control (death certifi-cates)	33,509 cases, 117,794 controls	Breast cancer mortality in women	Died 1984-1989 in state where job and industry were coded, 4 controls with noncancer death/case	Job-exposure matrix: probability of exposure & level of exposure (0-4)	30 other substances	Whites: 5,416 deaths exp level 1 adj OR 0.95 (0.90-0.98), 1,298 deaths level 2 adj OR 1.04 (0.97-1.1), 1,713 deaths level 3 adj OR 1.17 (1.1-1.3). Blacks: 1,552 deaths level 1 adj OR 1.01 (0.9-1.1), 238 deaths level 2 adj OR 1.12 (0.9-1.3), 232 deaths level 3 adj OR 1.46 (1.2-1.7)	"Usual" job reporting could be biased. No data about known breast ca risk factors. Exp inferred from job & industry coding. Low number with high probability exp 5 cases 37 controls (same numbers as for C tet)
Cocco et al. 1999	US NCI	Case-control (death certifi-cates)	12,980 cases, 51, 920 controls	CNS cancer in women	Died 1984-1992 in state where job and industry were coded, 4 controls with noncancer nonneurological COD/case	JEM for probability (0-3) & intensity (0-3) of exp	Solvents, chlorinated aliphatic hydrocarbons, EMF, lead, benzene, PAH, nitrosamines, pesticides, public contact	Any/none: CNS cancer OR 1.2 (1.1-1.3), meningioma OR 1.2 (0.7-2.2)	"Usual" job reporting could be biased. Exp inferred from job & industry coding
Dosemeci et al. 1999	US NCI	Case-control	438 cases, 687 controls	RCC diagnosis	RCC dx July 1988-1990, population-based controls	Industry, job linked to JEM	Other chlorinated solvents	Any/none OR adj age, gender, smoking, hyper-tension, diuretics, BMI: all 0.87 (0.6-1.2), men 0.85 (0.6-1.2), women 0.95 (0.4-2.2)	Gives % exp but not number exp. Est 70 cases 124 controls exp. Multiple exp

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Heineman et al. 1994, Gomez et al. 1994, Cocco et al. 1994	US NCI	Case-control	300 male cases, 320 male controls	Brain tumor mortality	Died 1978-1981 hospital-confirmed astrocytic brain tumor or other causes (minus CVD, epilepsy, suicide, homicide, cirrhosis, some ca), NOK interviewed	JEM for probablity of exp, cum= duration weighted by probability. 38% estimated exp. to Me Cl	JEMs also for other chlorinated solvents	Low cum exp OR adj age, location 0.9 (0.5-1.5), med adj OR 1.9 (1.1-3.2), high adj OR 1.2 (0.6-2.5), test for trend not statistically significant Using detailed coding: Exp probability low OR adj age, era exp, location 1.2 (0.7-3.0), med adj OR 1.5 (0.3-9.0), high adj OR 6.1 (1.1-43.8), test for trend not reported *test for trend calculated as p< 0.001)*	Low participation rate, Interviewed <50% (300/ 741 cases, 320/ 741 controls) Adjusting for exp by decade & doing detailed coding reduced numbers—24 exp cases v 104 or 121—but apparently also reduced exp misclassification & refined risk estimates
Dumas et al. 2000 (update of Siemiatycki et al. 1991)	Québec Institute Research Occup Health Safety & Health Research Funds; Canada Health, Natl Health Res-earch & Development, NCI	Case-control	257 cancer patients, 533 population controls	Cancer diagnosis	Males age 35-70, dx 9/79-6/85, resident in Montreal metropolitan area on electoral list	Reanalysis (rectal cancer only) adjusts for other exposures	Many	Rectal cancer: any exp OR 1.2 (0.5-2.8) substantial exp OR 3.8 (1.1-12.9)	Multiple exp. Low number with any/substantial exp (7/5 rectal ca cases)
Seidler et al. 2007	German Federal Office for Radiation Protection	Case-control	710 lymphoma cases, 710 population-based controls	Lymphoma	Cases dx in 6 German regions age 18-80; controls matched on region, gender, and age ± 1 year	Complete occupational history assigned intensity & frequency of DCM by case-status blinded industrial physician	TCE, carbon tet, Perc, benzene, toluene, xylene, styrene	RR for cumulative exposure RR for ppm-yr: 0 - 1.0 (ref); > 0–≤26.3 – 0.4 (0.2-1.0); 26.3–≤175–0.8 (0.3-1.9); >175–2.2 (0.4-11.6); test for trend p=0.4	No exposure measurements. Mixed exposures?

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Blair et al. 1998, Stewart et al. 1991 , Radican et al. 2008	US NCI	Retro-spective cohort mortality & cancer incidence	14,457 workers, 1,222 ever exposed to methylene chloride	Death/cancer diagnosis	Civilian aircraft maintenance workers s employed >1 y between 1952-956 at Hill AFB, VS to 1990	JEM (ever/never methylene chloride)	Also exposed to TCE, other solvents, all but 3,739 workers exposed to 1-25 chemicals	1998 update Any v. none: NHL 6 male deaths RR 3.0 (0.9-10.0); multiple myeloma 5 male deaths RR 3.4 (0.9-13.2); breast cancer 4 deaths RR 3.0 (1.0-8.8) 2008 update Any v. none: NHL 8 male deaths RR 2.0 (0.8-5.4); multiple myeloma 7 male deaths RR 2.6 (0.9-7.7); breast cancer 6 deaths RR 2.6 (1.0-5.7)	Mixed exposures. Evaluation by job title, not person
Gibbs et al. 1996	Hoechst Celanese	Retro-spective cohort	2187 male, 1024 female	Cause of death	Worked >3 mons cellulose triacetate fibers, on payroll 1970 or later, VS through 1989	High (350-700 ppm), low (50-100 ppm	methanol, acetone, finishing oils, cellulose fibers	Overall no excess mortality Prostate ca: 13 deaths high exp SMR 1.8 (0.95-3.1), 9 deaths low exp SMR 1.4 (0.6-2.7). SMR 2.1 among those with 20 y latency. Cervical ca: 6 deaths among exp SMR 3.2 (1.2-7.3)	Exposure 0-50 ppm & 100-350 ppm? Pre-NDI followup complete? % with 20 yrs since 1st exposure?

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Hearne & Pifer 1999 (update and expansion of Hearne et al. 1990)	Eastman Kodak	Retro-spective cohort	1311 men starting 1946, 1013 men working 1964-70	Cause of death	Cohort 1 began work in cellulose triacetate mfg ≥1946, median F/U 34 ys to 1994. Cohort 2 worked ≥1 yr 1964-70 in roll coating, median F/U35 ys	Cohort 1 mean exp 39 ppm 8 hr TWA, cohort 2 mean exp 26 ppm	Acetone, methanol	Brain ca, Hodgkins, elevated both cohorts. Combined: 10 deaths SMR 2.2 (1.0-4.1), 4 deaths SMR 2.4 (0.6-6.5), respectively Cohort 1- also excess mortality for stomach SMR = 1.5 (0.5-3.0), and leukemia (SMR 2.0 (0.9-4.1); Cohort 2- also excess cancer mortality for pancreas, SMR = 1.6 (0.7-3.1), esophageal, SMR = 1.3 (0.4-2.9), stomach, SMR = 14 (0.4-3.6). and leukemia, SMR = 1.4 (0.5-3.0) dose/response with career exposure, test for trend, p= 0.01 for leukemia (both cohorts), and 0.08 for pancreas (cohort 1)	Combined SMRs not presented
Lanes et al. 1993	Hoechst Celanese	Retro-spective cohort	1271 workers	Cause of death	Cellulose fiber production prep/extrusion depts. ≥3 mo between 1954-76, VS through 1990	8 hr TWA range LOD-1700 ppm. Medians in 3 areas 140, 280, 475 ppm	Acetone, methanol	172 deaths SMR 0.9 (0.8-1.0), 4 biliary-liver ca SMR 3.0 (0.8-7.6). 43 IHD SMR 0.9 (0.7-1.2)	No healthy worker effect. Can't compare to unexp (latter excluded from cohort)

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Goldberg & Thériault, 1994a, 1994b	Celanese Canada	Retro-spective cohort & nested case-control (colorectal cancer)	7,487 men, 2,724 women	Cause of death, colo-rectal cancer diagnosis	Synthetic textile production	Methylene chloride in 2/30 cellulose triactetate extrusion machines for 2/22 y & in closed-circuit cellulose acetate process. Est. low levels	Acetone, methanol	RR (v referents) all ca: cellulose triacetate unit 1.18 (0.4-2.0) all cancers, 4.28 (1.18-14.89) NHL; cellulose acetate fiber 1.32 (1.0-1.7) all cancers; all extrusion unit 3.85 (1.07-13.42) NHL Test for trend with employment duration, p < 0.001 for NHL for both cellulose acetate fiber and all extrusion units. OR colon ca: <5 y cellulose triacetate 7.43 (0.8-65), 5+ y 9.21 (1.3-64), <5 y cellulose acetate 0.59 (0.2-2.3), 5-9 y 2.16 (0.4-13), 10+ y 1.74 (0.4-7.9).	Multiple comparisons, no exp measurements
Ott et al. 1985	Dow Chemical	Retro-spective cohort	1,919 men total cohort 226 in chlorinat-ed methane group	Cause of death	Chemical manufacturing 1994-1969	Chlorinated methane employment category	Methyl chloride, chloroform, carbon tetrachloride,	20 deaths, 9 cancer deaths, Elevated mortality for digestive cancers 1.8 (0.7-4.0), and pancreatic cancer 3.3 (0.7-9.7)	Expected deaths obtain from company rates
Shannon et al. 1988	McMaster University Medical Center	Retro-spective cohort	203 females and	Cause of cancer (incidence)	Canadian lamp-manufacturing workers. Incidence cases: 1964 to 1982	Coiling and wire drawing	Trichloroethylene, strong acids (e.g., sulfuric, nitric acids) and metals (e.g., arsenic, chromium)	19 cancer cases, excess breast SIR = 2.0 (0.9-4.0), > 15 greater employment 3.2 (95% CI 1.1-7.5)	Initiated because of a cluster of 5 cancer cases in coiling wire drawing
Tomenson et al. 1997	ICI Chemicals & Polymers	Retro-spective cohort	1473 exp, 312 unexp	Cause of death	Cellulose triacetate production, men working 1946-88. VS to 1994	JEM to est cum exp for 70%	Not reported	47 deaths SMR 0.7 (0.5-0.99) in unexp, 287 deaths SMR 0.7 (0.7-0.8) in exp;, 15 IHD deaths SMR .7 (0.4-1.2) in unexp, 114 IHD deaths SMR 0.9 (0.8-1.1), increasing with increasing cum exp *Overall RR death exp/unexp 1, IHD RR 92/74=1.2*	30% no cum exp assignment

Trichloroethylene (TCE)

Jane Caldwell PhD, Ruth Lunn DrPH, and Avima Ruder PhD

Citation for most recent IARC review
IARC Monographs 63, 1995

Current evaluation
Conclusion from the previous Monograph: Trichloroethylene (TCE) is *probably carcinogenic to humans (Group 2A)* based on limited evidence in humans for the carcinogenicity of TCE and sufficient evidence in experimental animals for the carcinogenicity of TCE. In making the overall evaluation, the Working Group considered the following evidence: (i) although the hypothesis linking the formation of mouse liver tumors with peroxisome proliferation is plausible, trichloroethylene also induced tumors at other sites in mice and rats. (ii) several epidemiological studies showed elevated risks for cancer of the liver and biliary tract and for non-Hodgkin lymphoma (NHL).

Exposure and biomonitoring
TCE is a volatile compound with moderate water solubility. Most TCE produced today is used for metal degreasing, in a number of industries (Bakke et al., 2007). The highest environmental releases are to the air. Ambient air monitoring data suggests that levels have remained fairly constant since 1999 at about 0.3 μg/m^3. Indoor levels are commonly 3 or more times higher than outdoors due to releases from building materials and consumer products. TCE is one of the most common groundwater contaminants and the median level based on a large study by the U.S. Geological Survey for 1985-2001 is 0.15 μg/L (USGS, 2006). It has also been detected in a wide variety of foods in the 1-100 μg/kg range. None of the environmental sampling has been done using statistically based national surveys. However, a substantial amount of air and groundwater data has been collected allowing reasonably well supported estimates of typical daily intakes by the general U.S. population: inhalation - 13 μg/day and water ingestion - 0.2 μg/day. The limited food data suggests an intake of about 5 μg/day, but this must be considered preliminary (U.S. EPA, 2009a)

High exposures have occurred to various occupational groups. Bakke et al. (2007) reviewed occupational exposure to TCE and reported that the arithmetic mean (AM) of the measurements across all industries and decades was 38.2 ppm. The highest personal and area air levels were reported in vapor degreasing (AM of 44.6 ppm). Past studies of aircraft workers have shown short-term peak exposures in the hundreds of ppm (>500,000 μg/m^3) and long-term exposures in the low tens of ppm (>50,000 μg/m^3). Occupational exposures have likely decreased in recent years due to better release controls and improvements in worker protection. However, some of that protection relies on personal protective equipment, not always consistently used, rather than engineering controls.

Exposure to a variety of TCE-related compounds, which include metabolites of TCE and other parent compounds that produce similar metabolites, can alter or enhance TCE metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. Available estimates suggest that exposures to most of these TCE-related compounds are comparable to or greater than that to TCE itself.

Cancer in humans

(limited, vol 63, 1995)

Since the 1995 IARC review, there has been a plethora of publications evaluating TCE exposure and cancer in humans, including new cohort studies, updates of cohorts, case-control studies, review articles, and meta-analyses. Table 2 summarizes the case-control and cohort studies published since the IARC review in tabular format. [This is an updated version of the supplemental table to the review by Ruder (2006)]. Three reviews have summarized most of the recent literature (Ruder, 2006; Scott and Chiu, 2006; Wartenberg et al., 2000). Many of the new studies have more sophisticated exposure assessment and thus allow for more accurate classification of TCE exposed workers (Scott and Chiu, 2006).

Meta-analyses can be useful for evaluating risks for rare or uncommon cancers. Wartenberg et al. (2000) conducted a comprehensive review of over 80 studies and evaluated the evidence for over 20 cancer sites. The review categorized the cohort studies into tiers based on the quality of the exposure assessments. Average risks (separate for incidence and mortality) were calculated for multiple cancer sites for each tier as well as for the case-control studies. In addition, meta-analyses have been published for liver cancer (Alexander et al., 2007), pancreatic cancer (Ojajärvi et al., 2001), NHL (Mandel et al., 2007), and multiple myeloma and leukemia (Alexander et al., 2006). However, there are limitations in these meta-analyses. Scott and Chiu (2006) updated the literature since the Wartenberg et al. review for kidney, liver and NHL.

Overall, the body of literature provides convincing evidence of a causal association between TCE exposure in humans and site-specific cancers, particularly in the kidney. Wartenberg et al. (2000) found a significant increased incidence of kidney cancer among cohorts with the best exposure assessments. Since the latest IARC review, five case-control studies of renal cell carcinoma have been published, all reporting elevated adjusted odds ratios for estimated TCE exposure (from non-statistically significant to >3.00) (Brüning et al., 2003; Charbotel et al., 2006; Dosemeci et al., 1999; Pesch et al., 2000; Vamvakas et al., 1998). In addition, two high-quality cohort studies of TCE exposed workers have also found an excess of renal cancer (Rasschou-Nielsen et al., 2003; Zhao et al., 2005). Risks increased with employment duration (Rasschou-Nielsen et al., 2003), exposure score (Zhao et al., 2005) or cumulative exposure (Charbotel et al., 2006). However, no association between TCE exposure and kidney cancer was found in the update of the Rocketdyne study (Boice et al., 2006).

Associations were also observed for NHL and liver cancer. Since the last review, four case-control studies generally reported excess relative risk estimates for NHL (Hardell et al., 1994; Persson and Fredrikson, 1999; Wang et al., 2009; Seidler et al., 2007), the relative risks

increased with increasing TCE exposure in two studies (Wang et al., 2009; Seidler et al., 2007). Increased risks were also found in two cohort studies (Hansen et al., 2001; Rasschou-Nielsen et al., 2003), and a significant increased risk (summary relative risk estimates [SRRE] = 1.59, 95% CI = 1.21 to 2.08) among TCE subcohorts in the highest quality studies was found in the meta-analysis (Mandel et al., 2006). No increased risk was found in an Italian case-control study (Costantini et al., 2008).

For liver cancer, the evidence is more limited mainly because only cohort studies are available and most of these studies have multiple solvent and other exposures as well as small numbers of cases due the comparative rarity of liver cancer (Scott and Chiu, 2006). While high quality studies reported generally excess relative risk estimates, they were generally based on small numbers of cases or deaths, resulting in wide confidence intervals on the estimates. The low number of liver cancer cases in the available studies made assessing exposure-response relationships difficult. Significant increased risks across high-quality studies were reported by Wartenberg et al. (2000) (for incidence cohort studies) and Alexander et al. (2007) (SSRE, 1.41, 95% CI = 1.06 to 1.87). Associations have also been reported for cancer at other sites, including urothelial, bladder and esophageal cancer (Ruder, 2006; Scott and Chiu, 2006).

Recent studies have found also found statistically significant associations between high TCE exposure and breast cancer (Sung et al., 2007), and prostate cancer (Krishnadasan et al., 2007). Radican and colleagues updated the Hill Air Force Base study using a job-exposure matrix for TCE exposure and saw no statistically significant elevated hazard ratios (Radican et al., 2008).

Molecular epidemiology studies

There is limited information on genetic susceptibility and cancer risk from TCE exposure, which is a major research gap. Wiesenhütter et al. (2007) reported that there was no difference in the distribution of glutathione *S*-transferase (*GST)* polymorphisms (*GSTT1*, *GSTM1*, *GSTP1*), and N-acetyltransferase (*NAT2*) genotypes (slow and rapid acetylators) among TCE-exposed cases, TCE-exposed controls, non-exposed cases and non-exposed controls using subjects from the renal cell case-control study conducted by Brüning et al. (1997a). However, the authors were not able to conduct analyses at the individual level due to legal constraints.

Cancer in experimental animals

(sufficient, vol 63, 1995)

TCE exposures in animals have been associated with effects in a number of targets that are relevant to human cancer targets as well. The central nervous system, the kidney, the liver, the immune system, the male reproductive system, and the developing fetus have been identified through epidemiological and experimental animal studies with more limited evidence for TCE toxicity to the respiratory tract and female reproductive system (U.S. EPA, 2001, U.S. EPA 2009a).

There are several other lines of supporting evidence for TCE carcinogenicity in humans. Multiple chronic bioassays in rats and mice have reported increased incidences of tumors with TCE treatment, including tumors in the kidney, liver, and lymphoid tissues – target tissues of

TCE carcinogenicity also seen in epidemiological studies. Of particular note is the site-concordant finding of low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors in multiple strains of rats treated with TCE by either inhalation or corn oil gavage (Maltoni et al., 1988; NTP, 1988; 1990). The increased incidences were greater in male rats than female rats, though, notably, pooled incidences in females from five rat strains tested by National Toxicology Program (NTP, 1988; 1990) results in a statistically significant trend (U.S. EPA, 2001, U.S. EPA 2009a).

With respect to the liver, TCE and its oxidative metabolites chloral hydrate (CH), trichloroacetic acid (TCA), and dichloroacetic acid (DCA) are clearly carcinogenic in mice, with strain and sex differences in potency that appear to parallel, qualitatively, differences in background tumor incidence (NCI, 1976; Maltoni et al., 1986; Anna et al., 1994; Herren-Freund et al., 1987; Bull et al., 2002; George et al., 2000; Leakey et al., 2003; Bull et al., 1990; DeAngelo et al., 1996; 1999; 2008). Data in other laboratory animal species are limited. Except for DCA, which has been reported to be carcinogenic in rats (Richmond et al., 1995; DeAngelo et al., 1996), inadequate evidence exists to evaluate the hepatocarcinogenicity of these compounds in rats or hamsters but TCE is clearly less potent in the strains of rats tested than in mice. Evidence for TCE-induced lymphatic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats (Henschler et al., 1980; NTP, 1990; Maltoni et al., 1986; 1988; NTP, 1988; Fukuda et al., 1983) is more limited (U.S. EPA. 2009a).

With respect to the lymphatic cancers, two studies in mice reported increased incidences of lymphomas in females of two different strains, and two studies in rats reported leukemia in males of one strain and females of another. These tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. Rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE, and non-statistically significant increases in mice exposed orally. Pulmonary tumors were not reported in other species tested (i.e., rats and hamsters). Increased testicular (interstitial or Leydig cell) tumors have been observed in multiple studies of rats exposed by inhalation and gavage. Therefore, TCE is clearly carcinogenic in rats and mice. The apparent lack of site concordance across laboratory animal studies may be due to limitations in design or conduct in a number of rat bioassays and/or genuine inter-species differences in qualitative or quantitative sensitivity (i.e., potency). However, these studies show carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance with humans.

Mechanisms of carcinogenicity

Since 1995, a large body of literature has been published, on epidemiologic studies of TCE, various meta-analyses and criteria to proceed with appropriate meta-analyses, and studies describing the actions of TCE metabolites. During the course of development of the U.S. Environmental Protection Agency (EPA) draft TCE assessment, EPA's Scientific Advisory Board and the National Academy of Sciences (NAS) have provided insights regarding the large database with inferences about its carcinogenic hazard. EPA staff have published a mini-monograph outlining some of the outstanding science issues to be addressed in an assessment of TCE (Chiu et al., 2006a,b: Keshava and Caldwell, 2006; Scott and Chiu, 2006) as well as a number of subsequent publications (e.g., Caldwell et al., 2008; Guyton et al.,

2009; Evans et al., 2009) on its potential modes of action. Ruder (2006) provides an assessment of the epidemiological literature as stated above. In general, the following areas have seen large increases in the database and greater understanding of TCEs carcinogenic risk to humans.

Available mechanistic data do not suggest a lack of human carcinogenic hazard from TCE exposure.

1) The understanding of the toxicokinetics of glutathione conjugation (GSH) metabolites and conjugation pathways in humans has been deepened. Physiologically based pharmacokinetic (PBPK) models have been developed that allow for predictions of metabolism and differences in metabolism between species for a number of key metabolites (Chiu et al., 2006b; 2009; Evans et al., 2009).
2) Genotoxicity studies have included investigations of mutations of the Von Hippel-Lindau (*VHL)* gene in renal tumors of TCE exposed workers. Metabolism of *S*-dichlorovinyl-L-cysteine (DCVC), a mutagenic metabolite that transports to the kidney, lends biological plausibility to kidney cancer associated with human exposure. The documentation of increases in enzyme levels in the kidneys of humans exposed to TCE also lends plausibility to the kidney as a target of TCE toxicity and carcinogenicity in humans; the increases were observed (in non-cancer studies) at exposure levels that occur in occupational settings.
3) The epidemiological data (see above) identify other potential cancer sites and clearer signals with the addition of more studies since the previous IARC assessment. There is site concordance for multiple tumor types in both humans and experimental rodent studies that was not recognized previously.
4) The mode of action (MOA) of peroxisome-proliferation activated receptor (PPAR) activation, previously considered to dismiss the human relevance of effects observed in laboratory animals, has been questioned (e.g., Caldwell et al., 2008; Melnick et al., 2001) and the review by Guyton et al. (2009) of this proposed MOA raise questions about whether the hypothesized PPARα activation is either necessary or sufficient for rodent hepatocarcinogenesis (see DEHP review).
5) There is additional information regarding the toxicity of metabolites of TCE with multiple metabolites shown on their own to induce a carcinogenic response in rodent by potentially multiple MOAs (Caldwell et al., 2008). However, the MOA(s) has not been established for a number of TCE-induced tumors.

Toxicokinetics

TCE attains high concentrations relative to blood in the brain, kidney, and liver - all of which are important target organs of toxicity. TCE is cleared via metabolism mainly in three organs: the kidney, liver, and lungs. The metabolism of TCE is an important determinant of its toxicity. Metabolites are thought to be responsible for toxicity at multiple sites, particularly in the liver and kidney. Initially, TCE may be oxidized via cytochrome P450 (*CYP*) isoforms or conjugated with glutathione by *GST* enzymes. There are conflicting data as to which *GST* isoforms are responsible for TCE conjugation, with one study in rats indicating α*GST*s and another (also in rats) indicating μ and π*GST*. The balance between oxidative and conjugative metabolites generally favors the oxidative pathway, especially at lower concentrations, and

inhibition of *CYP*-dependent oxidation *in vitro* increases GSH conjugation in renal preparations. However, in humans, direct comparison of in vitro rates of oxidation and conjugation, as well as *in vivo* data on the amount of the TCE GSH conjugation to dichlorovinyl glutathione in blood, support a flux through the GSH pathway that may be much greater than that inferred from excretion of GSH-conjugation-derived urinary mercapturates (Chiu et al., 2006).

TCE carcinogenicity in humans is supported by toxicokinetic data indicating that TCE absorption, distribution, metabolism, and excretion are qualitatively similar in humans and rodents. Several metabolites and excretion products from both pathways have been detected in blood and urine from exposed humans as well as from at least one rodent species. Therefore, humans possess the metabolic pathways that produce the TCE metabolites thought to be involved in the induction of rat kidney and mouse liver tumors, and internal target tissues of both humans and rodents experience a similar mix of TCE and metabolites.

Quantitative interspecies differences in toxicokinetics do exist, and are addressed through PBPK modeling. Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE. Recently, EPA and the U.S. Air Force jointly sponsored an integration of the Fisher, Clewell, and Bois modeling efforts (Hack et al., 2006). Different efforts have been published (e.g., Evans et al., 2009; Chiu et al., 2009; 2007; 2006; Hack et al., 2006) for PBPK model analyses or empirical analyses of toxicokinetics of TCE and its metabolites in mice, rats, and humans. Such analyses have considered a wider range of physiological, chemical, *in vitro*, and *in vivo* data than any previously published analysis of TCE. PBPK analysis should support high confidence in the model predictions and should provide appropriate characterization of the uncertainty in metabolic pathways for which available data were sparse or relatively indirect, such as GSH conjugation and respiratory tract metabolism. Key conclusions from the model predictions should include: (1) the extent of TCE metabolism at doses below saturation, and (2) GSH conjugation and subsequent bioactivation in humans and its relation to previous estimates. The predictions of the PBPK model could then be used in inter- and intraspecies extrapolation of toxicokinetics.

Genotoxicity and VHL Mutation

For the kidney, there is now a predominance of positive genotoxicity data for TCE metabolites derived from GSH conjugation (in particular DCVC). Together with toxicokinetic data, these data are consistent with their systemic delivery to and in situ formation in the kidney.

Studies have been conducted to determine the role of *VHL* gene mutations in TCE-induced renal cell carcinoma. Renal-cell carcinomas from workers occupationally exposed to high levels of TCE had a higher frequency of overall VLH mutations, and C to T transitions than renal cell-carcinomas from non-TCE exposed people (Brüning et al., 1997b; Brauch et al., 1999; 2004). Because of their limitation or lower mutation detection rate, the two other available studies (Schraml et al., 1999; Charbotel et al., 2007) neither add nor detract to the conclusions from the earlier studies. Inactivation of the *VHL* gene through mutations, loss of heterozygosity and imprinting has been observed in about 70% of renal clear cell carcinomas

(Alimov et al., 2000; Kenck et al., 1996). However, while supporting the biological plausibility of mutagenesis as a MOA for TCE-induced kidney tumors, available data on the *VHL* gene in humans or transgenic animals do not conclusively elucidate the role of *VHL* mutation in TCE-induced renal carcinogenesis.

Modes of Action:

Cytotoxicity and compensatory cell proliferation, presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the MOA for renal carcinogenesis. Human studies have reported markers for nephrotoxicity at current occupational exposures but data are lacking at lower exposures. Nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis as toxicity has been observed in both mice and rats at high doses but kidney tumors only observed in rats and nephrotoxicity has not been shown to be necessary for kidney tumor induction by TCE in rodents. It is not clear if nephrotoxicity is one of several key events in a MOA or TCE-induced kidney cancer or is a marker for an "upstream" key event that may contribute independently to both nephrotoxicity and renal carcinogenesis, or if it is incidental to kidney tumor induction. As no data suggest that any of the proposed key events for TCE-induced kidney tumors rats are precluded in humans, TCE-induced rat kidney tumors provide additional support for the human evidence of TCE-induced kidney cancer.

Data are insufficient to conclude that any of the other hypothesized MOAs are operant for other TCE-induced tumor sites. In the liver, there is evidence for genotoxic effects mediated through CH (e.g., micronuclei induction following exposure to CH is positive in most test systems in both i*n vitro* and *in vivo* assays, and most recently in humans (Ikbal et al., 2004)), or some other oxidative metabolite of TCE. The previous IARC evaluation considered the MOA hypothesis for TCE-induced liver tumors involving activation of the PPARα receptor. Clearly, *in vivo* administration of TCE leads to activation of PPARα in rodents and likely does so in humans as well. However, the evidence as a whole does, rather than support PPAR-α activation as the sole operant MOA mediating TCE hepatocarcinogenesis, support multiple TCE metabolites and multiple toxicity pathways contributing to TCE-induced liver tumors (Caldwell et al., 2008).

Furthermore, recent experiments have demonstrated that PPAR-α activation and the sequence of key events in the hypothesized MOA are not sufficient to induce hepatocarcinogenesis and that the events comprising the hypothesized MOA are not necessary for liver tumor induction (Guyton et al*.,* 2009; see DEHP review for further discussion of the PPAR- α MOA). For mouse lung tumors, a mutagenic MOA involving CH has also been hypothesized, but there are insufficient data to conclude that it is operant. A second MOA hypothesis for mouse lung tumors that involve cytotoxicity and regenerative cell proliferation has only limited experimental support with no data on proposed key events in experiments of duration 2 weeks or longer. A MOA involving *in situ* oxidative metabolism, whether leading to mutagenicity, cytotoxicity, or other key events, may also be relevant to other tissues where TCE would undergo P450 metabolism. For the testes, *CYP2E1*, oxidative metabolites, and protein adducts have been reported after TCE exposure and this has been identified as a tumor target in rodents. However, inadequate data exist to adequately define a MOA hypothesis for this tumor site.

Research needs and recommendations:

Human cancer studies

Pooled or Meta-analysis: The NAS stated that there were weaknesses in the available meta-analyses. As outlined by Scott and Chiu (2006) meta-analyses of high-quality studies should be able to determine if estimated relative risks or odds ratios in cohort and case-control studies are consistent, robust, and insensitive to individual study inclusion, with no indication of publication bias or significant heterogeneity. A meta-analyses approach is warranted given the modest relative risk estimates and the relative rarity of the cancers observed, and therefore the limited statistical power of individual studies. Pooled analyses of the biomonitoring studies (measuring TCA metabolites) (Anttila et al., 1995; Axelson et al., 1994; Hansen et al., 2001) should also be explored.

Primary studies: Any planned cohort studies should endeavor to obtain, at a minimum, current and retrospective department-specific measured exposure levels of TCE and other exposure agents. A cohort without multiple solvent exposures, such as the manufacture of kitchen utensils, using TCE for a final degreasing after assembly-line production, would be desirable. Scott and Chiu (2006) noted that known inaccuracies exist between cancer incidence and death certificate recording for some of the cancer sites that have been associated with TCE exposure such as liver and biliary cancer and NHL. Studies evaluating cancer incidence rather than mortality are desirable especially when looking at NHL. Scott and Chiu (2006) noted that evaluation of lymphomas and TCE exposure is complicated by (1) the use of different ICD codes in the different studies (ICD codes for lymphomas have changed over time, and different studies have used different ICD revisions and (2) understanding of the biology of NHL has changed; lymphomas can be either B cell or T cell and thus lymphomas in the past may have been diagnosed as multiple myeloma or leukemia. If possible medical records or data on molecular markers of lymphoma should be obtained to provide more information on the diagnosis of lymphohematopoietic cancers. Studies evaluating liver cancer should look for possible interaction with lifestyle factors.

Genetic susceptibility: Future human studies should include genotyping of *GST* variants. Since the glutathione conjugation pathway is not active in *GST*-null individuals, it can be hypothesized that kidney cancer risk will be low among *GST*-null individuals and high among *GST*-nonnull individuals. Where possible, retrospective *GST* genotyping could be done on stored specimens. Genetic variants in the *CYP2E1* and other *CYP* genes, as well as any other genes coding for enzymes that metabolize TCE or its metabolites, should also be investigated. Other candidate genes for genetic susceptibility includes those involve in regulating immune function. Studies should also be conducted using entire genome scans to identify new susceptibility genes.

Mechanistic considerations

Research is needed to determine whether there are specific metabolites that appear to be the agent of carcinogenesis for specific sites. The multiple MOAs from multiple metabolites make comparisons between chlorinated solvents difficult to study and can account for differences in exposures and pharmacokinetic and pharmacodynamic characteristics of exposed populations contributing to variable responses in a number of studies. Information is needed on pathway effects, especially epigenetic changes induced by TCE and its metabolites, Research is needed to determine whether the effects on particular pathways be key to the lack

of site concordance for some endpoints between animals and human data for TCE (e.g., a particular pathway disturbance will manifest as susceptibility to differing tumor sites between species). Studies evaluating epigenetic changes induced by TCE and its metabolites are also useful for determining potential MOAs involved with TCE-induced effects.

Immunologic mechanism may be involved in lymphomagenesis from solvents (Vineis et al., 2007) and this should also be an area of future research.

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TABLE 2. Studies (published since 1995 IARC review) Evaluating Trichloroethylene Exposure and Cancer Risk (Ruder 2006)

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Brüning et al. 2003	Deutsch For-schungs-geimein-schaft, US EPA	Case-control, hospital-based	134 cases, 401 controls	Renal cell cancer diagnosis	Case: nephrectomy 1992-2000 ; control: hospitalized 1999-2000 (no dementia, no cancer)	High exposure = narcosis during TCE job	PCE, C tet, other solvents, heavy metals, fuels, paints, welding, etc.	Longest job in industry with TCE exposure, OR 1.8 (1.0-3.2); any "metal degreasing" OR 5.6 (2.3-13.3); est high TCE exp OR 3.7 (1.8-7.5) (all smoking adjusted)	OR decreased with increasing duration of TCE or solvent exposure
Charbotel et al. 2006, Fevotte et al. 2006	European Chlorinated Solvents Association	Case-control, hospital-based	86 cases, 316 controls	RCC diagnosis	Case: dx 1993-2003; controls same MD (no urinary tract cancer, chronic kidney disease), matched to case on gender, birthyear $\pm$ 2	Expert assessment of occupational history	Other solvents, oils, welding fumes, lead, cadmium, asbestos	High cum dose TCE OR 2.16 (1.02-4.60); high dose + peaks OR 2.73 (1.06-7.07); excluding jobs with low confidence scores, high cum dose OR 3.34 (1.27-8.74); high dose + peaks OR 3.80 (1.27-11.4)	Hospital/urologist patient controls
Costantini et al. 2008, Miligi et al. 2006	US NCI, Europe Against Cancer Programme, Italian Alliance Against Cancer	Case-control, populatio n based	586 leukemia cases, 1278 controls; 263 multiple myeloma (MM) cases, 1100 controls; 1428 NHL & 304 HL cases, 1530 controls	Acute myeloid leukemia (AML), chronic lymphatic leukemia (CLL), MM, or lymphoma diagnosis	Cases: dx 1991-1993 age 20-74; Controls from municipal files, stratified by sex and 5-year age groups	Detailed occupation history, industrial hygienist blinded to case status assessed TCE exposure as very low-low (LO) or medium-high (HI)	Benzene, styrene, xylene, toluene, dichlorome-thane, tetra-chloroethylene. 1,1,1-trichloro-ethane	AML LO OR 1.0 (0.4-2.5), HI OR 1.1 (0.5-2.9); CLL LO OR 1.2 (0.5-.7), HI OR 0.9 (0.3-2.6); MM LO OR 1.5 (0.7-3.5), HI OR 0.9-2.4); NHL LO OR 0.8 (0.5-1.3), HI OR 1.2 (0.7-2.0)	Leukemia & MM ORs for higher doses were lower, possible latency/lag issues, no solvent specific results presented for HL
Dosemeci et al. 1999	US NCI	Case-control	438 cases, 687 controls	RCC diagnosis	RCC dx July 1988-1990, population-based controls	Industry, job linked to JEM	Other chlorinated solvents	Any/none OR adj age, gender, smoking, hyper-tension, diuretics, BMI: all 1.30 (0.9-1.9), men 1.04 (0.6-1.7), women 1.96 (1.0-4.0)	Gives % exp but not number exp. Est 57 cases 69 controls exp. Multiple exp

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Dumas et al. 2000, Siemiatycki et al. 1991	Québec Institute Research Occup Health Safety & Health Research Funds; Canada Health, Natl Health Research & Develop-ment, NCI	Case-case (different sites) and case-control	3730 cancer patients, 533 population controls	Cancer diagnosis	Males age 35-70, dx 9/79-6/85, resident in Montreal metropolitan area on electoral list	Low, medium, high intensity & no, low, med, high frequency assigned by coders; reanalysis (rectal cancer only 257 cases) adjusts for other exposures	Many	Rectal ca: any exp OR 2.0 (1.0-3.9) substantial exp OR 0.9 (0.3-3.2), melanoma any exp OR 2.6 (1.3-5.0), substantial OR 2.3 (0.9-5.8)	Multiple exp. Low number with any/high exp (12/3 rectal ca cases, 8/4 melanoma)
Greenland et al. 1994	U. Lowell Research Foundation General Electric Corpora-tion	Case-control nested in cohort	1821 deceased workers	Cause of death	Employed <1985, died 1969-1984 age 21-90, death reported to pension office, work history available, controls nonca, nonblood, nondigestive, nonmental, nongenito-urinary causes	JEM created for TCE & 6 other exposures	PCB, benzene, other solvents, machine fluids, astestos, resins	Any TCE: pancreas ca OR 1.64 (0.8-3.3), liver & biliary ca OR 0.54 (0.1-2.6), other sites ORs closer to 1 (NSS).	Work histories not available for 34% of deceased workers Multiple exp
Hardell et al. 1994	Umea Hospital (?)	Case-control	105 NHL cases, 335 population-based controls	NHL	Cases dx 1974-1978 age 25-85, controls matched for sex, age, residence, vital status	Occupational history, self-reported exposures	Phenoxyacetic acids, chlorophenols, bezine, turpentine, white spirit, degreaser	TCE OR 7.2 (1.3-42) based on 4 exposed cases & 4 exp controls	Almost all substances show statistically significant elevated OR but no occupations do
Heineman et al. 1994, Gomez et al. 1994	US NCI	Case-control	300 male cases, 320 male controls	Brain tumor mortality	Died 1978-1981 hospital-confirmed astrocytic brain tumor or other causes (minus CVD, epilepsy, suicide, homicide, cirrhosis, some ca), NOK interviewed	JEM for probablity of exp, cum= duration weighted by probability. 41% estimated exp. to TCE/	JEMs also for other chlorinated solvents	Low cum exp OR adj age, location 0.9 (0.5-1.6), med adj OR 1.3 (0.8-2.2), high adj OR 1.3 (0.7-2.5), test for trend not statistically significant	Interviewed <50% (300/ 741 cases, 320/ 741 controls). Low number with medium or high exp (50 cases, 40 controls)

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Krishnadasan et al. 2007	California Cancer Research Program	Nested case-control	362 cases, 1,805 controls	Prostate cancer	Employed 1950-1993 at Rocketdyne. Cases: ID by link to 8 cancer registries, Controls matched 5:1 on age at 1st employment ± 2, age at case dx ± 2	JEM created from company records, walk-throughs, interviews	PAHs, hydrazine, benzene, other solvents	Low moderate TCE OR 1.3 (0.81-2.1), high TCE OR 2.1 (1.2-3.9), P trend 0.02	Multiple exposures, some controls could have been dx with prostate ca before registries started
Nordstrom et al. 1998	Swedish Work Environment Fund, Orebro County Council	Case-control	121 male hairy cell leukemia cases, 484 population-based controls	Hairy cell leukemia	Cases diagnosed 1987-1993; controls matched 4:1 for age and county	Exposure assessed from occupational & lifestyle questionnaire	Numerous exposures	TCE OR 1.5 (0.7-2.6), 9 exposed cases, 26 exposed controls	Only ever/never exposure? so no way to assess exposure-response
Persson and Fredrikson 1999		Case-control (2 studies)	(1) 106 NHL cases, (2) 93 NHL cases, 479 population based referents	NHL	Cases age 20-80, Swedish-born, alive, (1) dx 1964-1986, Orebro Med Ctr or (2) dx 1975-1984, Linkoping Hospital	Lifestyle and occupational questionnaire, exposure ≥1 year 5-45 years pre-dx	Other solvents, etc.	TCE OR 1.2 (0.5-2.4)	
Pesch et al. 2000a	German Federal Ministry of Research & Technology	Case-control	935 cases, 4298 population-based controls	RCC	Cases: German nationals dx 1991-1995. Controls frequency matched by region, sex, & age (5-year groups)	Occupational questionnaire, job-exposure matrices, job-task exposure matrices	Metals, paints, mineral oils, PAHs, asbestos	TCE JEM ORs: Medium exp males 1.1 (0.9-1.4), females 1.2 (0.6-1.7); high males 1.1 (0.9-1.4), females 1.3 (0.8-2.0); substantial males 1.3 (0.9-1.8), females 0.8 (0.3-1.9). TCE JTEM ORs: Medium exp males 1.3 (1.0-1.8), females 1.3 (0.7-2.6); high males 1.1 (0.8-1.5), females 0.8 (0.4-1.9); substantial males 1.3 (0.8-2.1), females 1.8 (0.6-5.0)	Combined OR (both sexes) not presented, trend tests (med-high-substantial) not done

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Pesch et al. 2000b	German Federal Ministry of Research & Technology	Case-control	1035 cases, 4298 population-based controls	Urothelial carcinoma	Cases: German nationals dx 1991-1995. Controls frequency matched by region, sex, & age (5-year groups)	Occupational questionnaire, job-exposure matrices, job-task exposure matrices	Aromatic amines, paints & dyes, cutting fluids, PAHs, other chlorinated solvents	TCEJEM ORs: Medium exp males 1.1 (0.8-1.3), females 1.0 (0.6-1.7); high males 1.1 (0.9-1.4), females 1.6 (1.0-2.5); substantial males 1.3 (0.9-1.7), females 0.6 (0.2-2.3). TCE JTEM male ORs: Medium exp 0.8 (0.6-1.2), high 1.3 (0.9-1.7), substantial 1.8 (1.2-2.7)	Combined OR (both sexes) not presented, trend tests (med-high-substantial) not done
Seidler et al. 2007	German Federal Office for Radiation Protection	Case-control	710 lymphoma cases, 710 population-based controls	Lymphoma	Cases dx in 6 German regions age 18-80; controls matched on region, gender, and age $\pm$ 1 year	Complete occupa-tional history assigned intensity & frequency of TCE by case-status blinded industrial physician	PCE, carbon tet, dichloromethane, benzene, toluene, xylene, styrene	<4.4 ppm years adj OR 0.7 (0.4-1.1), 4.4-35 ppm years adj OR 0.7 (0.5-1.2), >35 ppm years adj OR 2.1 (1.0-4.8), trend p 0.14 B-cell NHL trend p 0.02	No exposure measurements. Mixed exposures?
Vamvakas et al. 1998	Institute Toxicology, U. Wurzburg, Germany	Case-control	58 cases, 84 controls	RCC	Cases dx Dec 1987-May 1992 in one hospital, controls hospitalized trauma 3 nearby hospitals 1993, had sonography to exclude kidney cancer	Occ history + TCE, PCE modules, exp ranked by time & freq, severity pre-narcotic symptoms	PCE, heavy medals, petro-leum products, benzene, asbestos, PCB, pesticides	19 cases & 5 controls exp (+2 controls exp PCE). Means in exp: duration exp cases 16$\pm$ 11.3 y, controls 8$\pm$7.7y (NSS); latency cases 33$\pm$ 10.4, controls 18$\pm$7.2, p<0.01 *Exp v. unexp* X^2*=5.36, p<0.025*	Interviewers not blinded, controls from different era & hospitals, younger than cases (p<0.05).
Wang et al. 2009	US NCI	Case-control	601 female NHL cases, 717 female population-based controls	NHL	Cases: dx 1996-2000 age 21-84, controls by random digit dialing or Medicare-Medicaid frequency matched by 5-year age groups	Structured questionnaire linked to job-expsoure matrix	Benzene, other chlorinated solvents	TCE ORs: ever/never 1.2 (0.9-1.8); intensity low 1.1 (0.8-1.6), med-high 2.2 (0.9-5.4), trend p 0.06; probability low 1.1 (0.7-1.8), med-high 1.4 (0.9-2.4), trend p 0.37	

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Anttila et al. 1995 #	Finnish Work Med Fund, US NIOSH grant	Linkage monitoring registry-cancer registry	1,698 men, 1,391 women	Cancer diagnosis	Monitored (urine) for exposure any time during 1965-1983; cancer diagnosed 1967-1992	Median 48 µmol TCA/L (men), 63 µmol TCA/L (women).	Small % also monitored for PCE, 1,1,1-trichloroethane	Overall: 208 ca SIR 1.1 (0.9-1.2), 8 cervix ca SIR 2.4 (1.1-4.8), 8 NHL SIR 1.8 (0.8-3.6). 20+ ys since 1st exp: 60 ca SIR 1.6 (1.2-2.0), 7 stomach ca SIR 3.0 (1.2-6.1), 3 liver ca SIR 6.1 (1.3-17.7), 8 prostate ca SIR 3.6 (1.5-7.0), 7 lymph-ohematopoietic SIR 3.0 (1.2-6.1)	74% only 1-2 measurements (short duration employment?) .
Hansen et al. 2001	Interna-tional Epi-demiology Institute	Linkage monitor-ing registry-cancer registry	803 workers	Cancer diagnosis	Monitored (urine) for exposure any time during 1947-1989; cancer diagnosed 1968-1996	Median 25 µg TCA/ ml (1947-64), 2 µg TCA/ ml (1980-89), overall 15 µg TCA/ ml (1947-89)	Not reported	Overall: 128 ca SIR 1.0 (0.9-1.2), 4 cervix ca SIR 3.8 (1.0-9.8), 8 NHL SIR 3.1 (1.3-6.3), 6 leukemia SIR 2.0 (0.7-4.6), 5 liver SIR 2.1 (0.7-5.2).	No data provided on ca risk by duration of employment
Axelson et al. 1994#	Occup Environ Med, U. Hospital, Linkoping, Sweden	Retro-spective cohort	1421 men, 249 women	Cause of death, cancer dx	Wked at 1 of 115 companies where urinary TCA monitored 1955-1975, F/U from 1st urine not DFE, VS to 1986	Mean TCA <50/µg/ ml urine for 80%, ~ TWA 20 ppm	Not reported	Men 229 deaths SMR 0.97 (0.9-1.1), 37 ca SMR 0.65 (0.5-0.9), 138 circulatory SMR 1.17 (1.0-1.4), 107 ca dx SIR 0.96 (0.8-1.2), 8 skin ca SIR 2.36 (1.0-4.7). Women 24 deaths SMR 1.55 (1.0-2.3), 10 ca SMR 1.53 (0.7-2.8), 10 circulatory SMR 2.02 (0.97-3.7), 22 ca dx SIR 1.32 (0.9-2.0). No dose-response gradients.	Incomplete cohort minus nonmonitored workers (n=?)

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Blair et al. 1998, Stewart et al. 1991	US NCI	Retro-spective cohort mortality & cancer incidence	14,457 workers, 7,282 ever exposed to TCE	Death/ cancer diagnosis	Civilian aircraft maintenance workers s employed >1 y between 1952-956 at Hill AFB, VS to 1990, ca incidence 1973-1990	Job-exposure matrix, quantified for TCE, tertiles of <5, 5-25, >25 unit-ys	other chlori-nated solvents All but 3,739 workers exp 1-25 chemicals	TCE/no chemicals: all death RR adj age, calendar time, sex 1.0, all ca RR 1.1 (1.0-1.3). Ca incidence in men: Colon RR 4.1, 1.4-11.8 (nonTCE chem/none); 2.9, 1.0-8.9 (<5 TCE/ none); 4.3, 1.4-13 (5-25 TCE/none); 5.7, 2.0-16.7 (>25 TCE/ (nonTCE chem/none); 1.2, 0.1-14 (<5 TCE/none); 1.0, 0.1-16 (5-25 TCE/none); 2.6, 0.3-25 (>25 TCE/ none). Multiple myeloma RR 3.7, 0.4-32 (non-TCE chem/none); 0.8, 0.1-12.7 (<5 TCE/none); 3.8, 0.4-37 (5-25 TCE/none); 5.1, 0.6-44 (>25 TCE/ none).none). Liver RR 0.8, 0.1-12	Mixed exposures. Evaluation by job title, not person
Radican et al. 2008	US NCI	Retro-spective cohort mortality & cancer incidence	14,457 workers, 7,282 ever exposed to TCE	Death/ cancer diagnosis	Civilian aircraft maintenance workers s employed >1 y between 1952-956 at Hill AFB, VS to 2000, ca incidence 1973-1990	Job-exposure matrix, quantified for TCE, tertiles of <5, 5-25, >25 unit-ys, TCE exposure categories: LI (low intermittent), LC (low continuous), PI (peak infrequent), PF (peak frequent)	other chlori-nated solvents All but 3,739 workers exp 1-25 chemicals	4320 deaths HR 1.04 (0.98-1.09), cancer 854 deaths HR 1.03 (0.91-1.17), no COD in SS excess	Mixed exposures. Evaluation by job title, not person

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Boice et al. 1999	Lockheed Martin Corporation	Retrospective cohort	45.323 factory workers, 32,642 non-factory workers	Cause of death	Aircraft mfg ≥1 year >1959, 2267 exposed to TCE VS to 1996	TCE primary degreaser to 1966 (12% factory wkrs exp	PCE from 1966, many other solvents, cutting fluids, asbestos, chromate	TCE exp: 1110 deaths SMR 0.83 (0.8-0.9), 277 ca SMR 0.86 (0.8-0.97), 7 pancreas ca SMR 0.41 (0.2-0.9), 78 lung ca SMR 0.76 (0.6-0.95), no other ca SMR significantly up/down *RR TCE exp/non-factory workers overall 0.83/ 0.76=1.09, all ca 0.86/ 0.8=1.08*	Multiple exp. Short latency for 11% factory , 24% non-factory workers who started after 1980. What about those employed 1928-1960?
Boice et al. 2006	Boeing Corporation	Retrospective cohort	41,351 Rocketdyne workers, 8372 Santa Susana Field Lab, 32,979 elsewhere	Cause of death	Rocket engine testing ≥6 mo 1948-1999, VS to 1999	TCE	Hydrazines, fuels, propellants, oxidizers, other solvents	SSFL 2251 deaths, SMR 0.83 (0.80-0.86), cancer 655 deaths, SMR 0.89 (0.82-0.96), kidney cancer 21 deaths, SMR 1.15 (0.71-1.76)	No latency for those recently employed, 4729 current (SSFL, 613) employees contribute PYAR but not deaths
Henschler et al. 1995 #	Toxicology Institute, U. Wurzburg, Germany	Retrospective cohort	169 men exp TCE, 190 men unexp	RCC incidence, mortality	Cardboard workers exp ≥1 y 1956-75, VS to 1992	TCE predominant solvent 1956-75	Pentachlorophenol, 1,1,1-trichloroethane, aromatic & chlorinated solvents	Exp 50 deaths SMR 0.68 (0.5-0.9), 15 ca SMR 0.96 (0.5-1.7), 2 kidney ca SMR 3.28 (0.4-11.8). Unexp 52 deaths SMR 1.03 (0.8-1.4), 15 ca SMR 1.16 (0.7-1.9), 3 brain ca SMR 9.38 (1.9-27.4) Exp 5 renal ca dx SIR 7.97 (2.6-18.6), unexp no renal ca.	

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Morgan et al. 1998	Hughes Aircraft Company	Retro-spective cohort	4,733 TCE exp, 15,975 unexp	Cause of death	Wked ≥6 mo 1950-1985 in aerospace mfg, VS to 1993	Degreasers (high) >50 ppm. JEM rated low (1), medium (4), high (9) exp, cum=mon x score	Not reported	All deaths 917 TCE SMR 0.84 (0.8-0.9), 3135 nonTCE SMR 0.85 (0.8-0.9); ca 270 TCE SMR 0.92 (0.8-1.0), 830 nonTCE SMR 0.85 (0.8-0.9); ovarian ca 8 TCE SMR 1.21 (0.5-2.4), 5 nonTCE SMR 0.39 (0.1-0.97); prostate ca 21 TCE SMR 1.18 (0.7-1.8), 55 nonTCE SMR 0.86 (0.7-1.1); kidney ca 8 TCE SMR 1.32 (0.6-2.6), 24 nonTCE SMR 1.10 (0.7-1.6) ; bladder ca 8 TCE SMR 1.36 (0.6-2.7), 15 nonTCE SMR 0.70 (0.4-1.2) *RR TCE/nonTCE: all deaths 1, all ca 1.08, ovarian ca 3.10, prostate ca 1.37, kidney ca 1.2, bladder ca 1.94*	Probably have multiple exp
Raaschou-Nielsen et al. 2003	Internat-ional Epi-demiology Institute	Retro-spective cohort	40,049 incl 14,360 higher exp	Cancer diagnosis	347 TCE-using companies, <200 employees in company, blue-collar jobs, worked ≥3 mons 1964-1997.	Higher exp: worked ≥1 y, started <1980	Not reported. Main industries metal, elec-tronics, painting, printing, chemical, dry cleaning	All ca men SIR 1.1 (1.0-1.1), women SIR 1.2 (1.0-1.3); RCC 1.2 (0.9-1.5); NHL 1.2 (1.0-1.5); esophageal SIR 1.8 (1.2-2.7). Higher exp RCC 1.4 (1.0-1.8); NHL 1.5 (1.2-2.0); esophageal SIR 1.7 (0.9-2.9).	RCC & NHL risk increased with increasing duration of employment, higher among higher exp
Ritz 1999	US NIOSH grant	Retro-spective cohort	3814	Cause of death	White males hired 1951-72, wked ≥3 mon U processing plant, monitored for radiation, VS to 1990	JEM for no, low, med TCE exposure x duration	Uranium, cutting fluids, kerosene	Exp >5 yrs med TCE v. no TCE, 15 yr lag : liver ca RR 12.1 (1.0-144), brain ca RR 14.4 (1.2-167) adj radiation, salary v. hourly, latency	Multiple exp

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Sung et al. 2007	Taiwan Dept. of Health, National Health Research Institutes	Retro-spective cohort	63,982 female workers	Breast cancer incidence	Employed ≥1 day at electronics factory 1973-1992, dx 1979-2001 (could have been employed starting in 1970?)	Duration of employment <June 1974 (TCE only) & later (15 other solvents)	Isopropyl alcohol, acetone, MEK, trichlorometh-ane, methylene chloride, toluene, petrol-eum naphta, N-hexane, ethyl acetate, methyl alcohol, 1,2-dichloroethylene, 1,1,1- & 1,1,2-trichloro-ethane, 1,2-dichlorethane, tetrachloroethylen e	Pre 1974 employment SIRs: <1 mo, 1.97 (0.98-3.52); 1-11 mo 1.22 (0.73-1.90), 1-4 years 1.38 (1.81-2.22), 5-9 years 1.14 (0.70-1.76), >10 years 1.62 (1.02-2.42), overall 1.38 (1.11-1.70) Post 1974 employment SIR overall 0.99 (0.85-1.14)	How could workers have pre 1974 employment of >4 years?
Zhao et al. 2005	California Cancer Research Program(?)	Retro-spective cohort	55,000 workers	Cancer incidence and mortality	Employed 1950-1993 at Rocketdyne, matched to National Death Index, cancer registries of California, Arizona, Arkansas, Florida, Nebraska, Nevada, Oregon, Texas, and Washington State	Industrial hygiene review of facility, job description manuals, no-low-med-high exposure assigned by job title	Hydrazine, mineral oils	Incidence: Kidney cancer, med TCE RR 1.87 (0.56-6.20), high RR 4.90 (1.23-19.6), trend p 0.02; bladder cancer, med TCE RR 1.54 (0.81-2.92), high RR 1.98 (0.93-4.22), trend p 0.07	

† Odds ratio OR, relative risk RR, standardized mortality ratio SMR, standardized incidence ratio SIR, 95% confidence interval ( ); The 95% confidence intervals (CI) are presented where reported (where not reported CIs were calculated, if possible).
* 90% confidence interval. *Calculations done for this paper are in italics*

Study included in the 1999 IARC review

Chemical abbreviations: Me Cl (methylene chloride). PCB (polychlorinated biphenyls), PCE (tetrachloroethylene), TCA (trichloroacetic acid--TCE metabolite), TCE (trichloroethylene), TCP (2,4,6-trichlorophenol)

Disease abbreviations: ca (cancer), dx (diagnosed), NHL (non-Hodgkin lymphoma), RCC (renal cell carcinoma)

Miscellaneous abbreviations: cum (cumulative), environ (environment, environmental), EPA (Environmental Protection Agency), est (estimated), exp (exposure, exposed), IH (industrial hygienist), JEM (job-exposure matrix), med (medical, medicine), Natl (National), NCI (National Cancer Institute), NIOSH (National Institute for Occupational Safety and Health), occup (occupational) TWA (time-weighted average), US (United States)

Statistical abbreviations: DFE (date 1st exposed), freq (frequency), F/U (followup), GM (geometric mean), IDR (incidence density ratio, chance of preganancy), mon (month), NSS (not statistically significant), p (probability), VS (vital status), y (year)

Tetrachloroethylene (perc, tetra, PCE)

by Jane Caldwell PhD, Ruth Lunn DrPH, and Avima Ruder PhD

Citation for most recent IARC review

IARC Monographs 63, 1995

Current evaluation

Conclusion from the previous Monograph:

Tetrachloroethylene (Perc) is *probably carcinogenic to humans (Group 2A)* with the working group finding limited evidence in humans for the carcinogenicity of Perc and sufficient evidence in experimental animals for the carcinogenicity of Perc. In making the overall evaluation, the Working Group considered the following evidence: (i) Although Perc is known to induce peroxisome proliferation in mouse liver, a poor quantitative correlation was seen between peroxisome proliferation and tumor formation in the liver after administration of Perc by inhalation. The spectrum of mutations in proto-oncogenes in liver tumors from mice treated with Perc was different from that in liver tumors from mice treated with trichloroethylene (TCE). (ii) The compound induced leukemia in rats. (iii) Several epidemiological studies showed elevated risks for esophageal and cervical cancer and non-Hodgkin lymphoma, which the working group stated was unlikely due to chance although confounding could not be excluded.

Exposure and biomonitoring

Perc is a solvent used for dry cleaning clothes and for metal cleaning and degreasing in a number of industries, including metal finishing, cleaning mining equipment, testing coal, cleaning animal coats in taxidermy, and cleaning and duplicating film (Gold et al., 2008). It is a volatile liquid at room temperature. The largest human exposure occurs indoors to workers in dry cleaning and metal finishing facilities. Near points of use, such as dry cleaners or industrial facilities, indoor exposure to Perc is more significant than outdoor exposure (U.S. EPA, 2001). Indoor air concentrations in apartments above a dry cleaning shop have been measured at up to 4.9 mg/m^3 (Verberk and Scheffers, 1980), whereas the historical overall arithmetic mean (AM) for personal Perc in the dry cleaning industry was 59 ppm (range 0 to 4636 ppm, n =1395) (Gold et al., 2008). Lower exposures since 1995 (McKernan et al., 2008) will necessitate new study designs to determine effects from lower exposures and apply methods to accommodate the reduction.

Perc can be converted to vinyl chloride under anaerobic conditions, which can contaminate soil and ground water, and vinyl chloride has been detecting in a dry cleaning facility (Vogel and McCarty, 1985; ATSDR, 2000).

The characteristics and uncertainties involved with Perc metabolism make establishment of biomarkers difficult. The Perc metabolite trichloroacetic acid (TCA) is not a good biomarker for Perc exposure. Better characterization of metabolism of Perc in the kidney is especially needed. End-exhaled alveolar air, for which a U.S. National Institute for Occupational Safety

and Health (NIOSH) method has been developed, integrates dermal and inhalation exposure (NIOSH, 1998).

Cancer in humans

(limited, vol 63, 1995)

Since the 1995 review one linkage and six case-control studies have been published and two cohort studies updated. Many of these studies have been reviewed (Mundt et al., 2003; Ruder, 2006). Table 3 below summarizes the case-control and cohort studies published since the IARC review in tabular format. [This is an updated version of the supplemental table to the review by Ruder (2006)]. The case-control studies provide some support for associations between Perc exposure and cancer of the breast, bladder, and lymphoma although most of the increases were not statistically sufficient. In work published since the reviews appeared, one case-control study found a statistically significant relative risk of bladder cancer among dry cleaners (Lynge et al., 2006), another found that the risks of lymphoma increased with increasing cumulative exposure to Perc, and that the trend was statistically significant for T cell NHL (Seidler et al., 2007). Another recent case-control study found no association between Perc exposure and risk of leukemia (Costantini et al., 2008).

The U.S. Environmental Protection Agency (EPA) recently published its draft Perc assessment (U.S. EPA, 2009). It concludes that overall, the epidemiologic evidence has associated Perc exposure with excess risks for a number of cancers, although a causal association has yet to be definitely established. Studies of Perc and cancer showed positive associations between exposure and cancer of the lymphoid system, esophagus, and cervix, with more limited evidence for cancer of the bladder, kidney, and lung. For both lymphoid and esophageal cancer, excess risk was observed in studies of human populations exposed to Perc and other solvents, including studies of exposures to dry cleaners or workers involved with degreasing metal parts. In these cases, average risks were doubled as compared with those of referents. Furthermore, studies of drinking water exposure also supported an association between lymphoid cancer and Perc and other solvents, as did case-control studies that assessed employment as dry cleaners. Chance and confounding by smoking were unlikely the sole explanations for the observed excesses in risks. Information was lacking on lifestyle and socioeconomic factors, which are indirect surrogates for human papilloma virus infection, a known risk factor for cervical cancer (U.S. EPA, 2009b).

More studies have been published since the 1995 IARC monograph regarding Perc but the type of meta-analysis suggested for TCE by various review panels and conducted for TCE have not been conducted for Perc. Ruder (2006) has published a compendium of Perc epidemiological studies to aid in this effort.

Cancer in experimental animals

(sufficient, vol 63, 1995)

Perc exposures have been associated with effects in a number of targets, which bring relevance to cancer targets as well. Targets of toxicity observed both in human and animal studies include the liver, kidney, CNS, reproductive system, and developing fetus. Affected organs are all sites of high metabolic activity. Humans were found to be particularly sensitive

for neurological effects, including decrements in vision or visuo-spatial function, and other neurobehavioral (cognitive) effects following inhalation exposure (U.S. EPA, 2009b).

The EPA laboratory animal database includes 10 lifetime rodent bioassay data sets that demonstrate increased cancer incidence. (Two additional study data sets, in male and female rats exposed orally, were inconclusive due to excessive mortality caused by pneumonia or Perc-related toxic nephropathy). Perc is a carcinogen in rodents in 10 of 10 lifetime bioassay data sets—including by oral and inhalation routes. It is reasonable to use these animal tumors as indicators of potential human cancer hazard (U.S. EPA, 2009b).

Hepatocellular adenomas and carcinomas in mice and mononuclear cell leukemia (MCL) in rats occurred in multiple lifetime rodent bioassays, and hemangioendotheliomas in male mice (JISA, 1993) and cancers of the kidney and brain (glioma) in male rats (NTP, 1986) occurred in single lifetime bioassays. Also known as hemangiosarcomas, hemangioendotheliomas are rare tumors of the epithelial lining of blood vessels. Although the dose-response relationships for kidney and brain tumors in male rats were not as strong as for the other cancers, and the increasing dose-response trend for kidney tumors was not statistically significantly, both tumor types were considered by EPA to be Perc-related and biologically relevant. The statistically significantly elevated incidences of hepatocellular carcinomas and adenomas in male and female mice and MCL in male and female rats are considered to be indicators of potential human health hazard, despite questions regarding high background incidences of these tumors in controls and mode of action (MOA) hypotheses. Kidney cancer and MCL in rats as indicators of a potential human cancer hazard appear reasonable, given the observations in the epidemiologic studies (U.S. EPA, 2009b).

Therefore, Perc is clearly carcinogenic in rats and mice. The apparent lack of site concordance across laboratory animal studies may be due to limitations in design or conduct in a number of rat bioassays and/or genuine inter-species differences in qualitative or quantitative sensitivity (i.e., potency). The data gaps in the epidemiology database may prevent identification of further site concordance between rodents and humans although there is some site concordance indicated by the current database.

Mechanistic issues

The large body of literature available for TCE is not available for Perc but the TCE literature can help inform mechanistic issues for Perc. The same mechanistic studies that inform TCE hypothesized MOAs apply to Perc as well (Caldwell et al., 2008; Guyton et al., 2009). Both TCE and Perc affect multiple targets. There are similarities in some of the targets identified for TCE that can help guide further study as well.

There are two major routes of metabolism for Perc: (1) the predominant oxidative pathway, which results in TCA and other urinary metabolites, as well as reactive intermediates and carbon dioxide; and (2) the glutathione (GSH) conjugation pathway, which results in *S*-(1,2,2-trichlorovinyl)- glutathione (TCVG) and *S*- (1,2,2-trichlorovinyl)-L-cysteine (TCVC) that are further processed to other chemically reactive products that can bind to tissue macromolecules. Further, metabolism of the GSH metabolites, including activation by beta lyase, occurs in the kidney. A potential exists for extrahepatic metabolism and formation of reactive metabolites at sites other than the liver and kidney. Although many steps in the oxidative metabolism of

Perc are well characterized in both animals and humans, not all proposed intermediates have been identified or detected (U.S. EPA, 2009b).

Less Perc (approximately 1 to 37%) than TCE is metabolized by the body (Zewdie et al., 2008). The concentrations of Perc metabolites and their contributions to toxicity are harder to determine than those of TCE metabolites. More studies needs to be done of the GSH pathway. There are fewer data on Perc GSH metabolites and those metabolites are less well characterized than TCE metabolites. Adequate physiologically based pharmacokinetic (PBPK) models have not been developed that allow for predictions of metabolism and differences in metabolism between species for a number of key metabolites. For genotoxicity, there is not adequate information describing the toxicity of the GSH pathway and there is not a full genotoxicity battery for such metabolites. Other endpoints such as epigenetic changes have not been studied. While systemic metabolism is small, local metabolism could drive toxicities. The studies of the TCE GSH pathway may help provide information on the types of studies needed for Perc. The toxicokinetic and genotoxicity data for GSH metabolites available for TCE that suggest systemic delivery to and *in situ* formation in the kidney are not as well developed for Perc.

Although a wealth of new data related to understanding the toxic effects caused by Perc exposure has been published over the past decade, the MOA is not yet sufficiently characterized, tested, or understood for any one of these adverse effects (U.S. EPA, 2009b). A number of alternative hypotheses are identified and examined as possible MOAs for liver and kidney toxicity. Hypothesized MOAs for MCL, neurotoxicity, and developmental/ reproductive effects are indirect and are based on experimental observations of exposures to agents other than Perc. The available evidence points to multiple hypothesized MOAs as being involved, and, in each case, no one MOA can be uniquely identified.

Both Perc and TCA have been shown to activate the peroxisome-proliferation activated receptor (PPAR)-α, however, metabolism to TCA does not fully explain Perc-induced liver tumors, suggesting that other metabolites or intermediates contribute to Perc liver toxicity. The same issues regarding the PPAR-α activation MOA described for DEHP and TCE apply to Perc (see TCE and DEHP reviews). For the kidney, induction of alpha-2μ-globulin occurs only at doses higher than the doses that induce kidney cancer in male rat bioassays, and it is not likely to have an important role in toxicity or tumor induction. Scientific evidence is more supportive of the possibility that reactive metabolites from the GSH conjugation pathway are in some way responsible for kidney toxicity. The MOA of Perc-induced leukemogenesis in rats is not well understood; specifically whether the parent compound, a metabolite, or several metabolites are involved (U.S. EPA, 2009b).

Heterogeneity among humans is another uncertainty associated with extrapolating the results between animal and human databases. The extent of inter-individual variability in Perc metabolism has not been characterized. Several enzymes of the oxidative and GSH metabolism, notably those coded by *CYP2E1, CYP3A4, GSTZ, GSTA, GSTM,* and *GSTT,* show genetic polymorphisms with the potential for variation in metabolite production. Such heterogeneity should be incorporated into the design of epidemiological studies to further clarify the association between Perc exposure and carcinogenesis.

Lifestyle will also affect Perc metabolism, especially activities that induce P450 enzymes. A number of inducers of *CYP* enzymes have been shown to affect Perc metabolism.

Research needs and recommendations:

Human cancer studies

The chief venue of Perc exposure is dry-cleaning shops, which generally have fewer than ten employees, so assembling an occupational cohort could be difficult. Dry-cleaning workers could be recruited through the media, rather than shop by shop with shop management as the gatekeeper. Morning exhaled-breath specimens on a Saturday, highly correlated with the previous day's time-weighted average (McKernan et al., 2008), could be the criterion for inclusion in a study. Assembling a new occupational cohort could be difficult. In the United States, dry-cleaning workers could be recruited by various means (see discussion of overarching issues for further discussion). Morning exhaled-breath specimens on a Saturday, highly correlated with the previous day's time-weighted average (McKernan et al., 2008), could be the criterion for inclusion in a study. Data from the two U.S. dry-cleaning cohorts could be pooled for analysis (Blair et al., 2003; Ruder et al., 2001) and for a cancer incidence study. Identification of cohorts of workers outside the United States should also be explored. The Nordic study could be expanded into a cohort (Lynge et al., 2006).

Brain tumor is also a potential target for Perc. The working group is aware of several large brain case-control studies (NCI, NIOSH, Interphone), which will be analyzed in the next year or two for an association between exposure to chlorinated solvents and risk of brain cancer (Inskip et al., 2001; Ruder et al., 2006; Cardis et al., 2007)

Genetic susceptibility studies

Future human studies should include genotyping of *GST* variants. Since the glutathione conjugation pathway is not active in *GST*-null individuals, it can be hypothesized that the liver and kidney cancer risk will be low among *GST*-null individuals and high among *GST*-nonnull individuals. Where possible, retrospective *GST* genotyping could be done on stored specimens. Genetic variants in the *CYP2E1, CYP3A4* and other genes coding for enzymes that metabolize Perc or its metabolites, should also be investigated. Studies should also be conducted using entire genome scans to identify new susceptibility genes.

Mechanistic studies

With respect to mechanistic data, a major research gap is that MOAs are not sufficiently characterized or tested, for any of the Perc-induced adverse effects. Studies are needed that evaluate the GSH pathway of Perc metabolism, including genotoxicity studies, and that identify intermediates in the oxidative pathway. Lastly, adequate PBPK models have not been developed that allow for predictions of metabolism and differences in metabolism between species for a number of key metabolites.

Immunologic mechanism may be involved in lymphomagenesis from solvents (Vineis et al., 2007) and this should also be an area of future research. Brain tumor is also a potential target for DCM, and Perc, and has not been adequately studied for TCE.

Clearly more epidemiological study and analysis of existing epidemiological literature is need for Perc. However, the further development of the types of mechanistic data and toxicokinetic data that have been elucidated for TCE needs to be developed for Perc and will aid in the design and interpretation of epidemiological studies.

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Table 3. Studies Evaluating Tetrachloroethylene Exposure and Cancer Risk

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Aschengrau et al. 2003, Vieira et al. 2005 [Aschengrau, et al. 2003; Vieira, et al. 2005)	US NIEHS grant, US EPA grant	Case-control	672 female cases, 616 female controls	Breast cancer	Residents of 8 Cape Cod, Massachusetts, towns. Cases dx 1987-1993, Controls selected by random digit dialing or from Medicare roster	Relative delivered dose (RDD) or personal delivered dose (PDD) of PCE-contaminated piped-in water	Controlled for residence near dry cleaner, occ exp to PCE	RDD: with 0 latency adj OR 1.1 (0.8-1.4), PCE exp>75th percentile, adj OR 1.6 (1.1-2.4); with 5 years latency adj OR 1.2 (0.9-1.6), PCE exp>75th percentile, adj OR 1.6 (1.0-2.6); with 15 years latency adj OR 1.4 (0.9-2.3), PCE exp>75th percentile, adj OR 1.7 (0.7-4.3) PDD: with 0 latency adj OR 1.1 (0.8-1.4), with 5 years latency adj OR 1.2 (0.9-1.6), with 15 years latency adj OR 1.4 (0.9-2.3)	Exposure estimated wth model, no measurements
Bond et al. 1990 [Bond, et al. 1990]	Dow Chemical	Case-control nested in cohort	44 cases, 1,888 controls	Liver & biliary tract ca death	All employed in chemical production 1940-82, VS to 1982, controls randomly selected from cohort of 21,437	Work histories to classify by exp potential to 11 substances (any/none)	Carbon tet, chloroform, methylene chloride, 1,1,2-trichloro-ethane, vinyl chloride, PCB, dioxins, TCP, ethylene dibromide & dichloride	Exp: 13.6% cases, 11.3% controls RR 1.8 (0.8-4.3)	Workers could have had multiple exp, exp not quantified or qualified

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Costantini et al. 2008, Miligi et al. 2006 [Costantini, et al. 2008; Miligi, et al. 2006]}	US NCI, Europe Against Cancer Programme, Italian Alliance Against Cancer	Case-control, population based	586 leukemia cases, 1278 controls; 263 multiple myeloma (MM) cases, 1100 controls; 1428 NHL & 304 HL cases, 1530 controls	Acute myeloid leukemia (AML), chronic lymphatic leukemia (CLL), MM, or lymphoma diagnosis	Cases: dx 1991-1993 age 20-74; Controls from municipal files, stratified by sex and 5-year age groups	Detailed occupation history, industrial hygienist blinded to case status assessed PCE exposure as very low-low (LO) or medium-high (HI)	Benzene, styrene, xylene, toluene, dichlorome-thane, TCE, 1,1,1-trichloro-ethane	Leukemia LO OR 0.6 (0.2-1.6), HI OR 1.0 (0.4-2.7); NHL LO OR 0.6 (0.3-1.2), HI OR 1.2 (0.7-2.0)	possible latency/lag issues, no solvent specific results presented for HL
Heineman et al. 1994, Gomez et al. 1994 [Gomez, et al. 1994; Heineman, et al. 1994][37,42]	US NCI	Case-control	300 male cases, 320 male controls	Brain tumor mortality	Died 1978-1981 hospital-confirmed astrocytic brain tumor or other causes (minus CVD, epilepsy, suicide, homicide, cirrhosis, some ca), NOK interviewed	JEM for probablity of exp, cum= duration weighted by probability. 35% estimated exp. to PCE	JEMs also for other chlorinated solvents	Low cum exp OR adj age, location 0.8 (0.5-1.4), med adj OR 1.3 (0.8-2.2), high adj OR 1.5 (0.7-3.2), test for trend not statistically significant	Low participation rate, interviewed <50% (300/ 741 cases, 320/ 741 controls). Low number with medium or high exp (35 cases, 35 controls)
Lynge et al. 2006 [Lynge, et al. 2006]	Halogenated Solvents Industry Alliance & Danish Medical Research Council	Case-control nested in retro-spective cohort	46,768 laundry & dry-cleaning workers	Cancer incidence	Listed in 1970 census as "laundry and dry-cleaning worker" in Denmark, Finland, Norway, or Sweden. Cases dx 1970-2001, controls frequency matched by country, sex, 5-year age group, 5 year calendar period	For known dry cleaners, duration of employment (1964-1979) in shop where they worked in 1970 as PCE surrogate (90% of shops used PCE)	No way to separate PCE use from white spirit or chlorofluoro-carbon use	Bladder cancer RRs: dry-cleaner 1.44 (1.07-1.93), "other in dry-cleaning" 1.08 (0.55-2.11). Cervical cancer RRs: dry-cleaning 0.98 (0.65-1.47), "other in dry-cleaning" 1.73 (1.00-2.97).	18% unclassifiable as to dry-cleaning status

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Pesch et al. 2000a [Pesch, et al. 2000a]	German Federal Ministry of Research & Technology	Case-control	935 cases, 4298 population-based controls	RCC	Cases: German nationals dx 1991-1995. Controls frequency matched by region, sex, & age (5-year groups)	Occupational questionnaire, job-exposure matrices, job-task exposure matrices	Metals, paints, mineral oils, PAHs, asbestos	PCE JEM ORs: Medium exp males 1.4 (1.1-1.7), females 0.7 (0.4-1.3); high males 1.1 (0.9-1.4), females 1.1 (0.7-1.9); substantial males 1.4 (1.0-2.0), females 0.7 (0.3-2.2). PCE JTEM ORs: Medium exp males 1.2 (0.9-1.7), females 2.2 (0.9-5.2); high males 1.1 (0.7-1.5), females 1.5 (0.6-3.8); substantial males 1.3 (0.7-2.3), females 2.0 (0.5-7.8)	Combined OR (both sexes) not presented, trend tests (med-high-substantial) not done
Pesch et al. 2000b [Pesch, et al. 2000b]	German Federal Ministry of Research & Technology	Case-control	1035 cases, 4298 population-based controls	Urothelial carcinoma	Cases: German nationals dx 1991-1995. Controls frequency matched by region, sex, & age (5-year groups)	Occupational questionnaire, job-exposure matrices, job-task exposure matrices	Aromatic amines, paints & dyes, cutting fluids, PAHs, other chlorinated solvents	PCE JEM ORs: Medium exp males 1.1 (0.9-1.3), females 1.8 (1.0-3.0); high males 1.2 (1.0-1.5), females 1.0 (0.6-1.9); substantial males 1.4 (1.0-1.9), females 0.7 (0.2-2.5). PCE JTEM male ORs: Medium exp 1.0 (0.7-1.5). high 1.2 (0.8-1.7), substantial 1.8 (1.1-3.1)	Combined OR (both sexes) not presented, trend tests (med-high-substantial) not done
Seidler et al. 2007 [Seidler, et al. 2007]	German Federal Office for Radiation Protection	Case-control	710 lymphoma cases, 710 population-based controls	Lymphoma	Cases dx in 6 German regions age 18-80; controls matched on region, gender, and age ± 1 year	Complete occupational history assigned intensity & frequency of PCE by case-status blinded industrial physician	TCE, carbon tet, dichloro-methane, benzene, toluene, xylene, styrene	<9.1 ppm years adj OR 1.1 (0.5-2.3), 9.1-78.8 ppm years adj OR 1.0 (0.5-2.2), >78.8 ppm years adj OR 3.4 (0.7-17.3), trend p 0.12 T cell NHL trend p 0.01	No exposure measurements. Mixed exposures?

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Anttila et al. 1995 [Anttila, et al. 1995]	Finnish Work Med Fund, US NIOSH grant	Linkage monitoring registry-cancer registry	292 men, 557 women	Cancer diagnosis	Monitored (serum) for exposure any time during 1974-1983; cancer diagnosed 1967-1992	Median 0.7 µmol PCE/L (men), 0.4 µmol PCE/L (women).	Small % also monitored for TCE, 1,1,1-trichloroethane	31 ca SIR 0.9 (0.6-1.3), 3 pancreas ca SIR 3.1 (0.6-9.0), 5 lung ca SIR 1.92 (0.6-4.5), 2 cervix ca SIR 3.2 (0.4-11.6), 2 kidney ca SIR 1.8 (0.2-6.6), 3 NHL SIR 3.8 (0.8-11.0)	60% only had 1-2 measurements (short duration employment?) . Low statistical power
Lynge & Thygesen 1990 [Lynge and Thygesen 1990]	Danish Cancer Registry	Linkage census-cancer registry	8567 women, 2033 men	Cancer diagnosis	Working in dry cleaning or laundry in 1970, diagnosed 1970-80	PCE most common from 1950s (0-100 ppm)	TCE for workclothes, chlorofluorocarbons for fur	No increase overall. Men: 131 ca SIR 1.3 (1.1-1.5), 9 pancreas ca SIR 2.4 (1.1-4.6). Women: 7 liver ca SIR 3.4 (1.4-7.0), 13 pancreas ca SIR 1.4 (0.7-2.4)	Because of industry coding cannot separate dry cleaners & laun-derers. Short latency for those who began work close to 1970 (duration employment unknown)
Travier et al. 2002 [Travier, et al. 2002]	IARC	Linkage census-cancer registry	>500,000 PYAR	Cancer diagnosis	Working in dry cleaning-laundry industry or as launderer-dry clearner or presser in 1960 and/or 1970, diagnosed 1971-89	Stratified by presumed start date based on age: if 60+ in 1960, C tet, Stoddard, TCE; <40 in 1960, PCE & C tet; 40-60, all four	Stoddard solvent, C tet, TCE	RR (vs non dry cleaners launderers) adj for age, era, region, urban/nonurbal) if in both censuses 389 ca RR 0.99 (0.9-1.1), 4 Hodgkins disease RR 2.7 (1.0-7.2), 15 leukemia RR 1.8 (1.1-3.1)	Industry & job coding could not separate dry cleaners, launderers. Short latency for any who began work close to 1970 (duration employment unknown)
Blair et al. 2003 [Blair, et al. 2003]	US NCI	Retro-spective cohort	5,369	Cause of death	Joined dry-cleaning union 1948-1978, VS to 1993	Exp score based on literature: operators 40; pressers, etc. 7; drop shops 0	Not reported	590 ca SMR 1.2 (1.1-1.3), 12 bladder ca SMR 1.3 (0.7-2.4), 27 cervix ca SMR 1.6 (1.0-2.3), 26 esophageal ca SMR 2.2 (1.5-3.3), 21 emphysema SMR 1.7 (1.0-2.5)	No strong relationship with duration or estimated solvent exposure

Reference	Study support/ affiliation	Study design	N	Outcome studied	Eligibility criteria	Exposure level	Other solvents, other exposures	Outcomes, ratio† (CI)	Study design issues
Boice et al. 1999 [Boice, et al. 1999]	Lockheed Martin Corporation	Retro-spective cohort	45.323 factory workers, 32,642 non-factory workers	Cause of death	Aircraft mfg ≥1 year >1959, 2267 exp to PCE VS to 1996	13% factory wkrs exp PCE	TCE primary degreaser to 1966, many other solvents, cutting fluids, asbestos, chromate	PCE exposed: 476 deaths SMR 0.90 (0.8-0.98), 141 ca SMR 1.07 (0.9-1.3), no ca COD SMR significantly up or down *RR non factory workers overall 0.9/0.76=1.18, all ca 1.07/0.8=1.34*	Multiple exp. Short latency for 11% factory , 24% non-factory workers who started after 1980. What about those employed 1928-1960?
Ruder et al. 2001, Ludwig et al. 1983 [Ludwig, et al. 1983; Ruder, et al. 2001]	NIOSH	Retro-spective cohort	1,708, incl 625 exp only to PCE	Cause of death	Member of dry-cleaning union, ≥1 y PCE exp <1960, no C tet	1977-79 level PCE: operators GM 22 ppm, pressers GM 3.3 ppm, counter workers 3.1 ppm.	1,083 exp "other" solvent, probably Stoddard solvent	271 ca SMR 1.3 (1.1-1.4), 10 bladder ca SMR 2.2 (1.1-4.1), 12 cervical ca SMR 2.0 (1.0-3.4), 14 esophageal ca SMR 2.5 (1.4-4.1), 82 respiratory disease SMR 1.3 (1.0-1.6)	No increase in liver or hematopoietic ca, bladder, esophageal increase by duration employment, latency

† Odds ratio OR, relative risk RR, standardized mortality ratio SMR, standardized incidence ratio SIR, 95% confidence interval ( ); The 95% confidence intervals (CI) are presented where reported (where not reported CIs were calculated, if possible).

* 90% confidence interval. *Calculations done for this paper are in italics*

Chemical abbreviations: Me Cl (methylene chloride). PCB (polychlorinated biphenyls), PCE (tetrachloroethylene), TCA (trichloroacetic acid--TCE metabolite), TCE (trichloroethylene), TCP (2,4,6-trichlorophenol)

Disease abbreviations: ca (cancer), dx (diagnosed), NHL (non-Hodgkins lymphoma), RCC (renal cell carcinoma)

Miscellaneous abbreviations: cum (cumulative), environ (environment, environmental), EPA (Environmental Protection Agency), est (estimated), exp (exposure, exposed), IH (industrial hygienist), JEM (job-exposure matrix), med (medical, medicine), Natl (National), NCI (National Cancer Institute), NIOSH (National Institute for Occupational Safety and Health), occup (occupational) TWA (time-weighted average), US (United States)

Statistical abbreviations: DFE (date 1st exposed), freq (frequency), F/U (followup), GM (geometric mean), IDR (incidence density ratio, chance of preganancy), mon (month), NSS (not statistically significant), p (probability), VS (vital status), y (year)

Chloroform

by David M. DeMarini PhD and Elsebeth Lynge PhD

Citation for most recent IARC review

IARC Monograph 73, 1999

Current evaluation

Conclusion from the previous Monograph:
There is *inadequate evidence* in humans for the carcinogenicity of chloroform. There is *sufficient evidence* in experimental animals for the carcinogenicity of chloroform. Chloroform is *possibly carcinogenic to humans (Group 2B)*.

Exposure and biomonitoring

Occupational exposure to chloroform (trichloromethane) may occur during its production and use as a solvent and chemical intermediate. The general population may be exposed as a result of the presence of chloroform in chlorinated drinking water, ambient air, and some foods.

Exposures to chloroform via showering, bathing, and swimming have been characterized much better since 1999, and these studies show that blood levels are usually higher via these routes than via drinking chlorinated water (Xu & Weisel, 2005; Caro & Gallego, 2007; Zwiener et al., 2007). Chloroform exposure has also been documented in various occupations (Ruder, 2006) and in microenvironments, such as restaurants (Loh et al., 2006). Chloroform exposure also may be occurring when people use triclosan-containing antibacterial soaps (Fiss et al., 2007).

Considerable efforts have been made to establish the levels of chloroform exposure in the general population using NHANES data (Riederer et al., 2009), and computational toxicology based on these data suggest that 95% of the U.S. population represented by NHANE III data had chloroform exposures ≤7 μg/L in tap water and ≤0.2 μg/L in ambient household air (Lyons et al., 2008). The levels of chloroform in alveolar air appear to be the most sensitive biomarker of exposure relative to ambient air levels; urinary levels of chloroform are less sensitive than breath levels (Caro & Gallego, 2008). Concentrations of chloroform in breath appear highly associated with levels in water, and blood and breath levels are also associated with each other (Gordon et al., 2006). New studies have attempted cancer risk assessments based on exposure assessments via oral and dermal/inhalation routes (Liao et al., 2007; Tan et al., 2006, 2007; Wang et al., 2007; Panyakapo et al., 2008; Hamidin et al., 2008).

A survey of levels of chloroform, as well as of other disinfection by-products (DBPs), in U.S. drinking water (Weinberg et al., 2002) showed that chloroform at high concentrations (>60 μg/L) likely reflects the presence of relatively high levels of other trihalomethanes and other DBPs. In contrast, low concentrations of chloroform (<20 μg/L) prevent any conclusions regarding the levels of other DBPs in the water. As indicated from some of the new epidemiology and mechanistic studies described below, chloroform is probably not an ideal

surrogate for assessing risk for bladder cancer for humans from chlorinated water. Other possibilities are total DBP, total trihalomethanes (THM), brominated THMs, or MX. The panel recommended future IARC evaluation of "water chlorination disinfection-by products."

Cancer in humans

(*inadequate*, Vol 73, 1999)
Drinking water is the main medium by which people are exposed to chloroform. However, drinking water is a complex mixture, and only a few epidemiology studies have had adequate exposure assessments to permit associations between cancer and specific compounds in the water.

Bladder cancer

Since IARC vol. 73, a number of epidemiological studies have been published on the association between water DBP exposure and the risk of bladder cancer, the two most important ones being a pooled analysis of previous case-control studies by Villanueva et al. (2004) (data from only one of the six pooled studies was included in IARC vol. 73), and a new case-control study from Spain (Villanueva et al., 2007). In both studies, interview data were collected on residential history and daily tap water consumption; and in the latter study data were also collected on bathing, showering, and swimming in pools. The investigators collected data on annual average THM levels, water source history, and year of start of chlorination from 123 municipalities. In addition, 113 chlorinated water samples were collected, and the THM levels were measured. The data sources were merged to create the exposure variables. In both studies there was a clear dose-response relationship between average residential THM levels and risk of bladder in men but not in women (see Table).

Average residential THM level µg/L*	Men		Women	
	Villanueva et al. (2004)	Villanueva et al. (2007)	Villanueva et al. (2004)	Villanueva et al. (2007)
0-1	1.00	1.00	1.00	1.00
>1-5/8.0	1.10 (0.92-1.31)		0.99 (0.72-1.36)	
>5/8.0 – 25/26.0	1.26 (1.05-1.51)	1.53 (0.95-2.48)	0.86 (0.63-1.18)	0.40 (0.13-1.27)
>25/26.0-50/49.0	1.25 (1.04-1.50)	2.34 (1.36-4.03)	1.04 (0.76-1.43)	1.14 (0.31-4.10)
>50/49.0	1.44 (1.20-1.73)	2.53 (1.23-5.20)	0.93 (0.67-1.28)	1.50 (0.26-8.61)
Trend p value	<0.001	<0.01	0.753	0.61

* Limits: Villanueva et al. (2004)/Villenueva et al. (2007)

Villanueva et al. (2007) reported detailed analysis of bladder cancer risk by average ingestion of THM, THM exposure in shower and bath, and life-time hours swimming in pools. Compared to men with the lowest level, men with the highest THM ingestion had a RR of 1.61 (95% CI 1.06-2.44), men with the highest shower/bath THM exposure had a RR of 2.01 (95% CI 1.23-3.28), and men swimming in pools most frequently had a RR of 1.59 (95% CI 1.01-2.51); all associations showed a dose-response pattern. For women the three relative risks were 0.47 (95% CI 0.15-1.51); 2.26 (95% CI 0.58-8.90); and 1.19 (95% CI 0.30-4.72);

with none of the associations showing a dose-response pattern. Further analysis showed that the dose-response relationship was found for persons with two deleted *GSTT1-1* alleles (-/-), but not for persons with at least one non-deleted *GSTT1-1* allele (+/+ or +/-) (Cantor et al., 2006). In all THM exposure categories, the risk of bladder cancer decreased with increasing water intake (Michaud et al., 2007).

Other cancer sites

Based on the observation of an increased risk of bladder cancer following dermal exposure in Spain, a re-analysis was undertaken of data from a case-control study on skin cancer from New Hampshire, U.S. High exposure to THM (>40 µg/L) versus low exposure (<1 µg/L) was associated with an increased risk of both basal cell carcinoma, OR 2.4 (95% CI 0.9-6.7) and squamous cell carcinoma, OR 2.1 (95% CI 0.7-7.0) (Karagas et al., 2008).

The risk of colon and rectal cancer in relation to THM exposure was investigated in a case-control study in southern Ontario, Canada. Interview data on residence history was collected and merged with water supply data. A positive dose-response association was found for THM-years and colon cancer in men; highest versus lowest, OR 1.74 (95% CI 1.25-2.43). No association was found for women, nor was there any association between THM-years and the risk of rectal cancer (King et al., 2000).

The risk of rectal cancer was studied also in Monroe County, Western New York State, U.S. No association was found between THM exposure (µg/L) and risk of rectal cancer, OR 1.01 (95% CI 0.98-1.03), while the risk associated with bromoform exposure (µg/L) was slightly increased, OR 1.20 (95% CI 1.05-1.35) (Bove et al., 2007).

A large Canadian case-control study showed a borderline significant increase in risk of chronic myelocytic leukemia with duration of exposure to chlorinated surface water. In contrast, this exposure tended to be protective against chronic lymphocytic leukemia and hairy cell leukemia (Kasim et al., 2006).

Cancer in experimental animals

(*sufficient*, Vol 73, 1999)

Two, 2-year rodent bioassays of chloroform had been performed at the time of the 1999 IARC monograph, and two additional studies have been published since then. Yamamoto et al. (2002) showed that inhalation exposure to chloroform was not carcinogenic to male or female F344 rats but did induce kidney tumors in male BDF mice and liver tumors in female mice. Nagano et al. (2006) found no increase in cancer at any organ in male F344 rats exposed to chloroform in their drinking water (1000 ppm w/w) or in rats exposed by inhalation to chloroform at 25, 50, or 100 ppm (v/v) for 6 h/day and 5 day/week. However, male F344 rats exposed by both routes had increased frequencies of renal-cell adenomas and carcinomas, atypical renal-tubule hyperplasia, increased cytoplasmic basophilia, increased dilated tubular lumens of the kidney, and increased urinary glucose. Thus, the combined exposure enhanced carcinogenicity and chronic toxicity in the proximal tubule of the male rat kidney.

In summary, four studies in rats showed that chloroform induced kidney tumors by gavage, kidney tumors by drinking water in one study but no tumors in another study, no tumors in

two studies by inhalation, and kidney tumors by a combined drinking water/inhalation exposure. Three studies in mice showed that chloroform induced liver tumors by gavage, liver and kidney tumors by inhalation, and no tumors by drinking water. Thus, chloroform induced kidney tumors in three studies in rat, liver tumors in two studies in mouse, and kidney tumors in one study in mouse. A small case-control study of dogs showed no association between chlorinated household water and risk of bladder cancer (Backer et al., 2008).

Mechanisms of carcinogenicity

Chloroform is an anomaly among the trihalomethanes in that its mechanism of action is entirely different from that of the other THMs. All of the other trihalomethanes are activated to mutagens by *GSTT1-1*, and as discussed below, there is emerging evidence that these other trihalomethanes (i.e., brominated THMs) be the relevant causative agents for the bladder cancer associated with chlorinated drinking water. In contrast, chloroform is not mutagenic, and a likely mechanism for its carcinogenicity has been summarized by Schoeny et al. (2006).

The postulated mechanism involves oxidative metabolism primarily by CYP2E1 to produce cytotoxic metabolites, especially phosgene. Although reductive metabolism could produce mutagenic metabolites, this pathway would likely operate only under conditions of high exposure, as in the chronic bioassay. These metabolites injure and kill cells, resulting in regenerative cell proliferation. If this tissue injury and consequent proliferation is sustained, as it is in the chronic rodent studies, mutations, epigenetic changes, etc. likely occur that, with selection, lead to the kidney tumors observed in rats.

The concept that increased cellular proliferation in response to cellular toxicity is enough to produce tumors is an oversimplification of other crucial events taking place within transformed or initiated cells. Events such as increased growth advantage of initiated cells either by increase in cellular proliferation or decreased apoptosis are important determinants in the pathway to carcinogenesis. For instance, at an approximately 18% increase in cellular proliferation, carbon tetrachloride produced an 85% hepatic tumor incidence in mice (Nagno et al., 2007). At a similar frequency of cellular proliferation, chloroform produced a 12% hepatic tumor incidence (Yamamoto et al., 2002). The difference in tumor incidence between the two chemicals suggests that cellular processes downstream of increased cellular proliferation may be crucial. Furthermore, several studies reviewed in IARC Vol 73 reported that chloroform has the ability to inhibit the development of tumors, although the exact mechanism is not known.

As noted by Schoeny et al. (2006), the postulated mechanism for chloroform described above is presumed to be relevant to humans because all three key steps (metabolism to phosgene, toxicity, and cell proliferation) are known to occur in humans. However, children are not expected to have increased susceptibility because CYP2E1 is expressed minimally in fetal and neonatal tissue, and the developing organism is not especially sensitive to cytotoxic agents at low doses.

Philip et al. (2006) showed that repeated exposure of Swiss Webster mice to 150 mg of chloroform/kg/day by gavage for 30 days, followed by a single, normally lethal dose of chloroform (750 mg/kg) by gavage 24 h after the last exposure, permitted 100% of the mice to

survive, whereas 90% of the mice given only the lethal dose died. The authors noted that the pre-exposure resulted in 40% lower chloroform levels in the blood and increased tissue repair in the kidney (but not the liver) and that the protective effect of pre-exposure was not associated with enhanced detoxification or decreased bioactivation of chloroform.

Fabrizi et al. (2003) found that phosgene, one of the metabolites of chloroform, forms a covalent bond with human histone H2B in vitro. Because modified histones could alter gene expression, among other cellular functions, this epigenetic process could play a role in the carcinogenic mechanism of chloroform.

Chloroform does not induce colorectal tumors in rodents, but some of the brominated trihalomethanes do. A mechanistic study by DeAngelo et al. (2002) in which mice were exposed to various trihalomethanes in drinking water for 13 weeks found that the brominated trihalomethanes, but not chloroform, induced aberrant crypt foci in the colon. These potentially pre-neoplastic cells may develop into colon tumors, and studies such as this and others suggest that chloroform is unlikely to be the cause of such tumors.

As noted above, chloroform is usually the most prevalent DBP in drinking water; however, it is not necessarily a surrogate for the levels or presence of other DBPs. In addition, other DBPs, most likely the brominated trihalomethanes, are more probable as potential causes of the bladder cancer associated with drinking water than is chloroform. Richardson et al. (2007) have summarized the carcinogenicity, occurrence levels, and mutagenicity of more than 80 DBPs, and their current hypothesis, which is based on a set of studies in transgenic bacteria, rodents, humans, and the epidemiology study of Villanueva et al. (2007), is that exposure to the brominated trihalomethanes via dermal/inhalation routes through swimming/showering/bathing may result in systemic distribution of the compounds via the blood, and in the bladder these could be activated to mutagens by *GSTT1-1* and initiate bladder cancer. In contrast, THMs consumed via the oral route would likely be inactivated in the liver by CYP2E1 (Richardson et al., 2007).

Research needs and recommendations

To date, neither chloroform nor any other DBP has been tested in animals for any health effects whatsoever, including cancer, via the dermal route. Given the extensive and well-documented exposure to DBPs, including chloroform, via this route, and the emerging evidence that dermal/inhalation exposure to trihalomethanes (especially the brominated ones) may be most associated with bladder cancer, this type of experiment in rodents is necessary to explore the role that route of exposure may play in carcinogenicity of chloroform.

Additional epidemiology studies are needed such as that of Villanueva et al. (2007) in which route of exposure is stratified. In addition, detailed DBP exposure assessment, especially of chloroform and the brominated trihalomethanes, is needed to explore better any link between chloroform in drinking water and the risk for bladder or colon cancer. A large New England bladder cancer case-control study is currently being undertaken. A pooled analysis is under way of bladder cancer case-control studies from Spain, France, and Finland, and of colorectal cancer case-control studies from Spain and Italy (Nieuwenhuijsen et al., 2009). Epidemiological studies of high-exposure groups, such as competition swimmers, are

warranted. Pool attendants and indoor life guards are other potentially exposed groups worth investigating. Finally, follow up is recommended of cohorts of nurses and doctors exposed to chloroform when it was used as an anesthetic gas.

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Polychlorinated biphenyls (PCBs)

by Larry W. Robertson PhD and Avima Ruder PhD

Citation for most recent IARC review

IARC Supplement 7, 1987

In IARC Monograph Volume 100 F (in press), PCB 126 is *carcinogenic to humans (Group 1)* (Baan et al 2009). In making the evaluations, the Working Group considered the following mechanistic arguments: There is strong evidence to support a receptor-mediated mechanism for PCB 126 carcinogenesis in humans based upon evidence of carcinogenicity in experimental animals and upon extensive evidence showing activity identical to 2,3,7,8-TCDD for every step of the mechanism described for 2,3,7,8-TCDD carcinogenesis in humans including receptor binding, gene expression, protein activity changes, cellular replication, oxidative stress, promotion in initiation-promotion studies and complete carcinogenesis in laboratory animals. (http://monographs.iarc.fr/ENG/Meetings/vol100F-evaluations.pdf)

Current evaluation

Conclusion from the previous review:
There is *limited evidence for the carcinogenicity to humans of polychlorinated biphenyls (Group 2A).* The available studies suggest an association between cancer and exposure to PCBs. The increased risk from hepatobiliary cancer emerged consistently in different studies. Since, however, the numbers were small, dose-response relationships could not be evaluated, and the role of compounds other than PCBs could not be excluded, the evidence was considered to be limited.

Environmental Exposure and Biomonitoring

PCBs were widely used from the 1930's through the 1980's and later, with an estimated total production of about 1.3 million metric tons (Breivik et al., 2002). Exposure continues from leaks from transformers and capacitors, volatilization of PCBs in cites, in buildings, from sewage, landfills and waste sites, and combustion of materials containing PCBs (Dyke et al., 2003).

Occupational Exposure

PCB exposures associated with occupational settings have greatly diminished since the 1970's, due to the ban on new uses for PCBs. Since the production of PCBs ended worldwide in 1993 (Breivik et al., 2002), new occupational exposure has been confined to four groups of workers: personnel replacing or repairing transformers and capacitors still containing PCB dielectric fluid (Altenkirch et al., 1996; Hay and Tarrel 1997; Shalat et al., 1989; Wolff et al., 1992); first responders to incidents where a transformer has exploded (Kelly et al., 2002); construction workers removing old paint, plaster, caulk, or floor finishes containing PCBs (Fromme et al., 1996; Herrick et al., 2004; Herrick et al., 2007; Piloty and Koppl 1993; Rudel and Perovich 2009; Rudel et al., 2008), and workers at hazardous waste disposal sites (Gonzalez et al., 2000; Zhao et al., 2006). The serum levels of workers engaged in sealant removal were 2-10 times higher at the end of these activities than they had been one year before (Kontsas et al., 2004). Workers exposed occupationally while PCBs were still in general use have a body burden of PCBs from their former exposure (Schecter et al., 2002; Wolff 1985). A large number of capacitors and transformers filled with PCBs is still in use

(Environmental Protection Agency and Environment Canada 2005) so potential occupational exposure continues.

Foodborne Exposure

Food chain exposure incidents by accident or malice have also occurred; one is reminded of Yusho (1968), Yu-Cheng (1979), the French cheese contamination episode (1976) and the Belgium "Dioxin" Crisis of 1999 (Covaci et al., 2008). Lower levels of PCBs are broadly prevalent in foodstuffs, and a great deal of attention has been paid to these PCBs, especially higher chlorinated ones, those more resistant to metabolic transformation, and those from certain food sources, especially in fish (Ludewig et al., 2007). People living on or near PCB-contaminated soil or near PCB-contaminated water, those eating contaminated foods, and those living in old homes being renovated continue to be exposed (Patterson et al., 2009; Rudel et al., 2008; Weintraub and Birnbaum 2008; Zheng et al., 2008).

Airborne Exposure

Air as a source of environmental PCB exposure was nearly completely ignored until about a decade ago. Systematic measurements of atmospheric PCBs started only in the 1990's. The first urban monitoring site was installed in Chicago in 1995. The level of PCB contamination in the air is strongly influenced by temperature. In Chicago air concentrations between 100-300 pg/m^3 in winter and up to 5,000-16,000 pg/m^3 on hot summer days were reported (Green et al., 2000). Inhalation exposure is considered to be a major route of occupational exposure to PCBs, and it was estimated that in capacitor workers, for example, a maximum of 80% of adipose PCBs may have been absorbed by inhalation exposure (Wolff 1985). Recently even higher levels of PCBs were measured in indoor air in buildings constructed in the 1970's using joint sealants that contained 4-9% PCBs. Indoor air concentrations up to 13,000 ng/m^3 were measured in some classrooms of a contaminated school (Kohler et al., 2002), which is more than an order of magnitude above the NIOSH guideline of 1 $\mu g/m^3$ (NIOSH, 2004) for occupational settings. Other possible sources for indoor PCBs are believed to be data screen terminals (Digernes and Astrup 1982), ceiling tiles and fluorescent light capacitors (Harris 1985). It was reported that the concentration of PCBs in indoor air can be at least an order of magnitude higher than in outdoor air (Balfanz et al., 1993; Vorhees et al., 1997; Wallace et al., 1996); however, regional outdoor levels can be very high due to activities like building renovations, dredging, or contamination from cement factory exhaust. Thus under certain circumstances the intake from inhalation exposure exceeds PCB intake from food.

PCBs in foods, like fish or mothers' milk, and in human adipose tissue are usually the higher chlorinated ones, where congeners like PCB153, PCB180, PCB183 and others predominate. Airborne PCBs are very different, since they require volatilization. Major congeners in Chicago air are PCB5/PCB8 (co-elute), PCB18, PCB28, PCB44, PCB52, PCB77/PCB110 (co-elute), PCB95, PCB101, to name a few (Hu et al., 2008; Zhao et al., 2009). Of two populations in Italy the more urban group had significantly higher levels of lower chlorinated PCBs (PCB52 was about 100-fold higher) than the population in a more rural environment (Turci et al., 2006). In Germany, PCB28 and PCB52 were the prevailing congeners in indoor air of contaminated schools (Kohler et al., 2002; Schwenk et al., 2002). Elevated levels of PCB28 and PCB52 were measured in the blood of teachers from these schools compared to non-contaminated schools, whereas the mean blood levels of higher chlorinated PCBs, i.e. PCB138, PCB153 and PCB180

were almost identical (Schwenk et al., 2002). Children in schools with 690-20,800 ng PCB/m^3 air had median levels of 6, 9, and 5 ng/l PCB28, PCB52, and PCB101, respectively, whereas children in non-contaminated schools had levels below the detection level of 1 ng/L (Liebl et al., 2004). Both groups had no significant differences in PCB138, PCB153, and PCB180 levels, indicating that indoor air exposure contributed to the PCB body burden. In Germany the non-occupational tolerable indoor total PCB concentration was limited to 300 ng/m^3 (PCB Guideline 1995) based on a tolerable daily intake (TDI) of a total of 1 µg/kg body weight. Not only were these levels exceeded in several schools, but this TDI was based on a chronic toxicity study with a commercial PCB mixture, which measured hepatic enzyme induction as endpoint (Chen and Dubois 1973). Airborne PCB profiles are distinctly different from those of commercial PCB mixtures like Aroclor 1254, and enzyme induction in the liver is most likely a completely inappropriate endpoint of toxicity for inhalation exposure.

The importance of airborne PCBs is now understood. Very little is known, however, about the toxicity of these airborne PCBs and the consequences of exposure by inhalation compared to ingestion. Airborne PCBs are lower chlorinated. Our daily exposure to these airborne PCBs may be low under most circumstances, but children playing near Superfund sites in hot summer days, workers moving dried dredging material, or families living unknowingly in buildings with high indoor PCB concentrations, may be exposed to significant amounts of airborne PCBs for extended times.

Cancer in humans

(*limited***,** Supplement 7, 1987)

The previous IARC review included three occupational epidemiology cohort studies. All three of those studies have been updated (one was expanded) and an additional nine cohort studies have been published. There have also been twelve case-control studies published since the previous IARC review. Many of these used industrial hygienist reviews of participant occupational histories to estimate relative PCB exposure. The strongest studies determined PCB levels in plasma or serum from case and control specimens stored before the cases were diagnosed. No relation was observed between Janus Serum Bank specimen PCB levels and breast cancer diagnoses from the Norwegian Cancer Registry (Ward et al., 2000). Engel and colleagues found a statistically significant trend of higher odds ratios (OR) for non-Hodgkin lymphoma with higher total stored serum PCBs, in three different populations (Engel et al., 2007; Rothman et al., 1997). A significant inverse relation between total PCBs and risk of testicular germ cell tumors was seen in a study linking PCB levels of stored U.S. Department of Defense (DOD) blood specimens with DOD Medical Surveillance (McGlynn et al., 2009).

Recent papers have reviewed much of the epidemiologic literature (Faroon et al., 2003; Faroon et al., 2001; Golden and Kimbrough 2009). The lack of congruity in the cohort results may be due to all occupational PCB exposure having been to mixtures of congeners, with the proportion of each congener varying from batch to batch (Hopf et al., 2009). Because there are so many PCB congeners, some co-planar and some not, some estrogenic and some not (Fiedler 1998), it seems plausible that a variety of tumor types could arise from exposure to various congeners, or their metabolites.

Cancer in experimental animals

(*sufficient*, Supplement 7, 1987)

Studies indicate that PCB mixtures with a higher chlorine content are more potent in inducing nodular hyperplasia and hepatocarcinomas than mixtures with lower chlorination (Silberhorn et al., 1990), especially in male rodents. In a comprehensive chronic toxicity and carcinogenicity study, the effects of four Aroclor products (1016, 1242, 1254, and 1260) were investigated at multiple dietary concentrations, ranging from 25 to 200 ppm, for 24 months in male and female Sprague–Dawley rats. Statistically significant increases in hepatocellular carcinomas were noted in male rats only for the higher-chlorinated mixture Aroclor 1260, while all four commercial products produced an elevated incidence of hepatocellular carcinomas in female rats. It should be noted that Aroclor 1016 averages only three chlorines per biphenyl. These data indicate that commercial mixtures of chlorinated biphenyls are complete carcinogens, especially in the female rat (Mayes et al., 1998).

Mechanisms of carcinogenicity:

Metabolic Activation of Lower Chlorinated PCBs to Reactive Intermediates

It has long been recognized that biphenyl and halogenated biphenyls, particularly the lower halogenated congeners, are hydroxylated *in vivo* and *in vitro* (see review by (Safe 1989)). These hydroxylation reactions are primarily catalyzed by isoforms of cytochrome P-450. Experiments with PCB3 (4-chlorobiphenyl) and rat liver microsomes showed that five **mono-** and three **di-hydroxy metabolites** were formed (McLean et al., 1996a). The metabolism of PCB3 by cytochrome P-450 probably involves an **arene oxide intermediate** (McLean et al., 1996b; Safe 1989). Other arene oxides could be involved in the oxidation of the mono- to the di-hydroxy forms. Arene oxides are strong electrophiles which may react with critical cellular targets. The dihydroxybiphenyls can be further oxidized by various enzymes like peroxidases, prostaglandin synthase and cytochrome P-450s to the corresponding **quinone** with the formation of a **semiquinone intermediate** (Wangpradit et al., 2009). The formation of *ortho*- and *para*-quinones from diOH-PCB3 *in vitro* has been demonstrated as has their reactivity toward nitrogen and sulfur nucleophiles (Amaro et al., 1996). Other experiments demonstrated that the microsomal metabolism of PCB3 resulted in the formation of adducts with nucleotides *in vitro*, preferentially with purines rather than pyrimidines (McLean et al., 1996b). Most likely at least 1 of the 4 adducts seen is derived from an arene oxide intermediate, the 3 other adducts after further oxidation probably from a (semi)quinone (McLean et al., 1996b). These results suggest that several metabolic pathways and chemical species could be involved in PCB-induced DNA adduction. Very recent publications on oxidative DNA adducts arising from PCB exposure have appeared (Jeong et al., 2008; Spencer et al., 2009).

Oxidative DNA Damage

Lower chlorinated PCBs produce reactive oxygen species (ROS) and intracellular oxidative stress (Oakley et al., 1996; Srinivasan et al., 2001). Free radicals, particularly hydroxyl radicals, may produce 8-oxodeoxyguanosine (8-oxodG), a DNA lesion that is highly mutagenic, producing G $\rightarrow$ T transversions (Marnett and Burcham 1993). Hydroxyl radicals

can also attack fatty acids (linoleic acid, linolinic acid, oleic acid, etc) and form lipid peroxidation-derived enals, such as acrolein, crotonaldehyde, *trans*-4-OH-2-nonenal (4-HNE), and malondialdehyde (MDA) (Nair et al., 1999). These products can then modify DNA bases, resulting in cyclic adducts by interaction of their difunctional groups with NH_2 group in dA, dG or dC residues in DNA (Chaudhary et al., 1994; Chung et al., 1996; Winter et al., 1986). These cyclic adducts are mutagenic, producing base substitutions and deletions, for example G → T mutations from propano-dG and C → A mutations from various etheno adducts (Basu et al., 1993; Marnett and Burcham 1993; Nath et al., 1996). Therefore the question of mutagenicity of PCBs, especially congeners that are prone to metabolic activation like airborne PCBs, should be re-analyzed.

Hydroxylated PCBs

Stable hydroxylated metabolites of PCBs (OHPCBs) are routinely found in human blood. The percentage of total OHPCBs to total PCBs in human blood ranges from 13 to 44% (Fangstrom et al., 2002; Masuda and Haraguchi 2004; Sandanger et al., 2004; Sandau et al., 2000). 4-OHPCB187, 4-OHPCB146, 4-OHPCB107, 3'-OHPCB138 and 3-OHPCB153 are the five metabolites with the highest concentration in blood. There is increasing evidence that OHPCBs are important in the toxicities associated with PCBs. Along with studies over the last decade or so establishing the presence of OHPCBs in humans and other animals, there is increasing interest in the further metabolism of OHPCBs (Sacco et al., 2008; Wang et al., 2005; Wang et al., 2006). While sulfation has often been considered as a detoxication reaction, the OHPCBs also inhibit sulfotransferases that are important in metabolism of hormones and other endogenous molecules. This has been seen for human estrogen sulfotransferases (Kester et al., 2000), sulfotransferases active with thyroid hormone sulfation (Schuur et al., 1998), and, most recently, for human hydroxysteroid sulfotransferase (Liu et al., 2006). Thus, these molecules may potentially be involved in endocrine disruption and other responses relevant to carcinogenesis. The roles of metabolism of OHPCBs (e.g., further oxidation, sulfation, and other metabolic reactions) in the disposition and toxicity of these compounds represent a significant gap in our knowledge about mechanisms for carcinogenesis and other toxic responses to PCBs.

PCBs as mutagens/genotoxins

(*In vitro* studies)
Comprehensive reviews of the genotoxic actions of PCBs have been published (Ludewig 2001; Ludewig et al., 2008; Silberhorn et al., 1990). Recently a series of PCB3 metabolites were tested in various genotoxicity assays to determine their activity and genotoxicity profile. Both the 3,4- and particularly the 2,5-quinone of PCB3 were efficacious and potent inducers of gene mutations at the HPRT locus. Neither the corresponding dihydroxy metabolites nor the phenols had any activity in this assay. The 2,5-quinone was also by far the most potent and efficacious inducer of chromosome breaks as determined by CREST-negative (immunofluorescent antikinetochore staining using the CREST antibody) micronuclei induction (Miller et al., 1991). This suggests that at least some of the HPRT gene mutations may be due to breaks in the X-chromosome. The *ortho*-quinone, 3,4- and 2,5-dihydroxy and 4-monohydroxy metabolites induced some chromosome breaks at the highest concentration tested, but their by far stronger activity was the induction of chromosome loss (CREST-positive micronuclei). In this respect 4-OH and 2-OH

were the most efficacious metabolites tested, while the dihydroxy and quinone metabolites produced significant chromosome loss at more than 10-fold lower concentrations (Zettner et al., 2007). Of all PCB3 metabolites, only the 3,4-catechol induced sister chromatid exchanges (SCE), and only the 2,5-hydroquinone caused tetraploidization of cells, and this with an efficacy of nearly 90% at 7.5µM concentration. The HQ of PCB2 had the same effect, while PCB1-HQ and a PCB3 catechol, 3,4-Cat, were completely inactive. These results illustrate a strict structure-activity relationship (coplanar ring position, *para*-orientation of the two OH-moieties) for this effect.

These results show that metabolites of PCB3 are indeed genotoxic and that each metabolite induces its own, specific type of DNA damage. What these results did not explain was 1) the mechanism of genotoxicity for the individual endpoints, 2) whether this is of any importance *in vivo* or 3) which metabolic activation pathway(s) lead to these effects. These questions were addressed in the following experiments.

(*In vivo* studies)
A series of experiments was conducted with synthetic PCB3 metabolites in an effort to determine the metabolic activation pathway and the ultimate initiating carcinogen. For these experiments a modified Solt–Farber protocol was used to test whether PCB3 and its metabolic progeny can initiate carcinogenesis in the livers of exposed rats (Farber et al., 1977). For this experiment a fasting/refeeding protocol and 20 mg/kg DEN as positive control was used. Test compounds included the 2-OH-, 3-OH-, 4-OH-, 2,3-diOH-, 3,4-diOH-, 2,5-diOH-, 2,3-quinone, 3,4-quinone, and 2,5-quinone metabolites of PCB3. To summarize the results: the 4-OH- and 3,4-quinone metabolites of PCB3 significantly increased the number of gamma-glutamyl transpeptidase (GGT)-positive foci/cm^3, the number of foci per liver and the focal volume (% of liver). In fact, 100 mol/kg 3,4-quinone of PCB3 was more active than 20 mg/kg DEN with respect to foci number. None of the other PCB3 metabolites had a significant effect on either foci number or foci volume (Espandiari et al., 2004). The conclusion is that the 3,4-ortho-quinone of PCB3 is the ultimate initiating metabolite. It is noteworthy that formation of 3,4-quinone-derived protein adducts in the liver and brain of rats treated with PCB52 were reported (Lin et al., 2000).

Male transgenic BigBlue rats were injected intraperitoneally with PCB3, 4OHPCB3, 3-methylcholanthrene (3-MC), or corn oil and the induction of point mutations analyzed in the lacI indicator gene. PCB3 increased the mutation frequency in the liver (significantly P=0.03) (Lehmann et al., 2007) and lung (non-significantly P=0.244) (Maddox et al., 2008) of BigBlue rats, and changed the mutation spectrum in both organs from predominantly transitions to predominantly GC→TA transversions. 4OHPCB3 had a similar, but smaller effect that was below the level of statistical significance (liver P=0.18; lung P=0.208) (Lehmann et al., 2007; Maddox et al., 2008). These data demonstrate that this PCB congener is mutagenic *in vivo* in the target organ liver and less active in the lung. However, these data do not explain the mechanism of genotoxicity (DNA adduction or ROS).

Very recently Jeong and colleagues (Jeong et al., 2008) identified M_1dG DNA adducts after chronic exposure to PCB126/PCB153. These and several additional biomarkers of oxidative DNA damage including 8-OHdG, N2,3-ethenoguanine, and 1N6-ethenodoxyadenosine

indicate a role for oxidative DNA damage in the carcinogenic action of PCBs in rodent liver. In general these increases are associated with the higher exposures, which are also where the increases in liver tumors occur. More research is needed with this mode of action and with cell proliferation, as the two could drive the induction of mutations and subsequent carcinogenicity. Specific attention needs to focus on dose-response.

PCBs as Promoters of Hepatocarcinogenesis

PCBs promote liver tumors in rodents (reviewed in Glauert 2001; Glauert et al., 2008b; Silberhorn et al., 1990). Studies have shown that PCBs increase oxidative stress in the liver, including lipid peroxidation, oxidative DNA damage, and NF-κB activation. Recent studies were conducted to determine if the promoting activities of PCBs could be inhibited by dietary antioxidants (vitamin E, selenium, or phytochemicals) or by knocking out the p50 subunit of NF-κB. In the antioxidant studies, female rats were first injected with DEN (150 mg/kg) and then administered four biweekly intraperitoneal injections (300 μmol/kg/injection) of PCB77, PCB153, or vehicle; the number and volume of placental glutathione S-transferase (PGST)-positive foci were then quantified. Vitamin E did not influence the promoting activities of PCBs (Glauert et al., 2005). Increasing dietary selenium above the recommended intake increased the number of foci induced but decreased their volume (Stemm et al., 2008). Most of the phytochemicals examined (N-acetyl cysteine, ß-carotene, resveratrol, EGCG) had no significant effect on the promoting activity of PCB77. Ellagic acid increased and lycopene decreased the number of foci; ellagic acid, CoQ10, and curcumin decreased the volume of foci (Tharappel et al., 2008). In the NF-κB knockout study, male mice were first injected with DEN (90 mg/kg); controls not receiving DEN were also studied. Both p50 −/− and wild-type mice were then injected biweekly 20 times with PCB153 (300 μmol/kg). In DEN-treated and DEN+ PCB-treated mice, the incidence of tumors was lower in the p50 −/− mice than in wild-type mice. In mice receiving PCB153, the tumor incidence and tumor volume were higher. The volume of tumors that were positive for glutamine synthetase was increased in mice administered PCB153. These studies show that the promotion of hepatocarcinogenesis in rodents by PCBs is largely unaffected by dietary antioxidants but is diminished when NF-κB activation is impaired by the absence of the p50 subunit (Glauert et al., 2008a).

Biomarkers of exposure:

The analytical capabilities for congener-specific quantitation of PCBs and metabolites have improved greatly in past decades (ATSDR 2000). Higher halogenated biphenyls are themselves relatively resistant to metabolic breakdown and appear to be stable indicators of past exposure, with half-lives of 2 – 6 years (discussed in (Martin-Jimenez and Hansen 2008)). For individuals who received historic, high-level exposures, PCBs levels have diminished over time. On the other hand, for most individuals PCB exposure is low-level and continuing. For this group, body burdens of these industrial chemicals generally increase with age.

Higher halogenated metabolites, OHPCBs with five or more chlorines, also persist in human blood but little is known about their kinetics.

On the other hand, PCBs like those found in the air of buildings, in cities on a hot day, and near waste sites, are generally much more labile, are susceptible to metabolic attack, and may

disappear from blood with half lives of less than 30 days. These short lived congeners have been termed "episodic" (Martin-Jimenez and Hansen 2008). The fate of these residues, whether excreted without causing harm, or converted to metabolites that are toxic, or if bound covalently to tissues, is unknown.

Biomarkers of effect

Individual PCB congeners are ligands for a number of cellular receptors. The binding of "co-planar" PCBs to the aryl hydrocarbon (Ah) receptor was demonstrated almost three decades ago (Bandiera et al., 1982). The receptor binding/activation leads to increased transcription of a number of proteins, including a range of carcinogen – metabolizing enzymes. One notable effect is the increased expression of a cytochrome P-450-dependent monooxygenase CYP1A1, and its associated enzyme activity ethoxyresorufin-O-deethylase (EROD), routinely reported as a bioindicator for PCB-exposure, especially for coplanar PCBs and related chemicals. Other PCBs may alter the expression of other CYPs through actions on other receptors (Bandiera 2001).

Dioxin-like and Non-Dioxin-like PCBs:

Both dioxin-like (Ah receptor agonists) and non-dioxin-like PCBs (NDL-PCBs) may greatly alter gene expression controlled by estrogen receptors. A series of PCB congeners were evaluated for their estrogenic or anti-estrogenic potencies using in vitro reporter gene assay. The results suggest that some lower-chlorinated congeners exhibit weakly estrogenic effects, while higher-chlorinated ones are primarily anti-estrogenic (Pliskova et al., 2005).

Activation of AhR by dioxin-like PCBs led to a release of epithelial cells from contact inhibition of cell proliferation. The disruption of cell cycle control in liver progenitor cells might be linked to tumor promotion (Vondracek et al., 2005).

Non-dioxinlike PCBs have been shown to exert a host of rapid non-genomic effects, possibly linked to cell membrane changes. These newly discovered processes may be closely related to tumor promotion and progression. PCB153, a model NDL congener disrupted gap junction intercellular communication in liver epithelial "progenitor-like" cellular model WB-F344, which was associated with connexin43 degradation (Simeckova et al., 2009a). PCB153 and PCB47 congeners were found to induce a significant release of arachidonic acid from membranes of WB-F344 cells (Umannova et al., 2008). Moreover, PCB153 decreased protein levels of several adherens junction proteins, including E-cadherin, beta-catenin and gamma-catenin; the enhanced beta-catenin degradation led to disruption of Wnt/beta-catenin-dependent signaling (Simeckova et al., 2009b).

Research needs and recommendations:

1. Gaps in understanding related to PCB sources and exposures:

a. Mechanisms for human exposure to Aroclor and non-Aroclor PCBs. Recently non-Aroclor PCBs have been identified in air. PCB11, for example, appears to be associated with paint (Hu et al., 2008). There is a need to identify sources, including volatilization from paint. Mechanisms that deserve study include inhalation, child consumption of paint chips and other

building materials, accumulation in food, accumulation in fish, occupational exposures, for example, during building demolition.

b. Distribution of airborne PCB sources in cities.

c. Storage of Aroclors, particularly in cities. There is a great need for reasonably accurate inventories of stored PCBs. Some countries do a much better job of tracking their old transformers than others.

d. Human exposure to PCB degradation products and metabolites (these compounds are not well studied but are found in human tissues and in the environment).

2. Research needs related to mechanisms of action/toxicity:

a. There is an overwhelming need to investigate the metabolic fate of lower chlorinated PCBs found in buildings, in cities, near waste sites, and in schools. What are the reaction products? Are they mutagenic? Are any of these accessible/stable such that they may serve as biomarkers of exposure/effect? Can we prevent or abrogate the negative impacts of exposure? The fate of these residues, whether excreted without causing harm, or converted to metabolites that are toxic, or if bound covalently to tissues, is unknown.

b. The roles of metabolism of OHPCBs (e.g., further oxidation, sulfation, and other metabolic reactions) in the disposition and toxicity represent a significant gap in our knowledge about mechanisms for carcinogenesis and other toxic responses to PCBs.

c. Many mechanisms of genotoxicity/carcinogenicity for PCBs appear to involve issue of reactive oxygen species/oxidative stress, including the appearance of oxidative DNA damage. The recent identification of M_1dG DNA adducts after chronic exposure to PCBs 126/153 (Jeong et al., 2008), and the recent report by Spencer and colleagues (Spencer et al., 2009) demonstrate a role for oxidative DNA damage in the carcinogenic action of PCBs in rodent liver. In general these increases are associated with the higher exposures, which are also where the increases in liver tumors occur. More research is needed with this mode of action and with cell proliferation, as the two could drive the induction of mutations and subsequent carcinogenicity. Specific attention needs to focus on dose-response. More PCB congeners need to be studied!!

3. Airborne PCB profiles are distinctly different from those of commercial PCB mixtures like Aroclor 1254, and enzyme induction in the liver is most likely a completely inappropriate endpoint of toxicity for inhalation exposure. There is a great need to identify appropriate **biomarkers for exposure /effect/susceptibility** for airborne PCB exposure. Issues of dietary/nutritional deficiencies, *in utero* exposures, and developmental impacts are all unknown.

4. The existing epidemiologic literature, most of it produced since the last review of PCBs in 1987, may suffice for a re-evaluation of the carcinogenicity by an IARC working group. Possible studies include cancer incidence of the large (>26,000 workers) NIOSH cohort, which is under way. Nested case-control studies within this cohort and/or those in Sweden and Italy, evaluating current PCB blood levels in cases and controls, might be informative. A large population of individuals living in Aniston, Alabama, around the former PCB manufacturing facility received high levels of exposure through various routes. This group may be a useful study population.

Selected relevant publications since IARC review:

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Di(2-ethylhexyl) phthalate (DEHP)

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The use of di(2-ethylhexyl) phthalate (DEHP) has been modified since the last Monograph. An estimated 90% of DEHP is used as a plasticizer for PVC (Toxics Use Reduction Institute

at the University of Massachusetts, Lowell, USA (2004); www.turi.org). Consumer products such as footwear, shower curtains and toys, medical devices (IV bags, tubing), and commercial/industrial uses such as resilient flooring, wall covering, roofing, aluminum foil coating/laminating, paper coating, extrudable molds and profiles, electrical component parts, and wire and cable coating are all main users of DEHP.

Current evaluation

Conclusion from the previous Monograph:
DEHP is *not classifiable as to its carcinogenicity to humans (Group 3)* because peroxisome proliferation has not been documented in human hepatocyte cultures exposed to DEHP nor in the liver of exposed non-human primates. Therefore, the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans (IARC, 2000).

Metabolism in rodents

DEHP absorbed in the body is first metabolized by the catalytic action of lipase to produce mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) (Albro et al., 1989). Some MEHP is then conjugated with UDP-glucuronide by UDP-glucuronosyltransferase (UGT) and excreted in the urine. The remaining MEHP is excreted directly in the urine or is oxidized by cytochrome P450 4A, then further oxidized by alcohol dehydrogenase (ADH) or aldehyde dehydrogenase (ALDH) to dicarboxylic acid or ketones. 2-EH is metabolized mainly to carboxylic acid (mainly 2-ethylhexanoic acid, 2HEA) via 2-ethylhexanal by catalytic action of ADH and ALDH (Albro PW and Lavenhar SR, 1989).

Species difference in metabolism of DEHP

Lipase may be a rate-limiting step in the metabolism of DEHP and therefore species difference in the lipase activity may indicate the difference in DEHP metabolism. Recently, the i*n vitro* activities of lipase, UGT, ADH and ALDH for DEHP metabolism in several organs were measured and compared among mice, rats and marmosets (Ito et al., 2005). Marmosets were used as a reference for human metabolic activity. Clear-cut species differences were seen in the activities of the four enzymes involved in the DEHP metabolism among mice, rats, and marmosets. Of these, the difference in the lipase activity was most prominent. The constitutive levels in lipase (Vmax) and the affinity of lipase for DEHP (Km) were as follows: mice > rats >> marmosets: rodents had higher levels with greater affinity than marmosets. Thus, MEHP and perhaps 2-EHA and other di-carboxylic acid concentrations in the body were higher in mice or rats than marmosets when the same dose of DEHP was administered (Ito et al., 2007). However, there may be limitation using *in vitro* data to extrapolate to an in vivo kinetics, and also from marmosets to humans by these available data alone.

Metabolism in human

DEHP is also oxidized to MEHP in human, which is secondarily oxidized to mono-(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), which reflect the short-term exposure, and mono-(2-ethyl-5-carboxypentyl)phthalate

(MECPP) and mono-(2-(carboxymethyl)hexyl]phthalate (2cx-MMHP) (Koch et al., 2004; Koch et al., 2005; Koch et al., 2006). They represent the major share of DEHP metabolites excreted in urine (about 70% for these secondary oxidized metabolites vs. about 6% for MEHP. Half-life times have been estimated to about 5 h for MEHP, 10 h for5-OH MEHP and 5oxo-MEHO, 24 h for 2ox-MEHP and 12–15 h for MECPP in humans (Koch et al., 2006). Thus, long half-times of elimination make MECPP and 2cx-MEHP excellent parameters to measure the time-weighted body burden to DEHP (Koch et al., 2006). These half-life estimates are based on oral exposures, and those after inhalation or dermal contact are not known. Oral exposures reflect the environmental exposures experienced by the general population, while inhalation and dermal exposures reflect typical occupational exposures. The ratios between secondary metabolites have been shown to differ depending on DEHP exposure levels; high exposure where MEHP is predominant (26% MEHP) and low exposures where the secondary metabolites (e.g., MECPP) are higher than MEHP (6%) (Dirven et al., 1993).

Exposure and biomonitoring

Exposure to DEHP is common in the general population and in occupational settings. Occupational exposure to DEHP may occur during its manufacture and its use mostly as a plasticizer of PVC (compounding, calendering and coating operations). In the current Monograph it is stated that urinary levels of DEHP, its metabolites and total phthalates have been shown in a few studies to be higher in DEHP-exposed workers than in non-exposed workers and in post-shift samples than in pre-shift samples. No standard method had been proposed for biological monitoring of exposure to DEHP (IARC, 2000).

Since the Monograph was published in 2000, a standard method for biological monitoring of DEHP metabolites has evolved (Adibi et al., 2003; Barr et al., 2003; Becker et al., 2004; Blount et al., 2000; Jonsson et al., 2005; Kato et al., 2003; Kato et al., 2004; Koch et al., 2003; Koch et al., 2003; Nuti et al., 2005; Preuss et al., 2005; Silva et al., 2003) and verified (Silva et al., 2008), showing reproducible and accurate results for DEHP metabolites. In addition, it was found that the phthalate metabolites in urine at −70 ◦C were stable for several years (Silva et al., 2008).

The metabolites of DEHP as biomarkers of exposures, are specific to DEHP but may stem from occupational and environmental sources because phthalates are used in consumer products as seen in Hines et al., (2009) where workers had urinary metabolite levels from phthalates not used in the workplace.

Occupational exposure

Gaudin et al. (2008) reported biological monitoring of three urinary metabolites from PVC factory workers (n=25) exposed to DEHP (33% in plasticol used) and controls (n=19) in pre- and post-shift urine samples 5 consecutive days. Median concentrations of pre- and post-shift urinary samples in the exposed workers (controls) were 16.1 and 55.9 (12.0 and 10.4) μg/l for MEHP, 37.6 and 103.7 (38.1 and 11.4) μg/l for MCEPP and 46.3 and 72.1 (31.9 and 46.0) μg/l for 2-EHA, respectively. The authors found a significant increase of post-shift excretion in the exposed workers versus unexposed controls and in post-shift versus pre-shift

concentrations only in the exposed workers. While MEHP and MCEPP are specific biomarkers of DEHP exposure (Gaudin et al., 2008), 2-EHA is *not*.- These results are lower than levels reported by Driven et al. (1993); however the sensitivity of the laboratory methods were different, and the lower level could be potentially explained by the difference in analytical methods applied.

In a cross-sectional study of 156 workers (Hines et al., 2009), DEHP exposures were assessed in eight industry sectors by comparing urinary DEHP metabolites in post-shift samples. Evidence of occupational exposure to DEHP was strongest in PVC film manufacturing, PVC compounding, and rubber boot manufacturing where geometric mean (GM) end-shift concentrations of DEHP metabolites exceeded general population levels (NHANES 2003-2005) by 8-, 6- and 3-fold, respectively. Using urinary metabolites, this study identified workplaces with likely occupational phthalate exposure. However, metabolites of some phthalates not used in the workplace were detected in urine. It is difficult to distinguish occupational from non-occupational sources in low-exposure workplaces. This conclusion is in accordance with Gaudin et al. (2008). No controls (i.e., subjects with no occupational exposures) were surveyed in this study.

In the Swedish PVC-processing factory (Hagmar et al., 1990) 2,031 workers employed for at least 3 months between 1945 and 1980 and were followed until 1985. Exposure to plasticizers was stable over the entire study period, and the time-weighted average (TWA) breathing-zone level of phthalates was > 0.5 to 3, > 0.1 to 0.5, and up to 0.1 mg/m^3 for highly, moderately and low exposed workers, respectively. Significant excesses of total cancer morbidity (SIR = 1.28, 95% CI = 1.01 to 1.61; 75 observed cases) and respiratory cancer morbidity (SIR = 2.13, 95% CI = 1.27 to 3.46; 17 observed cases) were seen among the PVC-processing workers, but these excesses were not significantly associated with cumulative exposure to *plasticizers*. Only 6% of the cohort was exposed only to plasticizers. Smoking and other phthalates were not included as confounders.

A case-cohort study (Selenskas et al., 1995) of U.S. workers in a plastics manufacturing and research and development plant included 28 men case subjects who died from pancreatic cancer and had held at least one job as an hourly worker, and 140 men as control subjects (5 per case) randomly selected from the cohort and matched to cases by year of birth and survival. Smoking was not considered. Workers were classified into major production and nonproduction areas. Individuals with potential exposure to phthalates worked in either the vinyl and polyethylene processing department or the fibers and fabrics department. Potential exposure to DEHP, specifically mentioned as being used in this plant, occurred in the production of flexible plastics. Quantitative exposure measures were not available; however, duration of exposure (from employment history) and time since first exposure (latency) were known. A significantly increased risk of pancreatic cancer was observed only for workers in the vinyl and polyethylene processing department (RR = 7.15, 95% CI = 1.28 to 40.1, 5 exposed cases) exposed longer than 16 years. Limitations of this study include the exposure assessment because workers in the vinyl process might be exposed to phthalates, while workers in polyethylene processing are not (phthalates are not used with polyethylene plastics). Also, there are small number of exposed case subjects and potential confounders not considered.

Environmental exposures

Median MEHP values for the controls in (Gaudin et al., 2008) were 10.4-12.0 μg/L (n=25). This result was about the same reported in two previous studies 10.3 μg/L (n=85) (Koch et al., 2003; Koch et al., 2003), and 9.8 (n=19) (Preuss et al., 2005), but higher than environmental surveys; 0.9 – 4.5 μg/L (Barr et al., 2003; Blount et al., 2000; CDC 2003, 2005; Kato et al., 2003). There is currently no defined level for "background" DEHP exposure in the general population. Based on the metabolite levels found in environmental monitoring of DEHP and in controls of the occupational study, levels above 12 μg/L MEHP can be considered to be above background levels, and can be used to identify possible occupational exposures. However, other sources of environmental exposure such as use of DEHP consumer products cannot be ruled out and cannot be default be contributed to occupational exposures.

Donors undergoing apheresis (blood transfusion) are exposed to DEHP from disposables used in this process. In a small study, DEHP exposures in 18 donors were measured using biological monitoring (Koch et al., 2005) pre- and post-plateletpheresis. Maximum concentrations of metabolites after the continuous-flow plateletpheresis procedure was 826 μg/l for 5OH-MEHP, 774 μg/l for 5oxo-MEHP and 266 μg/l for MEHP (mean of the six volunteers); all levels are well above what is found in the general population, and were the highest in samples taken shortly after plateletpheresis.

Peroxisome proliferator-activated receptor(PPAR) and DEHP

MEHP, not DEHP, is an exogenous ligand of PPARα and PPARγ (Maloney and Waxman, 1999; Hurst and Waxman, 2003). In rats, mice, and marmosets exposed to DEHP (rodents for 2 weeks and marmoset for 15 months), Ito et al. (2007) reported constitutive expression to be 5-7 times greater in the rodents and to induce peroxisome keto-acyl-CoA thiolase mRNA and protein expression in mice and rats, but not in marmosets. The treatment, however, did not influence mitochondrial enzymes in any animals.

Constitutive androstane receptor (CAR) and DEHP

Eveillard et al. (2009) reported that DEHP activates not only PPARα but also CAR. Wild–type and PPARα-null adult mice were exposed to different doses of DEHP (0, 20 and 200 mg/kg for 21 days by gavages). Cyp2b10, CAR target gene in mice, transcript was markedly up-regulated by DEHP in wild-type mice (6.6-fold-change at 200 mg/kg/day), and slightly in PPARα-null mice (2.8-fold-increase). Similar result was also found in an *in vitro* experiment when recombinant JWZ-CAR cell line. In this cell line, androstenol abolished the induction of Cyp2b10 by DEHP(100μM), supporting the involvement of CAR in the regulation. Interestingly, MEHP was unable to increase Cyp2b10 mRNA in the JWZ-CAR cell line. The authors also investigated whether the activation of CAR by DEHP could be extrapolated to humans. DEHP dose-dependently increased the expression of CYP2B6, target gene of CAR in human (Maglich et al., 2003), at 50 and 100 μM of DEHP in human primary hepatocyte cultures. Thus, CAR also represents a transcriptional regulator sensitive to DEHP. These effects may provide additional pathways for induction of endpoints of DEHP toxicity.

Biomarkers of effect

DEHP exposures have been reported to significantly increase of 8-OHdG with high dose of DEHP (1000 mg/kg) in rats (Seo et al., 2004). Limitations of this biomarker of effect are that it has not been used in humans exposed to DEHP and it is not specific to DEHP. The high levels of 8-OHdG found in animal studies might be from mitochondrial DNA, which is a confounder in the methods used (Rusyn et al., 2006).

Cancer in humans

(*inadequate***,** Vol 77, 2000)

The current IARC Monograph (IARC, 2000) states that there is only limited DEHP-specific human carcinogen data are available. It included one epidemiological study; a mortality study (Thiess and Fleig 1978) of 221 workers in a DEHP production plant in Germany followed between 1940 and 1976. The Monograph Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced, and that the methods for this study were poorly described.

No epidemiologic studies specifically of DEHP exposure have been published since the last Monograph (IARC, 2000).

There are studies from the plastics industry with possible DEHP exposures focusing on different types of cancers. A Swedish case-control study (Hardell et al., 2004) from 1993 to 1997 included 791 cases of germ cell tumors and 791 controls matched by 5-year age group. A non-statistically significant increased risk was reported for exposure to soft plastics (containing plasticizer) (OR = 1.48, 95% CI = 0.94 to 2.34, 54 cases and 37 controls) but not to rigid plastics (containing little plasticizer) (OR = 1.06, 95% CI = 0.55 to 2.01, 23 cases and 26 controls).

A population-based case-control study among Danish men (Heineman et al., 1992) evaluated the relationship between multiple myeloma and exposure. There were 1,098 cases and 4,169 control subjects matched to the case by age. Exposure to phthalates was associated with nonsignificantly elevated ORs for multiple myeloma, with a higher estimated risk for probable exposure (OR = 2.0, 95% CI = 0.9 to 4.4, 11 cases and 21 controls) than possible exposure (OR = 1.3, 95% CI = 0.9 to 2.0, 34 cases and 99 controls). Stratified analysis conducted to separate the effects of exposure to phthalates from exposure to vinyl chloride, showed a non-statistically significant increased risk.

Cancer in experimental animals

(*sufficient***,** Vol 77, 2000)

The current IARC Monograph (IARC, 2000) concluded that DEHP produces liver tumors in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation; and

peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of DEHP in rats and mice.

However, recent animal studies suggest two additional cancer sites in rats of pancreatic acinar-cell adenoma (David et al., 2000) and testicular Leydig cell tumors (Voss et al., 2005). David et al.(2000) treated male and female Fisher 344 rats with 0-12,5000 ppm DEHP in the diet for up to 104 weeks and reported that incidences of bilateral aspermatogenesis in the testes increased after exposure to ≥ 500 ppm DEHP in male rats, and spongiosis hepatis in males exposed ≥ 1,250 ppm DEHP. David et al. (2000) also reported that incidences of castration cells in the pituitary gland and pancreatic acinar cell adenomas increased at 12,500 ppm DEHP in male rats; no adenomas were seen in female rats. Voss et al. (2005) reported that chronic exposure of DEHP at 300 mg/kg increased the incidences of hepatocellular tumors but that DEHP-induced testicular tumors developed earlier in lifetime than hepatocellular tumors, and their multiplicity increased with time. In addition, 300 mg/kg dose of DEHP showed a significantly increased rate of testicular atrophy. Although PPAR agonists have been hypothesized to induce Leydig cell tumors by inhibiting testosterone biosynthesis and/or by inducing aromatase, thereby increasing estradiol levels (Klaunig et al., 2003), DEHP was found to induce high-levels of gonadotropin-lutenizing hormone and to increase serum concentrations of estradiol and testosterone in Long-Evans rats exposed to 10 and 100 mg/kg per day DEHP for up to 100 days (Akingbemi et al., 2004).

Mechanisms of carcinogenicity:

The use of evidence of the PPAR-α activation to dismiss the human relevance of effects observed in laboratory animals has been questioned (Guyton et al., 2009; Caldwell et al., 2008; Melnick, 2001) based on the lack of experimental studies empirically challenging the mode of action hypothesis. Guyton et al. (2009) explain that PPAR-α activating compounds are pleiotropic and have been reported to exhibit a diversity of responses in addition to the hallmark effect of peroxisome proliferation, including genotoxicity, epigenetic alterations, oxidative stress, and effects on other receptors and other organelles within parenchymal cells. Importantly, DEHP reportedly affect non-parenchymal liver cells that do not express PPAR-α as well as other organ systems. Rusyn e al. (2006) also suggested that combination of molecular signals and pathways rather than a single hallmark event (such as induction of PPAR*α* and peroxisomal genes, or cell proliferation that contribute to tumors should be focused.

Two studies conducted after the IARC monograph suggest that DEHP can induce PPAR-*α* independent tumors without any loss of potency (Ito et al., 2007), and a robust hepatocyte and peroxisome proliferative response in itself is insufficient to cause tumorigenesis in transgenic model of PPAR-*α* activation in hepatocytes (Yang et al., 2007). Ito et al. (2007) reported that DEHP tumorigenesis in mice was not dependent on the PPARα pathway as both wild-type and *Pparα*-null mice fed diets containing 0, 0.01 or 0.05% DEHP for 22 months showed the incidence of liver tumors to be higher in *Pparα*-null mice exposed to 0.05% DEHP (25.8%) than in similarly exposed wild-type mice (10.0%).

Takashima et al. (2008) explored potential differences in the mechanisms of tumorigenesis between wild-type mice and *Pparα*-null mice using hepatocellular adenoma tissues of both

genotyped mice. Microarray profiles showed that the up- or down-regulated genes were quite different between hepatocellular adenoma tissues of wild-type mice and *Pparα*-null exposed to DEHP, suggesting that the mechanism of tumorigenesis might be different from each other. The authors suggested that DEHP may induce hepatocellular adenomas, partly via suppression of G2/M arrest regulated by Gadd45α and caspase 3-dependent apoptosis in *Pparα*-null mice but that these genes may not be involved in DEHP-induced tumorigenesis in wild-type mice. However, more study is needed whether DEHP promoted the spontaneous liver tumor in *Pparα*-null mice, because spontaneous hepatocellular tumors are known to occur in these mice at 24 months of age (Morimura et al., 2006)

To determine the difference in PPARα activation between mice and humans by PPAR agonists, two kinds of humanized PPARα mouse lines have been developed, hPPARα^{TetOff} mice (Cheung et al., 2004; Morimura et al., 2006) and hPPARα^{PAC} mice (Yang et al., 2008). The former line expresses the human receptor in liver in a *Pparα*-null background by placing the hPPARα cDNA under control of the Tet-Off system of doxycycline control with the liver-specific LAP1 (C/EBPβ) promoter. The hPPARα^{TetOff} mice express the human PPARα protein at levels comparable to or greater than that expressed in wild-type mice

Another transgenic mouse has the complete human PPARα gene on a P1 phage artificial chromosome (PAC) genomic clone, introduced onto the mouse *Pparα*-null background (Yang et al., 2008). This new line, designated hPPARα^{PAC}, expresses human PPARα not only in liver but also in kidney, heart, intestine and brown adipose tissue, that is, tissues with high fatty acid catabolism. hPPARα^{PAC} mice exhibited responses similar to wild-type mice when treated with fenofibrate lowering of serum triglycerides and induction of PPARα target genes encoding enzymes involved in fatty acid metabolism. Treatment of hPPARα^{PAC} mice with fenofibrate did not cause significant hepatomegaly and hepatocyte proliferation similar to hPPARα^{TetOff} mice, suggesting that the resistance to the hepatocellular proliferation found in the hPPARα^{TetOff} mice is not due to lack of expression of the receptor in tissues other than liver.

In a recent review (Guyton et al., 2009), available DEHP data were reviewed and meta-analyses performed on published data. The result raise questions about whether the hypothesized PPAR-*α* activation is either necessary or sufficient for rodent hepatocarcinogenesis. The authors question whether the proposed hepatocyte proliferation play a causal role in tumorigenesis or are merely correlated with cancer. The authors concluded the adequacy of the scientific basis for the conclusion that PPARα agonists pose no carcinogenic risk to humans requires re-examination. With regard to the hepatic, testicular, and pancreatic cancers associated with phthalates exposure, a recent National Research Council report (Committee on the Health Risks of Phthalates NEC, 2008) concluded that there is evidence that these cancer type may be mediated by mechanism independent of PPARα.

Research needs and recommendations

Possible cohort for future epidemiologic studies:

DEHP is commonly used in PVC and other plastics. Epidemiologic studies have been performed for a Swedish PVC-processing factory (Hagmar et al., 1990; Hardell et al., 2004), a mortality study of U.S. workers in a plastics manufacturing and research and development plant (Selenskas et al., 1995), and a population-based case-control study among Danish men (Heineman et al., 1992) exposed to PVC and phthalates.

Now that specific biomarkers of exposures for DEHP exist, these work populations identified above may serve as potential future DEHP biomarker epidemiological studies in addition to cohorts defined by Gaudin et al. (2008) and Hines et al. (2009). Also, in future biomarker epidemiological studies it would be necessary to identify what phthalates are used in the workplace and only count these metabolites as occupational exposure. DEHP metabolites should be determined in pre- and post-shift urine samples because an increase over the work-day would indicate occupational exposures, and a decrease a non-occupational exposure. In occupational and environmental settings, DEHP is often accompanied by other phthalates (i.e., to get the optimal flexibility in plastics a combination of phthalates are often used). Given that other phthalates may have similar carcinogenic properties; a cumulative phthalate exposure index should be used in future epidemiological studies.

The outcome variable could be cancer incidence, mortality, but preference would be to use an effect biomarker such as 8-OHdG with the exposure biomarkers may be useful (see section on oxidative stress).

In terms of human data, another suggested research need would be to look at studies of testicular germ cell cancer. DEHP causes reproductive effects (testicular dysgenesis, Leydig-cell dysfunction, cryptorchidism, and hypospadias) in male rodents, which are similar to risk factors for testicular germ cell cancer in humans. Furthermore, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient, and other modes, mechanisms, toxicity pathways and molecular targets may contribute to or be required for the observed adverse effects. Similarly, the epidemiologic data are inadequate to inform conclusions of human relevance of peroxisome proliferators as a class (Guyton et al., 2009).

Future toxicological studies

Previously, there have not been no reports concerning the effects of exposure of DEHP to transgenic mice with hPPARα^{TetOff} or hPPARα^{PAC}, but Ito and Nakajima (2008) reported that at a relatively high dose of DEHP (5.0 mmol/kg for 2 weeks) PPARα was activated in the liver of both genotyped mice. Although the magnitude of response was not large in hPPARα^{TetOff} mice from the standpoint of the target gene expression in the liver, the induction was beyond doubt. Hurst and Waxman (2003) reported a 5-fold lower sensitivity to the DEHP metabolite MEHP of human compared with mouse PPAR (see discussions in Guyton et al., 2009 regarding receptor activation). The results from the typical peroxisome proliferator, Wy-14643, may not always be similar to those of DEHP: Future studies are needed using hPPARα^{TetOff}, which expresses the human receptor only in liver, or hPPARα^{PAC}, which expresses the human receptor not only in liver but also in kidney, heart, intestine and

brown adipose tissues, mouse models to elucidate the role of human PPARα in DEHP carcinogenesis. Further characterization of DEHP exposures in industry is needed in order to reduce exposure misclassification in epidemiological studies. Since no epidemiologic studies have been published since the last Monograph (IARC, 2000) it is encouraged to use already established cohorts in the PVC-processing factories.

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Atrazine

by David M. DeMarini PhD and Shelia Hoar Zahm PhD

Citation for most recent IARC review

IARC Monographs 73, 1999

Current evaluation

Conclusions from the previous Monograph:
Atrazine is not classifiable as to its carcinogenicity to humans (Group 3). There is *inadequate evidence* in humans for the carcinogenicity of atrazine. There is *sufficient evidence* in experimental animals for the carcinogenicity of atrazine. The Working Group concluded that the animal mammary tumors associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism that is not relevant to humans.

Exposure and biomonitoring

Atrazine (6-Chloro-N-ethyl-N'-(1-methylethyhl)-1,3,5-triazine-2,4-diamine) is a triazine herbicide used widely on a variety of crops, especially maize, sorghum, and sugar-cane, for the pre- and post-emergent control of broad-leaved weeds. Occupational exposure may occur through both inhalation and dermal adsorption during the manufacture of atrazine, its formulation, and its application. It is found widely, together with its dealkylated degradation products, in rivers, lakes, estuaries, groundwater, and reservoirs. In drinking-water, the levels rarely exceed 1 μg/L. Surveys of various foods and feeds have generally found no detectable atrazine residue.

In water systems, atrazine is generally dealkylated to form desethylatrazine (DEA), desisopropylatrazine (DIA), and diaminochlorotriazine (DACT). As many as 8-12 metabolites of atrazine have been identified or postulated to occur in animals and humans, with some studies showing DACT as the primary metabolites and others showing atrazine mercapturate (AM) as the primary one (Barr et al., 2007). Typically, the level of AM in the urine is the only metabolite of atrazine that is measured in most biomonitoring studies, and this approach has generally found atrazine exposure in <5% of subjects at a detection level of <0.8 ng/ml (CDC, 2005).

A recent study evaluated the urinary levels of 9 atrazine metabolites in humans and concluded that DACT was the primary metabolite regardless of exposure scenario, and that exposure assessment based on measuring only AM or any single atrazine metabolite resulted in an underestimate of atrazine exposure (Barr et al., 2007). This study found that AM accounted for only 2-12% of the detected metabolites, that most of the subjects had detectable atrazine exposure, and that future biomonitoring studies would likely need to include analysis of at least AM, DACT, DIA, and DEA in order to characterize the extent of atrazine exposure in a population. The study also found that the proportion of these urinary metabolites varied considerably depending on whether the subjects had high or low acute exposures or whether the subjects had general environmental exposures (i.e., not agricultural workers).

Based on the observations above, Panuwet et al. (2008) have developed an analytical method that measures the 7 primary metabolites of atrazine in urine using an on-line solid phase extraction-high-performance liquid chromatography-tandem mass spectrometry (SPE-HPLC-MS/MS) and isotope dilution quantification method. Applying this method to maize farmers, Bakke et al. (2008) concluded that the amount of atrazine applied to the fields by an agricultural worker is likely to provide a valid surrogate of atrazine exposure in epidemiologic studies.

A study of pesticide urinary metabolite levels of farm worker children aged 1-6 found detectable levels of the metabolite atrazine mercapturate (Arcury et al., 2007). The authors noted that the children were most likely exposed to pesticide drift or contaminated water supplies.

Cancer in humans

(*inadequate*, Vol. 73, 1999)

At the time of the previous IARC evaluation of atrazine (Vol. 73, 1999), the most relevant epidemiologic studies consisted of two cohort studies of manufacturing workers, three population-based case-control studies of lymphatic and hematopoietic malignancies in agricultural areas of the U.S., and a population-based case-control study of ovarian cancer in a rice-growing area of Italy. Notable findings included a non-significant excess, based on small numbers, of non-Hodgkin lymphoma (NHL) among manufacturing workers, a significant association with NHL among exposed U.S. farmers, and a two- to threefold increase of borderline significance in the risk for ovarian cancer among women in Italy. The risk of NHL among U.S. farmers was attenuated when multiple exposures were considered.

Of note since the last evaluation, there have been further investigations among one of the manufacturing cohorts and the U.S. case-control studies of NHL, a new case-control study of ovarian cancer, analyses from the prospective U.S. Agricultural Cohort Study, and several ecological studies of environmental exposure.

Manufacturing

Approximately 2,000 workers manufacturing atrazine and other triazine herbicides at a plant in Louisiana were studied for cancer incidence during the time period 1985-1997 (MacLennan et al., 2002) and mortality during 1970-1997 (MacLennan et al., 2003). A nonsignificant excess of prostate cancer incidence was observed based on 11 cases (standardized incidence ratio = 175, 95 % confidence interval = 87-312). The plant had a prostate cancer screening program, which the authors and Hessel et al. (2004) suggest may be responsible for the apparent excess. The mortality study revealed a nonsignificant excess of deaths due to NHL (4 observed/1.1 expected, standardized mortality ratio = 372, 95 confidence interval = 101-952).

Agriculture

To evaluate the possible role of pesticides in the etiology of NHL, De Roos et al. (2003) pooled data from three population-based case-control studies in the Midwestern U.S. The resultant large sample size allowed for analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides. Atrazine was significantly associated with risk of NHL in both logistic and hierarchical regression analyses. Furthermore, there was an indication of a superadditive effect of atrazine in combination with carbofuran, diazinon, or alachlor. Using archival biopsies from the Iowa/Minnesota case-control study, Schroeder et al. (2001) found atrazine to be associated with risk of NHL among t(14;18) cases only.
The role of atrazine and cancer was investigated in the Agricultural Health Study, a large, prospective cohort study of licensed pesticide applicators and spouses from Iowa and North Carolina. Rusiecki et al. (2004), reported on cancer incidence among 36,513 applicators who ever used atrazine, based on follow-up through 2001. There were suggestive, nonsignificant excess risks for lung cancer, bladder cancer, NHL, and multiple myeloma. Rate ratios increase with lifetime days and intensity-weighted lifetime days of atrazine exposure, however confidence intervals were wide, and tests for trend were not significant. There were no excess risks for other cancers, including prostate, which was consistent with an earlier negative report on prostate cancer from the same study based on follow-up through 1999 (Alavanja et al., 2003).

A population-based case-control study conducted in Italy reported a nonsignificant association of triazines and the incidence of leukemia (odds ratio=1.7, 95 % confidence interval = 0.6 -4.7, six exposed cases) for men and women combined (Miligi et al., 2006). A population-based case-control study conducted in an agricultural area of central California evaluated the risk of ovarian cancer in relation to occupational and residential exposures to triazines (Young et al., 2005). Exposure indices were created using work and residential histories obtained by interviews along with the state Pesticide Usage Database. Ever having occupational exposure to triazines was associated with a nonsignificant excess risk (odds ratio = 1.3, 95% confidence interval = 0.8, 2.3). Exposure to atrazine specifically was not

associated with excess risk, based on two exposed cases. Residential exposure to atrazine also showed no increased risk (OR=0.9), based on eight exposed cases.

Environment

Since the last evaluation, the effects of environmental exposure to atrazine were evaluated in several ecological studies. Hopenhayn-Rich et al. (2002) investigated the relationship between atrazine exposure in drinking water to ovarian and breast cancer incidence rates for 1993-1997 in Kentucky, a slightly longer time period than a similar earlier report by Kettles et al. (1997). District exposure indices were based on public drinking water atrazine levels, acreage of corn planted, and atrazine sales. Atrazine levels were not associated with breast cancer and were inversely associated with ovarian cancer. Muir et al. (2004) observed an inconsistent but significant association between breast cancer incidence rates and kilograms of atrazine active ingredient applied in the rural areas of one agricultural county in England using spatial autocorrelation techniques; however, the association was not observed in a second county in the same study. Mills et al. (2006) found no increased risk of breast cancer incidence among California Latinas during 1988-1999 and county levels of atrazine use during 1970-1988. Van Leeuwen et al. (1999) reported that atrazine levels in drinking water in Ontario were positively associated with stomach cancer incidence and negatively associated with colon cancer incidence. The ecological approach is a relatively weak methodology because it uses county-level exposure information to impute "possible" exposure status for individuals instead of individual-level exposure data. In addition, some ecologic studies are cross-sectional whereby current exposure status is linked to measures of current cancer status, without any consideration of latency for tumor development.

Cancer in experimental animals

(*sufficient*, Vol 73, 1999)

Atrazine was tested for carcinogenicity in one study in CD-1 mice by oral administration in the diet, and no increase in tumor incidence was observed. Atrazine was also tested by oral administration in two studies in Fisher rats and in five studies in Sprague-Dawley rats, including a comparison of intact and ovariectomized females of the latter strain. No increase in tumor incidence was observed in the one adequate study in Fisher rats. In contrast, the incidence of mammary tumors was increased in intact Sprague-Dawley females in four studies, but no increase was seen in ovariectomized Sprague-Dawley females. Atrazine increased the incidence of lymphomas in one study in CD-1 mice tested by intraperitoneal injection.

Fukamachi et al. (2004) found that atrazine enhanced 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors in a transgenic strain of Sprague-Dawley female rat containing copies of the human c-Ha-*ras* proto-oncogene. In the comparable strain of male rat, atrazine decreased DMBA-induced skin tumor development (Fukamachi et al., 2004). The authors noted that the doses (5-50 ppm in the diet) were 10,000 times higher than those expected via environmental exposure in humans. The relevance to humans of the co-carcinogenicity study in transgenic animals is unclear. As summarized by Kandori et al. (2005), the observed suppression of prostate cancer in atrazine-treated transgenic rats expressing the probasin/SV40 T antigen was most likely caused by a decrease in calorie intake rather than by atrazine-related endocrine disruption.

Use of atrazine in the presence of nitrogen fertilizers has raised the possibility of *N*-nitrosation in soil. There may also be endogenous formation of *N*-nitrosoatrazine from precursors ingested in the diet and drinking water. A carcinogenicity study of *N*-nitrosoatrazine administered to female Swiss mice and female Wistar rats was cut short due to excessive toxicity, with insufficient duration of exposure for an adequate study (Weisenburger et al., 1990).

Mechanisms of carcinogenicity

IARC (1999), two reviews (Stevens et al., 1999; Cooper et al., 2007), a risk assessment b the California EPA (Gammon et al., 2005), and a U.S. EPA Scientific Advisory Panel (Schoeny et al., 2006) have reached similar conclusions regarding the mechanisms by which atrazine causes mammary tumors in female Sprague-Dawley rats. The mechanism involves an effect by atrazine on the hypothalamus, leading to decreased secretion of hypothalamic norepinenphrine. This results in decreased release of gonadotropin-releasing hormone (GnRH) and decreased levels of GnRH, which affects the pituitary gland (atrazine does not directly affect the pituitary gland). The pituitary gland then attenuates the release of luteinizing hormone (LH) (atrazine alters LH cycling), and disruption of LH cycling results in increased exposure to both endogenous estrogen and prolactin, affecting the function of the ovaries. These altered exposures to reproductive hormones enhance the growth of mammary tumors.

The prolonged atrazine exposure in the female Sprague-Dawley rats seems to accelerate aging within the brain-pituitary-ovarian axis, and this premature reproductive senescence (i.e., constant estrus) establishes the hormonal environment conducive to the development of mammary gland tumors. Because the causative factors associated with reproductive aging in the rat (i.e., impaired hypothalamic function) and human (depletion of primary follicles) are characteristically different, it seems unlikely that a similar process occurs in women.

Nonetheless, the hypothalamic regulation of LH and prolactin secretion is similar in the rat and human; thus, atrazine could possibly influence the secretion of these pituitary hormones in humans. Although the doses used in the rodent studies are extremely high, current estimates of exposure in humans may be too low because of the failure to analyze for the presence of various atrazine metabolites (Barr et al., 2007). Other chlorotriazine herbicides produce metabolites similar to those of atrazine; thus, the combined exposure to such agents may produce an exposure level higher than is currently appreciated or from atrazine alone.

Microarray analysis of 1185 cancer-related genes from RNA extracted from bone marrow of CD-1 mice exposed for 4 months to atrazine in drinking water (1 mg/L) found that atrazine did not alter expression of any of the genes (Cimino-Reale et al., 2008). Proteomic analysis of pituitary removed 5 days after exposure of ovariectomized female Wistar rats to a single dose of atrazine by gavage (200 mg/kg) detected the induction of several proteins that contained active-site or solvent-exposed cysteine residues, making them viable targets for covalent modification by diaminochlorotriazine (DACT), an electrophilic metabolite of atrazine (Dooley et al., 2008).

Atrazine is not mutagenic (IARC, 1999), and two studies published since the last review have confirmed this both in vitro (Kligerman et al., 2000a) and in vivo (Kligerman et al., 2000b).

Reports suggest that atrazine is an immune disruptor in frogs (Brodkin et al., 2007), suppresses immune function in male but not female Sprague-Dawley rats (Rooney et al., 2003), and alters expression of the *rag1* gene in zebrafish, which is involved in acquired immune system disruption ((Liedtke et al., 2008). However, this literature is inconsistent, and a critical review by Solomon et al. (2008) concluded that environmentally relevant concentrations of atrazine do not affect reproduction and/or reproductive development in fish, amphibians, or reptiles. Although some studies have argued that atrazine induces aromatase (Fan et al., 2007), which converts testosterone to estradiol, the review by Solomon et al. (2008) concluded that the literature does not support such a role for atrazine. The authors also concluded that the literature does not permit definitive conclusions regarding the ability of atrazine to affect immune function, stress endocrinology, parasitism, or population-level effects among fish, amphibians, or reptiles. The relevance of this literature to humans remains unclear.

Research needs and future recommendations

The finding that atrazine is carcinogenic at a single organ, sex, strain, and species of rodent makes it unlikely that atrazine is a human carcinogen, considering that most IARC Group 1 carcinogens are trans-species carcinogens. Nonetheless, clear mechanistic data are lacking to show that atrazine does or does not alter the secretion of luteinizing hormone (LH) and prolactin in humans. Thus, further studies are needed to characterize the ability of atrazine to interfere with the hypothalamic-pituitary-ovarian axis in women. Clarification of this issue would help to show whether atrazine is a mammary carcinogen in women.

The U.S. Agricultural Health Study team has conducted an intensive biomarker study among 30 male corn farmers and 10 agricultural extension agents exposed to atrazine and other pesticides (Vermuelen et al., 2005; Bakke et al., 2008), collecting blood and urine at six times during the year coinciding with critical periods in the growing season (e.g., prior, during, and after planting; prior and after harvest; off-season). Although the main focus is on immunological effects, the biospecimens may be suitable for studies of hormonal effects of exposure, which could pertain to prostate cancer risk. If a sufficient number of women who apply atrazine could be identified within the Agricultural Health Study or other populations, a similar biomarker study could shed light on atrazine's hormonal effects among women and its possible role in breast and ovarian cancer.

The epidemiologic report by Rusiecki et al. (2004) on 36,513 atrazine-exposed pesticide applicators within the Agricultural Health Study cohort found suggestions of trends for risk of lung cancer, bladder cancer, NHL, and multiple myeloma, based on follow-up through December 31, 2001. Follow-up of the Agricultural Health Study cohort has recently been extended through 2006. The final numbers are not available yet, but it appears that the number of incident cancer cases has increased by over 65% since 2001. Analyses incorporating these additional data may clarify some of the previously reported nonsignificant findings that were based on small numbers of events. Larger numbers may also allow better control for multiple exposures. In addition, it may be useful to include the spouse members of

the cohort (the majority of whom are female), who were not represented in the Rusiecki et al. (2004) report, to examine risk of breast and ovarian cancers.

The 1990 carcinogenicity study of *N*-nitrosoatrazine was terminated early due to excessive toxicity (Weisenburger et al., 1990). Conducting a study at lower doses might clarify its carcinogenicity.

More extensive microarray and proteomic studies in rodents and humans would also help to characterize the pathways disrupted by atrazine. It is important to verify all the steps in the putative pathway that might explain the unique effects in Sprague-Dawley rats, and the absence in other strains, species, and humans. In addition, investigation of possible effects of *in utero* exposure among humans and animals is needed. .

Although atrazine has been studied extensively for its ability to alter immune function and aromatase in amphibians, reptiles, and fish, only a limited literature has addressed this issue in mammals or humans. Thus, there is a need to explore atrazine's ability to alter these functions in species relevant to humans as well as in human molecular epidemiology studies.

Current biomonitoring methods that have evaluated just one metabolite of atrazine in the urine have probably underestimated exposure. Thus, exposure assessment is needed of a wide array of subjects by the method of Barr et al. (2007), which detects the urinary levels of seven metabolites of atrazine.

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Shift Work

by Richard Stevens PhD

Citation for most recent IARC review

IARC Monograph 98, in preparation

Current evaluation

Conclusion from the previous Monograph:
On the basis of "limited evidence in humans for the carcinogenicity of shift-work that involves nightwork", and "sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period (biological night)", the Working Group concluded that "shift-work that involves circadian disruption is probably carcinogenic to humans" (Group 2A) (Straif et al., Lancet Oncol, 8:1065-66, 2007)

Exposure and biomonitoring

Exposure to 'Shift Work' is common in the industrialized world (Costa, 2003), and increasing in prevalence worldwide. About 27% of the European Union work force works an evening shift 5 or more evenings per month, and about 10% work the night shift 5 or more nights per month (EWCS, 2005). The sectors with the highest percentage of workers on a non-day shift are Hotels and Restaurants, Agriculture, Health, and Transport and Communication. Of all workers, about 6% are on a permanent non-day shift whereas about 8% are on a rotating shift schedule. In the United States about 15% of workers are on non-day shifts, with 3.2 % on night shift and 2.5% on rotating shifts (BLS, 2004). Although there is less variability in number of hours worked per week among non-day shift workers compared to day workers, there is also considerably less autonomy on the job.

Occupational exposure

The 'exposure' is by definition occupational. However it is based on a theory that light at night (LAN) would disrupt circadian rhythms and that this disruption might increase cancer risk.

Environmental exposures

Other exposures to LAN are many and include short sleep duration, late-night reading or television, nocturnal awakening and consequent exposure to light for example in the bathroom, strong street lights at night shining thru the window shade of the bedroom.

Biomarkers of exposure

Mirick and Davis (2008) provide an extensive review of melatonin as a biomarker. Assay of melatonin in urine and blood can reveal disruptions of circadian rhythms, and melatonin itself has impact on the circadian rhythm; it can be both a biomarker of exposure and of effect. Burch et al. (2005) found that night workers had altered melatonin excretion, disrupted sleep, and greater symptom (e.g., 'feeling tired', 'not alert', etc.) prevalence compared to day workers and that when workers were ranked on their sleep to work urinary 6-sulphatoxymelatonin ratio, this ratio was a better predictor of adaptation than the shift worked. In a normal healthy day worker, the sleep:work ratio is between 5 and 20, whereas for non-day workers the ratio is often close to 1. A ratio close to or less than one was highly predictive of disrupted sleep and symptom prevalence in Burch's study. This innovative metric provides a new tool for investigating shift and personal factors that most strongly disrupt circadian rhythms and thereby, perhaps, risk of two of the most common cancers in people, breast and prostate.

Cancer in humans

(*limited***,** Vol 98, in preparation)

Very few new epidemiological studies have been published since the Monograph meeting that focus on non-day shift work and cancer. One study (Lahti et al., 2008) reported an increased risk of non-Hodgkin's Lymphoma in non-day workers in Finland. Another (Marino et al., 2008) found an elevated risk of endometriosis in shift workers, although this may not be directly relevant to cancer.

Epidemiological studies of other LAN exposures

Since the 2007 Monograph meeting several epidemiological studies have been published of other predictions of the LAN theory for cancer causation. These are not studies of shift work, but are based on the same idea that exposure to light-at-night (LAN) might increase risk of certain cancers (e.g., breast and prostate), and thereby these studies contribute to the rationale that non-day shift work might increase risk of cancer as well.

Two studies by Kloog et al. (2008, 2009) examined the co-distribution of nighttime light level of communities and risk of cancer. In Israel there was a significant association of community nighttime light level and breast cancer in women; and a global analysis found a significant association of nighttime light in 164 countries and risk of prostate cancer in men. In both studies, there was adjustment for per capita income and some other measures of affluence.

There have been three new prospective studies of sleep and cancer risk based on the idea that self-reported sleep duration would be a rough estimate of hours of exposure to dark at night. Wu et al. (2008) utilized the Singapore Chinese Health Study cohort and reported an inverse association of risk and sleep duration among post-menopausal women. Similarly, Kakizaki (2008a, 2008b) used the Ohsaki Cohort Study, a large prospective study in northeastern Japan, to show an inverse association of reported sleep duration and risk of breast cancer in women and prostate cancer in men.

There have been reported two more prospective studies of baseline urinary melatonin metabolite and breast cancer risk both of which reported significant inverse relationships (Schernhammer et al., 2008; 2009).

Bias and/or confounding

Known risk factors for breast cancer include reproductive factors and exogenous hormones, physical activity and BMI, as well as alcohol consumption. Some of the positive cohort studies adjusted for known risk factors for breast cancer and did not provide evidence for confounding. In the case-control studies, there is additional concern for selection bias since success to enroll potential controls who work shifts may differ from other controls. There is limited evidence for an increased risk of breast cancer with low serum 25-hydroxyvitamin D levels (IARC 2008) and a few studies have also suggested an inverse association between sun exposure and risk of breast cancer (IARC, in press). If these associations are corroborated by further studies, the potential lack of sun exposure in night-shift workers needs to be considered as a potential confounder for the association between shift-work and breast cancer or may act as on the causal pathway from shift-work to cancer.

Cancer in experimental animals

(*sufficient***,** Vol 98, in preparation)

The experimental work on cancer in experimental animals, (e.g. Blask et al., 2005), was judged to be sufficient by the Working group for a LAN role in cancer etiology.

Mechanisms of carcinogenicity

The cancer bioassays also explore a mechanism by which LAN-induced suppression of melatonin leads to release from growth inhibition of a small existing breast tumor. It has not yet been established if this mechanism also operates in exposed humans, and there may be other potential mechanisms that contribute to the carcinogenicity of shift-work. As it has become clear that the core circadian genes have many other functions including direct regulation of a large portion of the genome, a number of possible mechanisms for cancer causation are emerging (Stevens et al., 2007; Haus and Smolensky, 2006). These include circadian regulation of cell cycle checkpoint genes such as Cyclin D1 (Fu and Lee, 2003), histone-acetyl transferase (HAT) such as cmyc, and the WEE1 pathway (Matsuo et al., 2003). The Clock protein itself also has HAT activity (Sahar and Sassone-Corsi, 2007).

Also of potential importance is an effect of LAN on normal mammary tissue development. Based on Dimitrios Trichopoulos's (1990) hypothesis that early life experience, even beginning in utero, affects lifetime risk of breast cancer, LAN during these critical developmental periods could also affect lifetime risk perhaps by affecting melatonin and other hormones and/or altering circadian gene function (Stevens, 2005; Metz et al., 2006). This possibility could have serious implications for light exposure to pregnant women (e.g., shift work) and for the lighted environment of children.

Advancing understanding of the biology of circadian rhythms and of how light affects the rhythm (Brainard et al., 2008), the scientific and architectural lighting communities could help to design shift schedules, and the lighting of non-day shift environments that better accommodate circadian health. Although the suprachiasmatic nuclei (SCN) is the master circadian pacemaker in mammals, there are peripheral oscillators in tissues that can be decoupled from the SCN by, for example, feeding schedule (Stokkan et al., 2001).

Circadian disruption is characterized by at least two interrelated issues, melatonin suppression

(which may or may not induce phase shifting), and phase shifting and the attendant desynchrony of the master pacemaker with the sleep cycle and with the peripheral oscillators in tissues such as digestive system, breast, and prostate. The first, melatonin suppression, may be linked to alterations in hormone levels that directly increase risk of cancer and to the direct oncostatic effects of melatonin itself, and the second may be linked to clock gene influence on expression of genes in tissues for cellular processes (cell cycle regulation, DNA repair, apoptosis, etc.) that influence the chance that a normal cell will become transformed into a cancer cell. The two aspects might work together in which clock gene alteration results in a normal cell transforming into a cancer cell, and then melatonin suppression resulting in release of cancer cells from growth inhibition through estrogen signaling (Cos et al., 2006), or increased linoleic acid availability to cancer cells in a small tumor that would otherwise have remained indolent (Blask et al., 2005). Another related possibility is that the sleep disruption and deprivation in non-day workers contributes to cancer risk. This might occur from a couple of mechanisms including effects on immune function (van Leeuwen et al., 2009) or metabolism (Knutson and Van Cauter, 2008; Spiegel et al., 2009).

Biomarkers of effect

It is difficult to disentangle biomarkers of 'exposure' from biomarkers of 'effect', as one biological change can indicate both. A potentially important biomarker of effect may be gene promoter methylation (Weaver et al., 2004) which can occur from environmental exposures and may be reversible (Weaver et al., 2006). Although circadian gene expression and promoter methylation has been examined in cancers (Chen et al., 2005), it has not yet been investigated in normal tissues after environmental exposures.

Research needs and recommendations

An important limitation of the available epidemiological studies is that there have not been clear and uniform definitions of 'shift work' used. A manuscript is in preparation based on an IARC Workshop on defining 'shiftwork' which was held April 2 and 3, 2009 (Stevens et al., in preparation). There are several characteristics of a shift and shift schedule that the working group believes to be important to capture in epidemiological studies of cancer. These include 1) based on start and stop time, characterization of shift as 'day', evening', or 'night', 2) whether rotating, and if so, whether fast or slow, forward or backward, and 3) numbers of years on the shift. The approach to shift-work assessment will depend on the study design. Industry-based cohort studies may offer the opportunity to extract from company records clearly defined shift-schedules prevailing in a study plant. In most other study settings, such as population-based cohort studies or case-control studies in general, this information needs to be collected from the study subjects. Here, further criteria for exposure assessment, e.g. the strength and limitations of self-administered questionnaires vs. telephone interviews vs. face-to-face interviews need to be taken into consideration and validation of a questionnaire in a pilot study would be desirable. Preferably, data on shift-work should be collected separately for each job in an individual's lifetime occupational history. This would also allow assigning shift-work to certain periods of the individual's life history and may thus offer the opportunity to explore, for example, age-specific susceptibility to shift-work-related cancers.

Recommended are studies of the effect of non-day shift work on circadian biomarkers (e.g., melatonin and cortisol profiles), on circadian gene expression (e.g., promoter methylation),

and on expression of clock-controlled genes relevant to cancer risk such as of cell cycle regulation, apoptosis, and DNA repair.

Shift work and susceptibility to chemical toxicants

There are known genetic polymorphisms in detoxifying enzymes that changes an individual's sensitivity to exposure to a toxic chemical (Christiani et al., 2008). Similarly, there may be significant differences in susceptibility to adverse effects from chemical exposures in non-day workers compared to day workers. This is based on the known circadian variations in DNA excision repair (Kang et al., 2009), cell proliferation and activity of detoxifying enzymatic capacity (Schibler, 2007; Lévi et al., 2008). These variations by time of day have begun to be exploited in delivery of cancer chemotherapy to optimize killing of cancer cells while minimizing damage to normal cells (Lévi et al., 2008), but the important possibility that time of day of occupational exposures could affect risk has not been investigated to date.

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Overarching topics

During the discussions of the agents several issues were raised across agents. These issues were identified and discussed the last day of the meeting, and the results are presented in short paragraphs in the following.

Omics

By Martyn Smith PhD

In the biological sciences the suffix –omics is used to refer to the study of large sets of biological molecules. Through various omic technologies it is now possible to interrogate large sets of biological molecules. Although, the number of omic techniques is ever expanding, the most developed omics technologies are high throughput DNA sequencing, transcriptomics (focused on gene expression), epigenomics (focused on epigenetic regulation of gene expression), proteomics (focused on large sets of proteins, the proteome) and metabolomics (focused on large sets of metabolites, the metabolome). These technologies can be used as biomarker discovery tools in human observational studies or to elucidate mechanisms. Their application has become feasible in recent years due to an increase in the resolution and throughput of omics-based assays along with a lowering of cost. However, their application in occupational and environmental health research has been relatively limited to date. If omics-based assays were applied with appropriate study designs, thorough validation of the markers, and careful interpretation of study results then a bioinformatics database could be built of the human response to different chemical exposures and associated chronic diseases. This database could be useful in many ways for evaluating the risk posed by chemical exposures. For example, by comparing newly tested chemicals to the effects of established carcinogens we could identify potential carcinogens (hazard identification);

establish mechanisms of action by studying the effects of the same chemicals in experimental animals and on human cells *in vitro,* allowing for a better prediction of human carcinogenicity and assessment of carcinogenic mechanisms. Given the sensitivity of –omic analyses, low-dose adverse effects could also be observed and distinguished from high dose phenomena, and if exposures were accurately assessed, dose-response data could be incorporated into risk assessments.

Immune modulation

By Shelia Hoar Zahm PhD

Evidence of *in vivo* or *in vitro* genotoxicity often plays an important role in IARC carcinogenicity classifications. However, immunomodulation, hormonal activity, or chronic irritation (cytotoxicity/mitogenic activity) are properties of some substances known to cause cancer in humans. The importance of these latter modes of action needs to be kept in mind when evaluating compounds that appear to increase risk of cancer in humans but that are not classic genotoxins. For example, it is well established, primarily through studies of medical conditions and medications, that immunosuppression and immunostimulation can play a role in lymphogenesis. One of the compounds reviewed in this document, atrazine, is associated with increased risk of lymphoma, but is not genotoxic. A thorough investigation of its potential immunomodulatory effects may clarify its carcinogenicity potential. Identification and standardization of biomarkers of subtle changes in immune status that predict risk as reliably as gentoxocity markers, such as chromosomal abnormalities and sister chromatid exchanges, may make a valuable contribution to hazard identification.

Oxidative stress in carcinogenesis

By Jane Caldwell PhD, Eileen D. Kuempel PhD, Bernard D. Goldstein PhD

Oxidant damage to cellular DNA, proteins (including the epigenome), and lipids can occur when reactive oxygen species escape cell antioxidant and repair mechanisms. Oxidative stress has been implicated in the etiology of many diseases (e.g., cardiovascular, neurodegenerative, rheumatoid arthritis, diabetes, liver disease) and cancers (e.g., breast, colorectal, gastric, hepatic), including those attributed to exposure to exogenous chemical agents (Valavanidis et al., 2009). The mechanisms proposed include direct genotoxicity as well as tumor promotion; e.g. arsenic and perhaps other metals are thought to promote tumors by causing oxidative stress that interferes with apoptosis. (Shi et al., 2004).

A number of methodological issues present challenges to validation of an oxidative stress biomarker assay (Mayne, 2003). For example, 7,8-dihydro-8-oxo-2'deoxyguanosine (8-oxodG) is an extensively studied oxidative DNA damage lesion; however, the artifactual formation of 8-OHdG in cellular DNA during isolation and hydrolysis procedures has impeded its utility as a marker of oxidative stress (Mangal et al., 2009; Mayne, 2003). Recent advances in the development of a new immunoaffinity purification procedure reportedly now

provide a highly specific method of 8-oxodG analysis (Mangal et al., 2009). Additional methodological issues include highly variable background levels of 8-oxodG, differences in substrate affinities of various reactive oxygen species (relevant in chronic disease in which the key oxidizing species are rarely known), and the need to consider biomarkers of nitration as well as oxidation to assess oxidative stress (Mayne, 2003).

The collection of exhaled breath is a noninvasive procedure that permits repeated sampling of the respiratory tract for various biomarkers of oxidative and nitrosative stress, including nitric oxide (NO) and a number of markers in exhaled breath condensate (EBC), although standardization and validation are still needed especially for EBC (Horvath et al., 2005). Malondialdehyde (MDA) and isoprostanes are lipid peroxidation by-products that have been used widely as indicators of oxidative cell damage. Urinary MDA was reportedly stable under various storage conditions (Lee and Kang, 2008).

Although several studies in humans have shown associations between biomarkers of oxidative stress and airborne particulate exposures (Han et al., 2005; Risom et al., 2005; Barregard et al., 2007; Valavanidis et al., 2009), evidence is still lacking on the role of oxidative stress in human carcinogenesis (Loft and Møller, 2006). Lack of specificity and need for standardized and validated methods indicate that careful evaluation is needed in considering the use of oxidative stress biomarkers in epidemiological studies. As for any other biomarkers, research is needed to examine the relationship between exposure to toxic agents and oxidative stress biomarkers, and between these biomarkers and risk of cancer, while controlling for the many individual factors that contribute to oxidative stress. Guidelines on standardizing the collection and measurement of oxidative stress biomarkers in humans (Horvath et al., 2005; ATS, 1999) will facilitate their effective use in epidemiological studies of human cancers.

Exposure assessment

By Mary Schubauer-Berigan PhD

The agents in Group 2 are likely to require high-quality exposure assessment, conducted within the context of an epidemiologic study, in order to definitively assess their carcinogenicity. This need results from several, often concomitant, factors: 1) the low overall expected excess cancer risk compared to the external population, due to the use of industrial hygiene practices to reduce exposures; 2) the likelihood of exposure to multiple carcinogens with the same potential target organ as the agent of interest; 3) the ability to use biomarkers of exposure and effect to infer carcinogenicity (or lack thereof) based on mechanistic or pharmacokinetic information.

The first factor is illustrated by some Group 1 carcinogens; for example, crystalline silica exhibited relatively low standardized mortality ratios (e.g., 2 or less) for lung cancer compared to the general population, yet evidence for an exposure-response association within the cohort (e.g., Rice et al., 2001) greatly strengthened the evidence base for determining carcinogenicity (Straif et al., 2009).

The second factor is illustrated by the Group 1 agents nickel compounds and cadmium and cadmium compounds. Workers involved in metal refining are generally exposed to several potential carcinogens, but quantitative exposure-response information has permitted an evaluation of the contribution of these compounds to cancer risk, while accounting for other potentially carcinogenic exposures (IARC Monograph Vol. 100C, in preparation).

Di-2-ethylhexyl phthalate (DEHP) found in rubber and plastics manufacturing exemplifies the third factor. Using a biomarker of exposure such as DEHP's metabolite MEHP and its subsequent oxidative metabolites (Silva et al., 2006) gives an indication of internal exposure, a better surrogate for target organ dose than workplace measurements of external exposure. Such information about human exposure and metabolism may be used to infer that a mechanism operative in animals does or does not also operate in humans. Biomarkers of exposure, such as serum TCDD levels, have also been employed in lieu of external exposures to provide evidence of carcinogenicity.

Exposure assessments for epidemiologic studies often require the use of retrospective techniques to make use of historical measurement data to create a job-exposure matrix. To be most successful, this technique relies on the past collection and retention of comprehensive, relevant exposure data. Such data frequently consists of industrial hygiene measurements of air concentrations (either area-wide or in the workers' breathing zone). As mentioned above, cohort-wide collection and analysis of biological samples for exposure biomarkers can be employed to good effect; however, this can be expensive and impractical to conduct. Either technique requires consideration of whether adequate latency exists between the measured exposure and the cancer outcome (frequently, mortality) to permit useful evaluation of risk from the exposure. This limitation may be minimized by using validated biomarkers of early effect in lieu of cancer as an outcome (e.g., as discussed in this paper for indium). Practical limitations such as the inability of researchers to access populations or historical exposure information may hamper the ability to develop quantitative exposure estimates for epidemiologic studies of these Group 2 agents. Employers and government agencies should be encouraged to conduct and make available comprehensive exposure assessments that could be used for current or future epidemiologic studies.

Epigenetics

By David M. DeMarini PhD

Epigenetic events are modifications to DNA or chromatin that result in changes in gene expression or levels of translation of mRNAs to protein but do not involve changes in the nucleotide sequence of DNA. Epigenetic modifications involve three general mechanisms: modification (by methylation, acetylation, etc.) of DNA or histones in chromatin or the binding of microRNAs (non-coding RNAs that are 21-23 bases in length) to homologous sequences in mRNA, resulting in a double-stranded structure that can decrease the production of the corresponding protein. Alterations in gene expression and levels of key proteins are associated with carcinogenesis and are considered an essential component of the mechanisms by which most tumors arise. A number of the chemicals considered in this evaluation are not mutagenic, such as chloroform and atrazine, and others, such as lead or DEHP, are indirect

mutagens. These and other compounds considered here likely induce epigenetic changes that play a key role in their carcinogenesis. Chemicals are not routinely assessed for their ability to induce epigenetic events, and no standardized, validated assays are available for such assessments. However, research is needed to develop and validate assays to detect the various types/mechanisms by which agents induce epigenetic changes. Such assays then could routinely be included along with other toxicity endpoints, such as mutagenicity, to better characterize the carcinogenic mechanisms of agents.

Lymphohematopoietic cancer disease categorization

By Bernard D. Goldstein PhD, Ruth Lunn DrPH, and Elizabeth M. Ward PhD

An important methodological issue in designing epidemiological studies is disease categorization. Inconsistencies among findings from occupational cohort studies for a specific substance may be partially explained by misclassification of disease. Most occupational cohort studies measure cancer mortality, which rely on death certificate. Incidence studies using medical records or biological markers are preferred, especially for cancer such as lymphohematopoietic cancers (LHC), which have relatively high survival rates; the 5-year survival rate for leukemia is 51%, and for non-Hodgkin lymphoma is 65% (Jemal et al., 2009). Disease categorization of LHC is a special concern. Several of the substances discussed in this article are associated with increased risk of LHC cancers, for example the chlorinated solvents (DCM, TCE, and Perc), formaldehyde, styrene, and PCBs. For some substances, there is inconsistency in the types of LHC associated with exposure across studies. Some of this inconsistency may be partially explained by inaccuracies in disease characterization. Similarly, the interpretation of carcinogenic outcome in long term animal studies has generally viewed morphologically distinct hematological cancers as separate endpoints. International codes of diseases (ICD) and the understanding of the biology of these cancers have changed over time (Scott and Chiu 2006), raising questions as to the appropriateness of current approaches to hematological cancer characterization.

The ready availability of hematological tissues in the living human for microscopic and molecular study has led to a rich disease nomenclature based upon descriptive morphology. In recent years there has been growing recognition of the close relationship and overlap of disorders whose morphological diversity conferred seemingly distinct disease names. Examples include the clinical recognition that polycythemia vera, essential thrombocythemia and myelofibrosis are part of a myeloproliferative syndrome, which has in common a clonal origin and an increased likelihood of outcome in acute myelogenous leukemia (AML). More recently a specific mutation, known as JAK2 V617F has been found in almost all patients with these myeloproliferative disorders (Zhan and Spivak 2009). Further, deletions and mutations in the TET 2 gene were recently shown to be present in patients with myeloproliferative disorders, for which they preceded the JAK2 V617F mutation, as well as in some patients with secondary AML, with myelodysplastic syndrome and with chronic myelomonocytic leukemia (Delhommeau et al., 2009). Similarly, a variety of seemingly disparate disorders are now grouped together under the myelodysplastic syndrome, again each one having a large proportion of monoclonal blood cells and an increased likelihood of AML as an outcome. The seemingly straightforward distinction between myelogenous and lymphatic leukemias has been blurred by recognition of hybrid forms in which both

characteristics are present, at times one preceding the other. Initially, secondary leukemia following chemotherapy was thought to occur solely as AML, but modern molecular techniques have recognized that many are in fact acute lymphatic leukemia (ALL) (Snyder et al., 2005). Children with Down's syndrome are at increased risk of developing both ALL and acute megakaryocytic leukemia through a complex interplay of genetic events related to trisomy of chromosome 21 (Kearney et al., 2009). Further, in the most recent reclassification of lymphoproliferative disorders, chronic lymphocytic leukemia and multiple myeloma are now considered subclassifications of non-Hodgkin lymphoma; and individuals with myeloproliferative syndromes appear to be at higher risk for each of these lymphoid neoplasms.(Vannucchi et al., 2009)

The growing recognition of common genotypic origin of hematological malignancies with markedly different phenotypic manifestations suggests the need to re-examine current approaches to lymphohematopoietic disease categorization used in epidemiology and in the interpretation of animal toxicology.

Multiple mechanisms of chemical carcinogenesis

By Martyn T. Smith PhD

Recent advances in scientific understanding of cancer biology and increased appreciation of the multiple impacts of carcinogens on this disease process support the view that environmental chemicals can act through multiple toxicity pathways, modes and/or mechanisms of action to induce cancer. For example, the established Group 1 human carcinogens benzene and arsenic have been shown to cause many different effects from chromosome damage to epigenetic changes, underscoring the need to consider interactions among a carcinogen's multiple modes of action, which may in turn be highly informative of the complex interactions among different carcinogens (Guyton et al., 2009). In addition, the relative importance of a given mode of action may vary with life stage, genetic background, and dose. Recently Guyton et al. (2009) identified several key challenges. First, using even an abbreviated list of key cancer-inducing events, noting that the mechanistic information about even well-studied compounds is incomplete. Despite the large number of publications, covering decades of research, on the IARC Group 1 compounds (e.g.,>4000 publications on aflatoxin B1 with >200 specifically focusing on mechanisms), it is evident that information gaps still exist regarding their effects on some of the postulated key events in carcinogenesis. For other carcinogens, the information gaps are more pronounced; moreover, basic information is completely lacking for tens of thousands of chemicals. In summary, cancer in humans is far too complex a long-term process to conceptualize in terms of one simple mode of action and arises from multiple genetic and epigenetic changes, many of which are difficult to measure in vivo.

Nanoparticles

By Paul A. Schulte PhD

Some of the agents considered in this report consist of or may be produced as particles with at lease one dimension at the lower range of the nanoscale, particularly between 1 and 100 nm. Particles at this size have unique properties that are scientifically and commercially exploitable. They generally have more surface area per unit volume than larger particles of the same composition and are generally more biologically reactive, toxic and possibly carcinogenic than larger sizes. It will be important for investigators to consider particle dimensions in future research and to include particles in the range of 1-100 nm in research when appropriate (Schulte et al., 2009). In some cases, nanoscale materials may need to be evaluated separately from larger particles of the same chemical composition if the nanoscale materials could have different health effects. Critical in investigating the health effects of a nanoscale agent is attention to the metrics used in the research. It may be important to characterize exposure in various ways in addition to mass per unit volume. It may be important to use particle count and surface area as well. Also of importance is to consider the heterogeneity of nanoparticles. A large number of physio-chemical parameters can mitigate biological activity and toxicity and these should be considered in research and in comparing results of studies. Additionally, investigators should consider contaminants in nanoparticles and the degree of agglomeration in assessing exposure and biological effect (Schulte et al., 2009).

Polymorphisms/susceptible populations

By Paolo Vineis PhD

The issue of genetic susceptibility to carcinogenic exposures is complex and delicate for several reasons. First, in spite of the large amount of studies that have been performed on candidate genes, and of the recent wave of genome-wide association studies, the stable and reproducible associations are few. Only recently stringent criteria for a systematic evaluation of the genetic evidence ("Venice criteria") have been developed and, when applied to examples, tend to give rise to a small number of reproducible, stable findings (Ioannidis, 2008; Vineis et al., 2009). One of the best recent examples is ethanol/acetaldehyde and gene variants for ADH (Hashibe et al., 2008): in this case the carcinogenicity for upper aerodigestive cancer seems to be limited to those with the frequent genotypes (the variant genotypes being protective), for genes that are clearly involved in ethanol metabolism. This example is important because the observation ("mendelian randomization") strengthens epidemiological findings lending them credibility. Thus, in special cases genetic susceptibility can be used in the evaluation process to upgrade an exposure. In spite of limited examples, often genetic susceptibility to chemical carcinogens is invoked to claim that more sensitive subpopulations exist. When replicated, associations with gene variants tend to be weak, with relative risks in the order of 1.5.

It is currently almost impossible to establish how many cancers are attributable to genes viz. the environment. Researchers generally agree that less than 5% of cancers are attributable to high-penetrant genes, although little is known for other chronic diseases. In general, we can expect little from genetic screening of the population, apart from limited groups (usually families) with a concentration of high-risk mutations. Two key difficulties arise in genetic testing of populations. One is the availability of specific and effective preventive measures for

the screenees, the absence of which seriously detracts from any screening proposal. The second is the large Number Needed to Screen, which implies that very few people who are screened will benefit; a large NNS also implies a potentially large number of false-positive results and unnecessary treatments (Vineis et al., 2001). To assess the role of a gene-environment interaction and screening in a population we need to know the penetrance of the genetic trait and its frequency. A useful approach is to combine penetrance and frequency by computing the number needed to screen (NNS) in order to prevent one case of cancer. There are examples, in fact, of screening activities characterized by high, or very high, NNS: one is screening for phenylketonuria, a monogenic disease with a frequency of one in 10 000–12 000 in white people; population screening is successful in most western countries. However, this is a particular case, since there is a very effective and non-invasive preventive measure (dietary restriction). No similar example is available for carcinogens.

Small businesses

By Elsebeth Lynge PhD and Avima Ruder PhD

For several of the agents discussed at the meeting, accumulation of additional epidemiologic data is complicated by the fact that exposed workers come primarily from small businesses with a high turnover. As occupational cancer cohorts have traditionally been recruited from large factories, exploitation of new data sources is warranted. For historical cohort studies, the literature provides some examples of rosters for recruitment of workers from small businesses. These include union records (Ruder et al., 2001), workers participating in health surveys (Winter et al., 1990), or workers in biological monitoring programs (Anttila et al., 1998). Computerized census records may also provide information on workers from small businesses (Boffetta et al., 1994), and more detailed information may be obtained if the original census forms are available (Lynge et al., 2006). Agricultural census data may be used to identify farmers (e.g. Kristensen et al., 1996). In countries with national business and pension scheme registers, these data sources may be used for identification of workers from small businesses (Sorensen et al., 2007). Use of the listed rosters for epidemiological studies requires that they have sufficient information on identification (name, date of birth, address at time of recruitment, etc) for the later follow up of the registered workers.

For cross-sectional surveys, study participants may be recruited from numerous small workplaces (Calvert et al., 1998). Recruitment can be difficult because the shop or business owner may act as gatekeeper and effectively bar access to the workers (McKernan et al., 2008). One possible solution would be recruiting currently employed workers outside the workplace. If there were a good validated biomarker for recent exposure, such as end-exhaled breath levels of tetrachloroethylene (PCE) for dry cleaners, workplaces could be bypassed. Potential participants—for example, dry-cleaning operators and spotters--would be recruited through advertisements in the appropriate ethnic press/radio stations to come to a Saturday morning study site. PCE breath analysis would be used to determine eligibility (above a threshold for potentially exposed; nondetectable for potential referents). A half-day of tests and interviews would begin with a medical exam and, for example, for dry cleaners at increased risk of cervical cancer, a Pap test evaluated on site (so further tests could be offered the same day if dysplasia or other positive results were found). Blood would be drawn to test

for PCE level and biomarkers of exposure, genetic susceptibility, and effect. Testing for possible neurological, renal, liver, and other health effects would be included in the exam, depending on costs and funding. An occupational history would be taken (including asking the number of workers at the current job), and questions relative to health and lifestyle would be included. Approaches could be made to some of the shops whose workers participated to do environmental sampling and pre- and post-shift testing of the workers. Shops would only be approached if the workforce was large enough so the participating workers could not be identified, and if the exposures (based on the workers' breath levels) were high enough to merit testing. Data based on volunteers should be interpreted in light of the potential selection bias.

Resources

By Jack Siemiatycki PhD

The present document sets out some recommendations for improving our capacity to prevent cancer by identifying its causes. This report deals only with a small fraction of potentially carcinogenic agents, those for which there is some as yet inconclusive evidence of carcinogenicity; for most other agents there exists little or no evidence one way or another. But even the modest research agenda outlined here will be difficult to achieve given current trends in the research environment. In particular, there has over the past two decades been a precipitous decline in the amount of research produced to address issues of environmental/occupational risk factors for cancer. This has been most evident in epidemiologic research. The proximal cause is that there are fewer epidemiologists working in the area of environmental/occupational etiology of cancer. This is not a place to engage in analysis or speculation on the reasons for this unfortunate trend. Such analysis would have to include but not be limited to consideration of the role of training opportunities, career opportunities, funding opportunities and legal-ethical barriers to accessing human subjects and their personal data. If measures are not taken to stem the decline of this area of research, we will be stuck in the future with the same limited epidemiologic knowledge base we have today. It is imperative that appropriate authorities, and we believe national and international public health agencies should be the prime movers, should take stock of this problem to understand its causes and to find ways of solving it.

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Summary of all agents

Lead and lead compounds

Although the occurrence of lead in the environment has greatly decreased due to the elimination of most leaded gasoline, substantial occupational exposures continue primarily via lead in the battery industry and lead pigments in paints. IARC Monograph 87 in 2006 classified inorganic lead compounds are probably carcinogenic to humans based on sufficient evidence in animals and limited evidence in humans. IARC based its conclusions regarding humans primarily on six cohorts exposed to inorganic lead. Stomach cancer was consistently

elevated (30-50%) in four of the five cohorts where stomach cancers were reported. Lung, kidney, and brain cancer showed elevation in some studies but were not consistent. There have been four new epidemiologic studies since the 2006 monograph, which have been of limited importance and do not change the overall picture. Future work which could advance knowledge include new large cohorts with documented lead exposure such as the NIOSH ABLES lead surveillance cohort which include 50,000 workers with lead exposure over 25 mg/dl, and which is currently under study. Further followup of the existing lead cohorts would also be useful. This study could be strengthened by the addition of two components: 1) measurement of a sample of subjects for bone lead to determine the correlation of the blood lead measurements with cumulative exposure as measured by bone lead, and 2) assessment of whether *Helicobacter pylori* infection has been more common among those with higher blood leads. If so such infection could either be a mechanism by which lead caused higher rates of stomach cancer, although it could also be a confounder. In addition, given positive results from some studies (Keteleslegers et al., 2008, Rajaraman et al., 2006) it would be good if that future epidemiological studies of relationships between lead exposures and cancer should include evaluation of genetic susceptibility factors, such as the ALAD gene. Finally, further experimental research studies are needed to evaluate the complex mechanisms by which lead may cause cancer with particular emphasis on the roles of oxidative stress / apoptosis and the roles of cellular defense mechanisms, signaling pathways and intracellular lead binding patterns in mediating these processes.

Indium phosphide

Indium phosphide was evaluated as probably carcinogenic to humans (Group 2A) in Monograph 86, based on its carcinogenicity at very low doses in numerous animal studies (IARC 2006). The monograph considered indium phosphide alone; since then, use of other indium compounds [e.g., indium tin oxide (ITO), copper indium gallium diselenide (CIGS)] has burgeoned. More than 300,000 workers are employed worldwide in the semiconductor industry, but over 80% of indium is now used as ITO in flat panel displays, with a large number of workers employed either in display manufacture or in the production and reclamation of indium materials used to manufacture of the displays.

No epidemiologic studies to date have specifically evaluated indium compounds. Several studies of semiconductor industry workers have been conducted (e.g., Beall et al., 2005) and more are underway, including a large study of U.S. semiconductor workers sponsored by an industry trade association and a NIOSH study of circuit board manufacturing workers. While these studies are attempting to characterize risk associated with work in specific departments or operations, they are unlikely to inform on cancer risk of indium compounds, because: 1) little indium exposure likely occurred in past circuit board manufacturing, although its use may be growing; 2) wafer fabrication workers (those most likely to have indium phosphide exposure) are typically exposed to a wide variety of other carcinogens, including arsenic, trichloroacetic acid, tetrachloroacetic acid, and more than 20 others (Cullen et al., 2001); 3) little historical exposure monitoring information is likely available to provide estimates of exposure to indium phosphide or other potential carcinogens, which would be necessary to evaluate the contribution of indium to any observed carcinogenicity among wafer fabrication workers.

A better approach may be to conduct (if feasible) epidemiologic studies (e.g., retrospective cohort studies) of workers involved in primary (e.g., zinc smelting) or secondary refining industries. Most primary indium refining occurs in Asia. There are two large secondary refineries in the U.S. and several elsewhere. Studies in secondary refineries may be more informative because of the presence of cadmium in zinc smelting. Also, the focus of secondary refineries on indium production suggests that exposures to other carcinogenic substances may be lower than those to indium. Analogy exists to Group 1 carcinogenic metals (e.g., nickel, cadmium, beryllium), for which the most informative studies have generally been conducted among the refiners and production facilities for these metals and metal compounds (Straif et al., 2009). Recent reports have indicated that pulmonary effects may be occurring in indium workers in Asia (Chonan et al., 2007, Hamaguchi et al., 2008). Studies of current exposure and biomarkers of genetic damage (using the metrics described below) of such indium-exposed workers may be informative in identifying early precursors of cancer.

Further experimental research is needed into mechanisms of indium-compound-induced toxicity and carcinogenesis with particular focus on formation of oxidative stress, inhibition of protective protein synthetic mechanisms and DNA damage. DNA damage from indium exposures could be evaluated by measurement of validated biomarkers of genetic damage such as chromosomal aberrations in accessible cells (e.g., nasal epithelium, buccal cells, shed urinary cells, or circulating lymphocytes).

Cobalt with tungsten carbide

The evidence for carcinogenicity of cobalt with tungsten carbide in humans comes from two studies of workers in the hard-metal industry in France and Sweden. We understand that a study is about to begin or has recently begun at the University of Pittsburgh.
There is good experimental evidence that cobalt and cobalt with tungsten carbide produce cellular toxicity via formation of reactive oxygen species (ROS). This triggers a number of cellular regulatory pathways which may lead to cancer. This general mechanism is mediated by a number of protective cellular mechanisms which may define subpopulations at special risk. These factors include but are not limited to cellular anti-oxidant systems, the stress protein response, DNA excision and repair enzymes and genetic polymorphisms in the processes which maintain these protective systems. There is growing evidence of cobalt with tungsten carbide being formulated into nanomaterials. These have potential health consequences irrespective of chemical make-up.

We recommend that consideration be given to updating the French and Swedish studies in identifying other plants where hard-metal manufacturing is carried out in order that additional cohort studies might be established. Further studies of cobalt metal without tungsten carbide and soluble cobalt (II) salts would also be useful. The epidemiological studies could usefully include molecular biomarkers of early cellular effects. We also recommend additional research is needed to further understand the genetic factors which regulate the above cellular protective systems in order to better protect sensitive human sub-populations exposed to cobalt with tungsten carbide. Further research is needed into the potential health effects of cobalt with tungsten carbide as nanomaterials.

Titanium dioxide

Worldwide, over 5 million metric tons/year of bulk titanium dioxide (TiO2) is manufactured each year. The percentage of TiO2 manufactured as nanoparticles has been estimated as 2.5% in 2009 and about 10% by 2015. User industries include paints and pigment; plastics; paper; cosmetics, catalysts, ceramics, printing inks, roofing granules, glass, and welding fluxes (Robichaud et al., 2009). In 2006, IARC classified TiO2 as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies (Monograph 93, in press).

Since 2006, one new epidemiological study has been published – a re-evaluation of two previously conducted case-control studies found no association with lung cancer (Ramanakumar et al., 2008). There have been no new chronic studies in animals. New subchronic studies in rats (intratracheal instillation) confirm earlier findings that ultrafine TiO2 (nanometer particle diameter) was more potent in causing pulmonary inflammation and cytotoxicity than fine TiO2 (micrometer particle diameter) on a mass basis, whereas both particle sizes showed a consistent dose-response relationship when dose was expressed as particle surface area (Sager et al., 2008; Sager and Castranova 2009). Recent studies have shown that the crystal structure and coatings can also influence the pulmonary responses (pulmonary inflammation, cytotoxicy, and cell proliferation) to TiO2 (Warheit et al., 2006a,b; 2007). These recent studies are consistent with the mechanistic evidence that inhaled TiO2 and other poorly-soluble particles can be carcinogenic through a secondary genotoxic mechanism involving chronic inflammation and oxidative stress; although possible direct genotoxicity by nanoscale TiO2 cannot be ruled out (Schins and Knaapen 2007).

Epidemiological studies with well-characterized exposures and adequate follow-up are needed, especially for workers producing or using nanoscale TiO2. Exposure data should include information on particle size, crystal structure, and surface properties. A possible cohort for epidemiologic studies would include workers in industries using TiO2, particularly the ultrafine (nanoscale) TiO2 now used extensively in the cosmetics industry. Workers handling or mixing TiO2 powders with other ingredients would probably be at the greatest exposure. NIOSH is currently conducting exposure studies of TiO2 users and identifying possible cohorts.

Experimental studies are needed to elucidate the biological mechanisms between particle-induced inflammation and lung cancer. A study examining the relationship between TiO2 exposure in workers and validated markers of oxidative stress, with quantitative comparison in rodent studies, could provide data on interpretation of the animal studies for predicting lung cancer risk in humans. The observation of inhaled discrete nanoscale TiO2 particles inside rat alveolar epithelial cell organelles including the nucleus (Geiser et al., 2005) suggests that possible direct genotoxic mechanisms for lung cancer should be examined. Given the increasing applications of nano-TiO2 in consumer products (e.g., food or food packaging and skin care products), there is a need to develop better techniques to detect TiO2 in tissues and to examine possible carcinogenicity of nano-TiO2 by other routes of exposure (oral, dermal).

Welding fumes

Epidemiologic studies indicate an increased risk of lung cancer among welders in the order of 20% to 40% (Ambroise et al., 2006; Siew et al., 2008). Experimental studies are suggestive but not conclusive of lung carcinogenicity of welding fume exposure (Antonini 2003; Zeidler-Erderly et al., 2008). Genotoxicity of various types of welding fumes has been shown in many *in vitro* and in human *in vivo* studies (Antonini et al., 2003). Pulmonary effects consistent with oxidative stress and inflammatory responses have been observed in experimental animals. Research needs include re-examination of existing cohorts and new cohorts, or appropriate case-control datasets, with improved exposure assessment and smoking data. Experimental studies are needed using inhalation exposure to different types of welding fumes, and studies on ultrafine/nano-sized particles, epigenetic mechanisms, gene expression pathways, and functional level changes related to welding fume exposure (Ayers et al., 2008; Rim et al., 2007). In addition, the recent finding that welders have an increased risk of ocular melanoma (El Ghissassi et al., 2009, Lancet Oncology 10:751-752) should be pursued to determine whether this is due to ultraviolet or other forms of radiation, or to metal and chemical fumes.

Refractory ceramic fibres

Refractory ceramic fibers (RCF) are fibers used for high-temperature insulation. Their production and use is relatively rare compared to low-temperature insulation materials such as glass wool and rock wool. NIOSH has estimated that there were about 30,000 U.S. workers exposed to ceramic fibers in the 1980s, in both production and use. The general public is not usually exposed. Exposures to RCF are of concern because they are relatively more biopersistent than other fibers like low-temperature insulation wools. However exposures to RCF have been lower than historical levels of asbestos fibers which are known to cause cancer in humans. IARC last evaluated ceramic fibers in 2002 in Vol 81, and determined that evidence for cancer in humans was inadequate, while evidence in animals was sufficient, leading to an overall classification of 2B (possible carcinogenic in humans). Human epidemiology for cancer is confined to a single small U.S. cohort (n=942) which at time of last followup had only 9 lung cancer deaths. There were no mesotheliomas found in a review of 35% of the death certificates, although there has been an exposure-related excess of pleural plaques after controlling for past asbestos exposure. There is also a small English cohort (n=774) which showed some cross-sectional association between fiber exposure and lung function, but which has not been followed for cancer. Research needs in experimental animals include a study of the combination effects of RCF and granular, low biosoluble particles. The presence of granular dust retained in lungs could significantly aggravate effects of inhaled fibres. The impact of fibre length on carcinogenicity should be investigated. Fibres longer than 20 μm are supposed to be more carcinogenic than fibres in the range between 5 and 10 μm. Furthermore, the validity of dose response data in rats after inhalational exposure is potentially questionable as there are indications that the sensitivity of this assay is relatively low. More sensitive models for investigating carcinogenicity of man-made fibres should be developed. Regarding epidemiology, further followup of the U.S. cohort is recommended; more mortality follow-up is currently planned. Incidence follow-up would be useful. However it is unlikely that this small cohort will yield important results until many more

years of followup. Mortality to date is 13% for this relatively young cohort. Follow-up for cancer mortality or incidence in the European cohort would also be useful.

Diesel exhaust

In 1989, IARC classified diesel exhaust (DE) as probably carcinogenic to humans (Group 2A) because of limited evidence of carcinogenicity in humans coupled with sufficient evidence of the carcinogenicity of whole engine exhaust in experimental animals. Environmental exposure to DE is ubiquitous in urban areas and occupational exposure to DE is widespread, affecting 1.4 million workers in the United States and 3 million workers in the European Union. Because of the ubiquitous nature of environmental exposure to DE, it is difficult to measure environmental DE exposure for risk estimation in epidemiologic studies. In contrast, DE exposure is potentially quantifiable among the several DE-exposed occupational groups. With regard to lung cancer, much relevant research on the relation between DE exposure and risk of dying has been published since the last Monograph. Two meta-analyses have estimated the summary risk to range from 1.33 (95%CI = 1.24-1.44) (Bhatia et al., 1998) to 1.47 (95%CI=1.29-1.67) (Lipsett and Campleman, 1999). Although each meta-analysis was based on about 30 studies, most of the studies inferred DE exposure based on job title rather than from data on individual exposure, which may have led to misclassification of exposure and estimates of risk biased towards the null. A small number of studies in the past 20 years have included a retrospective assessment of DE exposure. Studies in miners and truck drivers are among the most informative in this regard (Neumeyer-Gromen et al., 2009; Steenland et al., 1990; Garshick et al., 2008). Epidemiologic evidence to date suggests that the relation between DE exposure and lung cancer risk may be causal, but the dose-response curve in humans remains unknown. To establish causality will require well-designed epidemiologic studies of large cohorts of DE-exposed workers with (a) quantitative estimates of DE exposure for individual study subjects, (b) with adequate latent period for the development of lung cancer, and (c) with information on potential confounders. Two ongoing studies satisfy these criteria and will estimate risk for a wide range of DE exposure: 1) a cohort and nested case-control study of lung cancer in U.S. nonmetal miners with heavy DE exposure (NCI/NIOSH, 1997); 2) additional retrospective exposure assessment in the truck driver cohort with light-to-moderate DE exposure (Garshick, personal communication). If these ongoing epidemiologic studies of nonmetal miners and/or truck drivers yield significant, positive exposure-response relationships, it will be important to conduct research into the underlying mechanisms of DE-induced carcinogenesis. Cross-sectional molecular epidemiological studies in DE-exposed human populations will be needed to evaluate the relationship between DE exposure and biomarkers of inflammation, genotoxicity, and other relevant early biological effects, and to study potential sources of genetic susceptibility. Such studies may help us identify the components of DE that are most biologically active in humans. In the long-term, the design and implementation of technologies for population surveillance of DE exposure coupled with biomarkers of effect merit consideration.

With the increased use of biodiesel in recent years, the potential carcinogenicity of biodiesel warrants future evaluation. Although several biodiesel fuels derived from rapeseed oil or rapeseed methyl ester have been found to be highly mutagenic (Bunger et al., 2007), the soy-oil-based biodiesel emissions are less mutagenic. It is premature to conduct epidemiologic

studies of biodiesel because the latent period for the development of solid tumors is currently inadequate. However, experimental laboratory studies of biodiesel should be a priority in view of the increasing prevalence of use of biodiesel in the United States and European populations.

Carbon black

Carbon black is a powdered form of elemental carbon that is used in rubber products, paints, plastics, and inks. In 2006, IARC affirmed its 1995 evaluation of carbon black as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.

Sorahan and Harrington (2007) reported elevated lung cancer in an update of the U.K. carbon worker cohort with an additional eight years of mortality follow-up (SMR 146; 95% CI 113-185). Elevated lung cancer risk was limited to workers employed in the most recent 15 years and a significant trend was seen with increasing cumulative carbon black exposure during that time ("lugged" analysis), suggesting that carbon black may be a late-stage carcinogen. SMRs also tended to decrease with time period since leaving employment. In two re-analyses of the German carbon black worker cohort, Morfeld and McCunney (2007; 2009) found no evidence that the risk of lung cancer declined following cessation of employment, or that recent carbon black exposure was related to lung cancer risk.

There have been no new chronic studies of carbon black exposure in animals. Several recent subchronic studies in rats or mice (intratracheal instillation of carbon black) have strengthened the evidence that particle size and surface area influence the dose-response relationships of early biological events considered to be important in particle-induced lung cancer (Schins and Knaapen 2007). These studies showed that ultrafine carbon black was more potent in causing pulmonary inflammation and cell damage than fine carbon black on a mass basis, whereas both particles sized showed a consistent dose-response relationship when dose was expressed as particle surface area (Stoeger et al., 2006; Duffin et al., 2007; Sager and Castranova 2009). In human alveolar or bronchial epithelial cell lines, carbon black particle surface area was associated with the generation of reactive oxygen species and with oxidative stress (Hussain et al., 2009); and ultrafine carbon black caused single-strand DNA breaks, whereas fine carbon black did not at the same mass dose (100 µg/ml) (Mroz et al., 2008).

Worker exposure data in relation to particle size and surface area are needed to better examine possible exposure-response relationships. The possible influence of worker age at exposure to carbon black as an effect modifier of lung cancer mortality should also be investigated. Basic work history data are needed in the study of U.S. carbon black production workers, which would permit further investigation of the hypothesis that carbon black is a late-stage carcinogen. There is also a need to confirm that the lung cancer SMR in this U.S. study is not artefactually depressed by incorrect allocation of person-years-at-risk. In addition, it would be worthwhile to identify other carbon black production facility workers for study. Experimental studies are needed that improve our understanding of the mechanisms of particle-elicited lung cancer. A study examining the relationship between occupational

exposure to carbon black and validated biomarkers of oxidative stress may provide information on the early biological responses relevant to particle-induced lung cancer mechanisms. These exposure-response relationships should be quantitatively compared in humans and rodents, and the role of particle size should also be examined.

Styrene-7,8-oxide and styrene

Styrene is a large volume industrial chemical, with over 50 billion pounds (23 billion kilograms) produced annually worldwide. Styrene is a key component in the manufacture of synthetic rubbers, many plastics, resins and fiberglass and as such is in a wide array of consumer products. Exposure is ubiquitous. For the general population, food packaging residues appear to be the largest source of exposure, followed by indoor air. Smokers are most exposed through tobacco smoke. Concentrations measured in occupational settings are orders of magnitude higher than environmental concentrations.

In 2002, IARC classified styrene as possibly carcinogenic to humans (Group 2B) with limited evidence in humans and experimental animals for the carcinogenicity. The styrene metabolite styrene-7,8-oxidewas classified in 1994 as probably carcinogenic to humans (Group 2A) with inadequate evidence in humans, sufficient evidence in experimental animals, and the following supporting evidence: (i) forms covalent adducts with DNA in humans, rats and mice; (ii) induces gene mutation in bacteria and rodent cells in vitro; (iii) induces chromosomal aberrations, micronuclei, and sister chromatid exchange in human cells in vitro; and (iv) induces chromosomal aberrations and sister chromatid exchange in mice in vivo.

In 2008 National Toxicology Program expert panel reviewed styrene and also found limited evidence in humans, but sufficient evidence of carcinogenic activity in animals from multiple studies in mice by multiple routes. A consistent finding across studies is the occurrence of lung neoplasia in mice. Genotoxicity of the styrene-7,8-oxide metabolite, evidence for styrene-related DNA adducts and cytogenetic effects in styrene-exposed workers were also afforded weight.

The two main epidemiological studies published since the 2002 IARC monograph did not conclusively address certain critical questions such as: why cancer risk is concentrated in cohorts apparently less exposed to styrene, the effect of potentially confounding exposures, the different types of hematolymphopoietic cancers found in different cohorts, and the potential for other than increases in non-hematolymphopoietic (e.g., pancreatic) cancers sometimes observed. Thus at this point the human evidence remains limited.

At least 70 research publications released since the styrene monograph explore various mechanistic aspects of potential carcinogenicity in humans and rodents, such as variability in enzymes involved in activation and detoxification, the possible role of enzymes besides the 7,8-oxide, potential for human extrahepatic metabolism to styrene oxide and other mutagenic metabolites, and dose response relationships between styrene exposure and DNA damage. Overall, the recent mechanistic research point support to all of carcinogenicity, but also leave questions unanswered. The evidence that has been generated since the last monograph together with the evidence in the monograph may support a re-evaluation of styrene.

Recommendations for new research are to: perform a pooled analysis of human studies on chromosome aberrations and other genotoxic effects, as well as the cancer studies; update the existing studies, while clarifying classification of hematolymphopoietic cancers and addressing the questions mentioned above; recruit new cohorts; for the largest cohorts, promote case-control studies of hematolymphopoietic, bladder, kidney and pancreatic cancers; further explore the extrahepatic metabolism of styrene by mammary, hematolymphopoietic, human lung and other tissue/cells; study the formation of other possible genotoxic metabolites such as the 2,3- and 3,4- oxides, their genotoxicity; further study the pharmacokinetic basis for inter-individual differences in susceptibility; and further explore the relationship between styrene exposure, potential increases in prolactin, and the potential for mammary carcinogenesis.

Propylene oxide

Propylene oxide (PO) (CAS 75-56-9) is used primarily as a chemical intermediate for glycols and glycol ethers and to a lesser extent as a fumigant, food additive, and in production of hydroxypropyl starch ethers. Occupational exposure can occur during production of PO and its derivatives. In 1994, IARC classified PO in group 2B. Only one case-control study was available at the time resulting in inadequate evidence in humans for the carcinogenicity of propylene oxide, while there is sufficient evidence in experimental animals for the carcinogenicity of propylene oxide.

Recent exposure and biomarkers studies have shown that PO form chemically stable adducts with the N-terminal valine of hemoglobin (Hb); N-(3-hydroxypropyl)valine (HOPrVal) adducts. Concentration of HOPrVal (Hb) is linearly related to air concentrations of PO (Boogaard et al., 1999). Hb-adducts are sensitive, not subject to repairs, and therefore a cumulative exposure dose over the past 120 days is achieved. The origin of minor background levels of OHPrVal measured in non-occupational control subjects is unknown; but a likely source is propene found in tobacco smoke or automobile exhaust, metabolically converted to PO in the body. Czene et al. (2002) analyzed the levels of specific DNA (1-hydroxypropyl-adenine) and hemoglobin adducts and sister chromatic exchanges in workers occupationally exposed to propylene oxide and in controls. All these outcomes were significantly increased in the exposed group.

Since the last IARC monograph, only one epidemiological study (Olsen et al., 1997) of PO manufacturing workers (USA) has been reported. This occupational mortality study did not show an increased mortality rate due to cancer by duration with or without latency or cancer risk by process (PO versus EO). To further understand the cancer risk of PO, a future prospective occupational biomarker epidemiology study using DNA and Hb-adducts as biomarker would be beneficial particularly in reference to dose-response and adduct appearance/disappearance kinetics. Possible cohorts for future epidemiological studies have been identified: PO manufacturing workers in the United States (Olsen et al., 1997), in France and the Netherlands (Jones et al., 2005), in China (Czene et al., 2008), processing workers where PO is used as a starting material in polyurethane polyols (NTP 11th RoC), surfactants for textiles (Schettgen et al., 2002) and glycol/glycol ether manufacturing (Boogaard et al.,

1999), and manufacturing of polyethylene (PE), which metabolizes to PO. Women should be included in the study as PO might be mammary carcinogen (Rudel et al., 2007). PO is also used in paint and automotive fluids. Workers of these manufacturing sites have never been assessed either for exposure or included in epidemiological studies. Generally, it is recommended that research be conducted to enhance the mechanistic evidence base in human hemoglobin and DNA adducts and cytogenetic changes.

Formaldehyde

Formaldehyde is a major industrial compound with many uses and is a ubiquitous indoor and outdoor air pollutant. At its last review in 2006 it was classified as a Group 1 carcinogen based upon sufficient animal and human evidence of nasal carcinogenesis. However the Working Group noted that the epidemiologic evidence provided "strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde". Further, after reviewing the toxicological and mechanistic data available, the Group concluded that 'Based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukemia in humans'.

Only one new report from an original epidemiology study in relation to leukemia induction by formaldehyde has been published since the last review. The NCI group has published a recent update of one of their studies, with an additional 10 years of follow-up, and it continues to suggest a possible link between formaldehyde exposure and mortality due to lymphohematopoietic malignancies, particularly myeloid leukemia (Beane Freeman et al., 2009). A recent meta-analysis using a "highest exposure" category to evaluate leukemia risk from formaldehyde exposure (Zhang et al., 2009) provides additional evidence of an association between formaldehyde exposure and human leukemia, especially for myeloid leukemia. Questions have been raised about the highest peak exposure metric used in the NCI studies which otherwise did not show a statistically significant association of hematological cancers with more standard dose metrics (Marsh and Youk 2004),

Reevaluation of previously published animal data has led to an additional focus on lymphoid as well as myeloid malignancies. Mechanism models for formaldehyde leukemogenesis have been proposed (Zhang et al., 2008) while other reviews have questioned the plausibility of formaldehyde as a cause of hematological diseases (Goldstein, 2009)

Particularly valuable in providing additional insight into whether formaldehyde is a cause of hematological neoplasms would be additional studies examining the genotoxic and the pancytopenic effects of formaldehyde in epidemiological studies or in laboratory animals, including assessment of biological markers of internal dose. Further evaluation of hematological effects reported in Chinese studies of formaldehyde-exposed workers is needed. Reassessment of the peak exposure dose metric associated with hematological neoplasms would be useful, as would understanding its implications to the toxicological mechanism of action and to risk considerations. Particular emphasis should be placed on clarifying whether formaldehyde fits into the pattern observed with other known myeloleukemogens, including why the formaldehyde latency period appears longer. The nose as a potential site of

formaldehyde leukemogenesis warrants exploration as do pathways by which inhaled formaldehyde or a formaldehyde derived intermediate can reach bone marrow or lymphatic tissue. Additional mechanistic studies should include evaluation of the interaction of formaldehyde or formaldehyde-derived intermediates on blood stem cells. Studies examining the role of FANC/BRCA repair pathway, or of other mouse models of susceptibility are needed. Closer evaluation of the hematological findings and tumor incidence in existing animal studies is warranted.

Acetaldehyde

Acetaldehyde is primarily used as an intermediate in the manufacturing of acetic acid, flavorings, aniline dyes, plastics and synthetic rubber, in some fuel compounds and in the manufacture of numerous other products. Acetaldehyde is also a ubiquitous air and water pollutant. Acetaldehyde is also an endogenous metabolite produced from ethanol. At its last review in Monograph 71 (1999), it was classified as *possibly carcinogenic to humans (Group 2B)* because of *inadequate evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals. Acetaldehyde binds to DNA, forming stable DNA adducts, and acetaldehyde DNA adducts have been found in alcohol consumers.

Epidemiologic studies of cancer in populations occupationally-exposed to acetaldehyde published after Monograph 71 were not identified. The most compelling evidence of the carcinogenicity of acetaldehyde is provided by studies of alcohol drinkers. Acetaldehyde is the first metabolite of ethanol oxidation. The conversion from ethanol to acetaldehyde is catalyzed by the enzyme alcohol dehydrogenase (ADH), and the subsequent oxidation from acetaldehyde to acetate is catalyzed by the enzyme aldehyde dehydrogenase (ALDH). The genes that code for these enzymes are polymorphic and result in low or fast metabolism of ethanol.

Numerous epidemiologic studies in alcohol drinkers with ALDH2 deficiency or low ADH1B activity strongly suggest that acetaldehyde derived from the metabolism of ethanol contributes towards causing upper digestive tract cancers. This notion is also supported by two meta-analyses that used a Mendelian randomization approach and a recent large-scale case-control study that reported a multiplicative combined risk for esophageal cancer among alcohol and tobacco consumers, who were low ADH1B and ALDH2-deficient carriers.

An epidemiologic study that evaluates the association between acetaldehyde exposure and upper digestive tract cancer will require evaluation of all potential sources of exposure to acetaldehyde, to address their contribution to the overall risk. Prospective studies could be designed to assess all sources of exposure using a combination of questionnaires and environmental and biological monitoring, as well as genotyping to identify individuals with ALDH2, ADH1C, and ADH1B deficiencies. However, given the long induction and latency of most cancers, such a study may not be feasible. Retrospective studies, conversely, have the limitation that exposures have to be evaluated retrospectively, increasing the potential for misclassification. Alternatively, acetaldehyde-derived DNA adducts could be used as biomarkers of exposure to acetaldehyde.

The IARC Working Group that evaluated the carcinogenicity of alcoholic beverages (2007, Monograph 96) concluded that “acetaldehyde derived from the metabolism of ethanol in

alcoholic beverages contributes to causing malignant esophageal tumors". Furthermore, recent risk assessments that consider individual sources of exposure have concluded that the lifetime cancer risks for many of these sources of exposure greatly exceed the usual limits for cancer risks from the environment ($1:10^4$-$1:10^6$). Acetaldehyde exposure is cumulative and in some cases synergistic (as occurs with alcohol exposure and smoking). Exposure scenarios that consider multiple sources of exposure and genetic deficiencies in alcohol metabolism convey increased risks. It was thus recommended that the IARC classification of acetaldehyde be reviewed in a Monograph meeting.

Chlorinated solvents

Trichloroethylene (TCE), tetrachloroethylene (perchloroethylene, Perc), and dichloromethane (methylene chloride, DCM) are used worldwide as degreasers, cleaning solvents for metals and fabrics, and as chemical intermediates. In 1998, the U.S., Europe, and Japan used 318,000 metric tons of TCE; 345,000 metric tons of Perc, and 506,000 metric tons of DCM (Chemical Economics Handbook Program 1999; Leder et al., 1999). TCE and Perc both were evaluated by IARC as probably carcinogenic to humans (Group 2A) (International Agency for Research on Cancer, 1995) and DCM was evaluated as possibly carcinogenic to humans (Group 2B) (International Agency for Research on Cancer, 1999).

Several common issues arise in consideration of research needs for TCE, Perc, and DCM. Each has widespread exposures (often as co-exposures), similar tissue targets observed in human and animal studies, and common epidemiological and mode of action (MOA) issues. The body of knowledge is more comprehensive for TCE than Perc and DCM, and is informative for identifying data gaps and research needs for the other solvents. The meta-analysis approaches of human epidemiological studies on TCE (Scott and Chiu, 2006) (see TCE, below) can be useful not only for future study of TCE, but also to help give a clearer signal and identify new targets of toxicity for Perc and DCM.

Each solvent has been associated with increased risk of lymphohematopoietic cancer (LHC). The evaluation of LHC has been limited by the use of mortality studies, changes in ICD codes over time and changes in the understanding of the biology of these cancers over time. For each of these solvents, studies on LHC should evaluate incidence data and if possible use improved diagnoses, such as biological markers to measure disease. Immunologic mechanism may be involved in lymphomagenesis from solvents (Vineis et al., 2007) and this should also be an area of future research. Brain tumor is also a potential target for DCM and Perc and has not been adequately studied for TCE. The working group is aware of several large brain case-control studies (NCI, NIOSH, Interphone), which will be analyzed in the next year or two for an association between exposure to chlorinated solvents and risk of brain cancer (Inskip et al., 2001; Ruder et al., 2006; Cardis et al., 2007).

Mechanistic data gaps for Perc and DCM include the (1) identification and role of metabolic pathways involved in carcinogenicity, (2) identification of modes of actions from the numerous toxicologically active metabolites of the solvents, and (3) development of physiologically based pharmacokinetic (PBPK) modeling. Similar to TCE, Perc and DCM are metabolized by both the cytochrome P450 (CYP) pathway to oxidative metabolites and by the glutathione (GSH) conjugation pathway to genotoxic metabolites. Genetic susceptibility

studies evaluating cancer risk from exposure to chlorinated solvents among individuals with polymorphisms in relevant metabolisms genes (GST and CYP2E1) as well as other genes in the disease pathway are needed. Since the GSH pathway is not active in glutathione S-transferase (GST)-null individuals, it can be hypothesized that cancer risk will be lower among GST-null individuals. Studies should also be conducted using entire genome scans to identify new susceptibility genes. As shown for TCE, multiple MOAs (Caldwell et al., 2008) from multiple metabolites can contribute to toxicity and make comparisons between chlorinated solvents difficult to study. These can account for differences in exposures and pharmacokinetic and pharmacodynamic characteristics of exposed populations contributing to variable responses in a number of studies. Issues and research needs for specific compounds are discussed below.

Chloroform

Human exposure to the trihalomethane (THM) chloroform is primarily from drinking water, where it is a predominant disinfection by-product (DBP). IARC monograph Vol. 73, 1999 evaluated chloroform as having inadequate evidence for carcinogenicity in humans but sufficient evidence for carcinogenicity in experimental animals; thus, it was classified as Group 2B, possibly carcinogenic to humans.

Since Vol. 73, several epidemiological studies have been published on the association between exposure to DBPs and risk of bladder cancer, with two being a pooled analysis of previous case-control studies and a new case-control study from Spain. Both studies found that the risk of bladder cancer in men, but not in women, increased with increasing THM level. In Spain, a dose response was found both for exposure via ingestion and via shower/bath/pools, and this was found only in persons having GSTT1-1.

Two reports of 2-year rodent studies of chloroform were considered in Vol 73, and two more have been published since. Four studies in rats showed that chloroform induced kidney tumors by gavage, kidney tumors by drinking water in one study but no tumors in another study, no tumors in two studies by inhalation, and kidney tumors by a combined drinking water/inhalation exposure. Three studies in mice showed that chloroform induced liver tumors by gavage, liver and kidney tumors by inhalation, and no tumors by drinking water. Thus, chloroform induced kidney tumors in three studies in rat, liver tumors in two studies in mouse, and kidney tumors in one study in mouse.

Chloroform is an anomaly among the THMs in that it is not mutagenic, whereas the other THMs (i.e., the brominated THMs) are activated to mutagens by GSTT1-1. A model has been proposed by which the bladder cancer associated with drinking water results from the dermal/inhalation exposure to the brominated THMs (not chloroform), followed by systemic distribution that largely bypasses the liver, and activation by GSTT1-1 to mutagens in the bladder. In contrast, oral consumption of the THMs (including chloroform) would result in inactivation by CYP2E1 in the liver. A postulated mechanism for chloroform carcinogenicity involves oxidative metabolism by CYP2E1 to produce cytotoxic metabolites, especially phosgene, which would injure and kill cells, resulting in regenerative cell proliferation.

Additional events, such as epigenetic changes and selection of mutations, could then result in tumors.

Future IARC evaluations should address the entire group of DBPs in drinking water, and chloroform. Other THMs/DBPs should be evaluated for biological effects in rodents via the dermal route. Additional epidemiology studies are needed that have information on route of exposure and detailed DBP exposure assessment. A large New England bladder cancer case-control study is currently underway. Also ongoing are pooled analyses of bladder cancer case-control studies from Spain, France, and Finland, as well as of colorectal cancer case-control studies from Spain and Italy. Epidemiological studies are warranted of high-exposure groups such as competitive swimmers and indoor pool attendants/lifeguards. There should be follow up of cohorts of nurses and doctors exposed to chloroform when chloroform was used as an anesthetic gas.

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) were widely used from the 1930's through the 1980's and later, with an estimated total production of about 1.2- 2 million metric tons. Exposure continues from leaks from transformers and capacitors, volatilization of PCBs in cites, in buildings, from sewage, landfills and waste sites, and combustion of materials containing PCBs. PCBs were evaluated as probable human carcinogens in Supplement 7 (1987).

Among the most important research since the last IARC evaluation: Considerable new data have been generated about mechanisms of toxicity, and routes of exposure, especially inhalation of airborne PCBs, which are more volatile, lower chlorinated and better substrates for xenobiotic metabolism/activation than commercial PCBs. Several studies confirm that in rodents, PCBs are complete carcinogens, initiating, promoting and progressing tumors (reviewed in Ludewig et al., 2008). In vitro genotoxic endpoints varied widely and were highly structure-dependent (Zettner et al., 2007). In vivo lesions (GC-TA transversions (Lehmann et al., 2007)) may arise from direct adduction of DNA bases or reactive oxygen species. Biomarkers of oxidative DNA damage have been described recently (Jeong et al., 2008). An updated expanded cohort study of 15,000 workers found a strong relationship between estimated cumulative PCB exposure and risk of prostate cancer mortality (Prince et al., 2006); a case-control study of testicular germ-cell cancer, nested in the U.S. military cohort, found a significant decreasing risk of disease with increasing prediagnostic serum PCB levels (McGlynn et al., 2009).

Gaps related to PCB sources and exposures include distribution of airborne PCBs, identification of sources, and mechanisms for human environmental exposure. Potential risks of such exposures are unknown, as are mechanisms of toxicity, protective strategies, and predictions about possible susceptibility factors and/or interactions with other compounds. Airborne PCB profiles differ from those of commercial PCB mixtures. Appropriate biomarkers of exposure/ effect/susceptibility for airborne PCB exposure need to be identified. Mechanisms that deserve study include inhalation, volatilization from paint, child consumption of paint chips and other building materials, accumulation in food, and possible occupational exposures, for example, during building demolition. Contaminated sites and

multiple chemical exposures often are found in poor neighborhoods, with medically underserved and nutritionally deficient children. Issues of in utero exposures, and developmental impacts are all unknown. Understanding and, if needed, ameliorating the risks is a matter of environmental justice and social responsibility. Reasonably accurate inventories of stored PCBs (in transformers and capacitors) are needed. Human exposure to PCB degradation products and metabolites is not well studied.

Research needs related to mechanisms of action/toxicity include investigation of the metabolic fate of lower chlorinated PCBs. What are the reaction products? Are they mutagenic? Could any serve as biomarkers of exposure/effect? Can we prevent or abrogate negative impacts of exposure? The fate of these residues, whether excreted, converted to toxic metabolites, or bound covalently to tissues, is unknown. The roles of metabolism of OH-PCBs (e.g., further oxidation, sulfation, and other metabolic reactions) in the disposition and toxicity represent a significant gap in our knowledge about mechanisms for carcinogenesis. Many mechanisms of genotoxicity/carcinogenicity for PCBs appear to involve reactive oxygen species, oxidative stress, oxidative DNA damage, and formation of DNA adducts. More research is needed with this mode of action and with cell proliferation, as the two could drive the induction of mutations and subsequent carcinogenicity. Specific attention needs to focus on dose-response. There has been only limited information available on PCB effects on metastasis formation. Clinical, epidemiological, and basic research studies are needed to address possible prometastatic effects of PCBs on tumor cells or how PCBs can alter the vascular endothelium to increase transendothelial migration of tumor cells and the development of metastases.

The existing occupational epidemiologic literature, most of it produced since the last review of PCBs in 1987, may suffice for a re-evaluation of the carcinogenicity by an IARC working group.

Possible studies include cancer incidence within the large (>27,000 workers) NIOSH cohort, which is under way. Nested case-control studies in this cohort and/or those in Sweden and Italy, obtaining and evaluating current PCB blood levels in cases and controls, might be informative. A useful study population might be residents of Aniston, Alabama, around the former PCB manufacturing facility, who received high levels of exposure through various routes.

Di(2-ethylhexyl) phthalate

Although extensive human exposure occurs to di(2-ethylhexyl)phthalate (DEHP) through its use as a plasticizer of polyvinyl chloride (PVC), definitive epidemiologic studies are not available due to the difficulty in identifying highly exposed workers in retrospective cohort or case-control studies. Since the previous Monograph review concluded that liver cancer observed in animals resulted from PPAR-*a* induction and peroxisome proliferation activation was not relevant to humans, several lines of evidence suggest that DEHP may have multiple mechanisms of carcinogenesis, some of which might be relevant to humans. A study of DEHP-induced tumorigenesis in wild-type and *Pparα*-null mice found that the incidence of liver tumors in *Pparα*-null mice exposed to 0.05% DEHP (25.8%) was higher than in

similarly exposed wild-type mice (10.0%). Microarray profile studies find that patterns of up- or down-regulated genes are quite different in hepatocellular adenoma tissues of wild-type mice and *Pparα*-null exposed to DEHP. Animal studies also suggest additional target organs in rats (pancreatic acinar-cell adenoma and testicular leydig cell tumors). Future studies in mouse models, using hPPARα^{TetOff}, which expresses the human receptor only in liver, or hPPARα^{PAC}, which expresses the human receptor not only in liver but also in kidney, heart, intestine and brown adipose tissues, may elucidate the role of human PPARα in DEHP carcinogenesis. Further characterization of DEHP exposures in industry is needed and could be done in established cohorts in the PVC-processing factories using DEHP metabolites, mono(2-ethylhexyl)phthalate and mono(5-carboxy-2-ethylpentyl) phthalate, as a sensitive biomarker of DEHP exposure.

Atrazine

Atrazine is a widely used herbicide to which populations are exposed occupationally, through drift, and in surface and ground water. In 1999, an IARC Working Group determined that atrazine was not classifiable as to its carcinogenicity to humans (Group 3), with inadequate evidence in humans and sufficient evidence in experimental animals. The Working Group concluded that the mammary tumors among Sprague-Dawley rats associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism that is not relevant to humans. Atrazine causes accelerated aging within the brain-pituitary-ovarian axis (i.e., constant estrus), thereby establishing the hormonal environment conducive to the development of mammary gland tumors in rats. Recent epidemiologic investigations include updated data from a manufacturing cohort study and U.S. non-Hodgkin lymphoma (NHL) case-control studies, a new case-control study of ovarian cancer, analyses from the prospective U.S. Agricultural Health Study (AHS), and several ecological studies of environmental exposure. The manufacturing cohort demonstrated a nonsignificant excess of prostate cancer incidence (MacLennan et al., 2002), which may have been due to the plant's intensive prostate cancer screening program (Hessel et al., 2004). The cohort also had a nonsignificant excess of deaths due to NHL (N=4) (MacLennan et al., 2003). A significant association of NHL with agricultural exposure was observed in pooled data from three population-based case-control studies in the Midwestern U.S. (de Roos et al., 2003), which controlled for potential confounding by other pesticides. Using archival biopsies from a case-control study, atrazine was associated with risk of NHL among t(14;18) cases only (Schroeder et al., 2001) Rusiecki et al. (2004), reported on cancer incidence among 36,513 applicators in the AHS who ever used atrazine, based on follow-up through 2001. There were suggestive, nonsignificant excess risks for lung cancer, bladder cancer, NHL, and multiple myeloma. New studies of general population exposure did not provide convincing evidence of risk (Young et al., 2005; Hopenhayn-Rich et al., 2002; Muir et al., 2004; Mills et al., 2006; Van Leeuwen et al., 1999).

The finding that atrazine is carcinogenic at a single organ, sex, strain, and species of rodent makes it unlikely that atrazine is a human carcinogen, considering that most IARC Group 1 carcinogens are trans-species carcinogens. Nonetheless, clear mechanistic data are lacking to show that atrazine does or does not alter the secretion of luteinizing hormone and prolactin in humans. Thus, further studies are needed to characterize the ability of atrazine to interfere with the hypothalamic-pituitary-ovarian axis in women and to clarify whether atrazine is a

mammary carcinogen in women. Further analysis of the AHS biomarker study among male corn farmers (Vermuelen et al., 2005; Bakke et al., 2008) and expansion of similar efforts to include women could shed light on atrazine's effects on hormonally-related cancers. Of critical importance is the need to analyze the extended follow-up data of the AHS through 2006, which is expected to have over 65% more cases than the last follow-up. Finally, more extensive microarray and proteomic studies in rodents and humans would also help to characterize the pathways disrupted by atrazine. There is also a need to explore atrazine's ability to alter immune function and aromatase in species relevant to humans as well as in human molecular epidemiology studies.

Shift work

Based on the theory that electric light at night might explain part of the international differences in breast cancer incidence, it was predicted in the late 1980s that women working at night would have elevated risk. Evidence has accumulated to the point where the IARC has classified 'shift work' as a probable human carcinogen (2A) based on limited epidemiology and sufficient animal data. Between 5 and 20% of people in the modern world work a non-day shift, so the possibility is important. Research needs in this area are: 1) better definition of what is meant by 'shift work', 2) studies of markers of circadian disruption in non-day workers, 3) better description of controls and their exposure to light-at-night, and 4) investigation of the impact of variations in expression of circadian genes on cancer in shift workers. An emerging area is the relative toxicity of occupational exposure to toxic chemicals depending on time of day of that exposure. There are marked circadian variations in cell division and DNA repair over the daily cycle, and these are changes are controlled by the circadian genes. Therefore, non-day workers may have very different sensitivity to occupational exposures.