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Benzene and human health: A historical review and appraisal of associations with various diseases

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Abstract

Over the last century, benzene has been a well-studied chemical, with some acute and chronic exposures being directly associated with observed hematologic effects in humans and animals. Chronic heavy exposures to benzene have also been associated with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) in humans. Other disease processes have also been studied, but have generally not been supported by epidemiologic studies of workers using benzene in the workplace. Within occupational cohorts with large populations and very low airborne benzene exposures (less than 0.1–1.0 ppm), it can be difficult to separate background disease incidence from those occurring due to occupational exposures. In the last few decades, some scientists and physicians have suggested that chronic exposures to various airborne concentrations of benzene may increase the risk of developing non-Hodgkin's lymphoma (NHL) (Savitz and Andrews, 1997, Am J Ind Med 31:287–295; Smith et al., 2007, Cancer Epidemiol Biomarkers Prev 16:385–391), multiple myeloma (MM) (Goldstein, 1990, Ann NY Acad Sci 609:225–230; Infante, 2006, Ann NY Acad Sci 1076:90–109), and various other hematopoietic disorders. We present a state-of-the-science review of the medical and regulatory aspects regarding the hazards of occupational exposure to benzene. We also review the available scientific and medical evidence relating to benzene and the risk of developing various disorders following specific levels of exposure. Our evaluation indicates that the only malignant hematopoietic disease that has been clearly linked to benzene exposure is AML. Information from the recent "Benzene 2009," a symposium of international experts focusing on the health effects and mechanisms of toxicity of benzene, hosted by the Technical University of Munich, has been incorporated and referenced.

Keywords: *Acute myelogenous leukemia; benzene; epidemiology; myelodysplastic syndrome; toxicology*

Contents

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Introduction

Benzene continues to be a widely used industrial chemical in the production of polymers, resins, and synthetic fibers (ATSDR, 2007). In the late 19th century and extending into the early 20th century, benzene played a key role in the growing synthetic rubber manufacturing industry, and was used by numerous industries as a solvent (Paustenbach et al., 1992). Early observations of benzene effects in humans in the workplace were difficult to correlate with specific levels of exposure. Such difficulty was due mostly to inadequate technology for exposure estimation, compared with contemporary methods. Dr. Alice Hamilton, who was perhaps the premier occupational physician of her era, noted in an early review of benzene toxicity that "an excellent precaution" was used by a steel manufacturer to prevent workers from being overcome by benzene vapors in their holding tanks.

> "After emptying, washing out, and steaming out the tank, they lower into it a cage of white mice, and if the mice are overcome by the vapors the process of flooding and steaming is repeated until the little animals can be lowered into the tank without showing any effect" (Hamilton, 1929).

During the early 20th century, there were many reports of acute human exposures to benzene in the workplace at concentrations of benzene relating to certain toxic endpoints. For example, exposure to 4700 ppm reportedly produced confusion within 30 minutes of exposure, whereas levels of 6000 to 9000 ppm produced symptoms of acute poisoning within a few hours (Lehmann, 1919). A listing of health effects on humans observed over time resulting from various levels of exposure to inhaled benzene appears in Table 1.

Benzene has been known to have a depressive effect on the bone marrow following chronic exposures, occasionally leading to complete failure of the blood-forming elements, or aplastic anemia (Hamilton, 1929). During the past 60 years, concerns about the adverse effects of benzene in exposed workers have led to a series of increasingly stringent regulatory standards in the United States for benzene in the workplace (Table 2), and in 1978, there was a voluntary withdrawal of benzene as an added ingredient to consumer products manufactured in the United States (National Research Council, 1981). Over the past 25 years, concentrations of benzene in various consumer products has generally been between zero and less than 0.1% (Williams et al., 2007).

Table 2. History of benzene regulatory standards.

Time period	TLV (ppm)	PEL (ppm)
≤1946	100	—
1947	100	—
1948	50	—
1949–1957	35	—
1964–1969	25 (ceiling)	—
1957–1976	25	—
1972–1986	—	10
1987–current	—	1
1977–1996	10	—
1997–current	0.5	—

TLV = threshold limit value, a recommended standard from the American Conference of Governmental Industrial Hygienists (ACGIH); PPM = parts per million; PEL = permissible exposure limit, an enforceable standard created by Occupational Safety and Health Administration (OSHA). All concentrations represent time-weighted averages (TWA) unless otherwise noted. *Sources*: ACGIH, 1976; OSHA, 1987; Paustenbach et al., 1992; ACGIH, 2001; Capleton and Levy, 2005.

Table 1. Noncancer human health effects of inhalational benzene exposure.

Benzene concentration (ppm)	Length of exposure	Health effect	Reference
19,000–20,000	5–10 minutes	Death	Flury, 1928; Browning, 1937; Goldwater and Tewksbury, 1941; Von Oettingen, 1940; Gerarde, 1963
6200–9300	30 minutes–1 hour	Immediate or subsequent death	Hamilton, 1934
4700	30 minutes	Confusion	Greenburg, 1926
3000	30 minutes	Endurable	Gerarde, 1963
1570–3130	Several hours	Slight symptoms	Browning, 1937
1550–3100	6 hours	No serious effects	Hamilton, 1934
1500–3000	Several hours	Slight symptoms	Bloomfield, 1951
1500	1 hour	Serious symptoms	Gerarde, 1963
500	1 hour	Symptoms of illness	Gerarde, 1963
300	30 minutes	Dizziness, headaches	Flury, 1928
150	4 months–1 year	Pancytopenia	Aksoy, 1978
50–150	5 hours	Headache, lassitude, weariness	Gerarde, 1963
60	1–20 days, 2.5–8 hours/day	Mucous membrane irritation, dyspnea	Midzenski, 1992
40	1 year	Leukopenia in first 4 months	Cody, 1993
25	8 hours	None	Gerarde, 1963
7.6	6.3 years avg	Reduced lymphocyte counts	Rothman, 1996
2.3	5–10 years avg	Reduced neutrophil and RBC counts	Qu, 2002

Sources: Paustenbach, 1995; ATSDR, 2007.

Over the years, there have been significant improvements in industrial hygiene practices, which have considerably reduced the risk to workers (Hamilton, 1929, 1945; Gafafer, 1943; McCord, 1931; Hemeon, 1955, 1963; Hamilton and Hardy, 1974; ATSDR, 2007; Gaffney et al., 2009). These have included the adoption of better skin/respiratory protection and engineering controls, substitution of other less toxic chemicals for benzene whenever possible, the advent of closed systems for performing chemical synthesis in manufacturing settings, and increased monitoring of the exposures to workers, as well as biomonitoring of those workers in industries that continue to have exposures to benzene at concentrations approaching the contemporaneous occupational exposure limits (OELs).

Although it was established in the 1920s that heavy exposures to benzene adversely affected the blood-forming organs, there have been several case reports since that time suggesting that certain chronic diseases could be associated with exposures to benzene or even causally linked. Early reports focused on abnormalities found in peripheral blood, but in the 1950s to 1960s, some scientists reported that chronic exposures to certain airborne concentrations of benzene seemed to increase the risk for developing leukemia (Cronkite, 1961; De Gowin, 1963; Vigliani and Saita, 1964; Goguel et al., 1967). In 1977, Infante, Rinsky, and colleagues clearly established a link between exposure to benzene and an increased incidence of leukemia in an analysis of workers occupationally exposed to benzene during the production of a natural rubber cast film called "Pliofilm" at two Ohio facilities (Infante et al., 1977). Following that report, industrial hygiene practices relating to benzene in the workplace changed significantly, resulting in a marked decrease in the use of benzene in manufacturing processes, as well as lesser degrees of exposure.

The major environmental sources of benzene exposure for most persons in Western society are active and passive smoking, gasoline vapor emissions, and automotive exhaust fumes (Wallace, 1996a, 1996b; Capleton and Levy, 2005). By and large, concentrations in the ambient air have decreased steadily since the early 1990s. The use of benzene in consumer products likewise has fallen off dramatically (Williams et al., 2008). Smokers have been found to experience about half of all human exposures to benzene in the United States, with 90% of their cumulative lifetime doses coming from smoking, with measured body burdens 6 to 10 times that of nonsmokers (Wallace, 1996b). In many countries, the permissible concentration of benzene in gasoline is less stringent compared to North America, Europe and Australia, and for many countries, no standards have been set at all (Figure 1) (International Fuel Quality Center, 2008).

Human exposure to benzene continues to occur due to its presence in ambient air, cigarette smoke, and some workplaces, and the range of possible adverse effects following acute or chronic exposure remains an issue of important interest to regulatory agencies and to the public. Over the last few decades, some have suggested that exposures to benzene could be responsible for an increased incidence of several other chronic malignant hematopoietic diseases, including multiple myeloma (MM), chronic myelogenous leukemia (CML), and non-Hodgkin's lymphoma (NHL), among others (Infante, 2006; Mehlman, 2006; Smith et al., 2007; Savitz et al., 1997). In our review, we evaluate the scientific and medical evidence supporting assertions that diseases other than acute myelogenous leukemia (AML) are attributable to benzene. We apply an evidence-based methodology to characterize our evolving state of knowledge with respect to hematopoietic effects and occupational exposures (Guzelian et al., 2005; US FDA, 2009; Greer et al., 2000).

State of the science regarding the effects of benzene (1880–2010)

Benzene has been used as an industrial solvent in a variety of different industries (Table 3). Since the late 1800s, significant exposures to benzene have been noted to result in suppression of one or more of the blood-forming elements and with a sufficient dose and duration have led to the development of aplastic anemia (Santesson, 1897; Hamilton, 1929). Our knowledge regarding the hematopoietic effects of benzene at various levels of exposure has significantly evolved over the years. A summary of the various reported hematopoietic effects of benzene is presented in Table 4. The two widely acknowledged effects following high level, chronic exposures to benzene are aplastic anemia and AML.

The following represents a simplified overview, by decade, of the evolution of our understanding (state of the science) of the health effects related to benzene from both toxicology and epidemiology studies. A number of other reviews have been written over the past 20 years (Snyder, 2002; Schnatter, 2005; Pyatt, 2004; Lamm et al., 1989; Austin et al., 1988).

1880s

Autopsies of deaths following incidents of acute inhalational benzene poisoning were first reported (Sury-Bienz, 1888).

1890s

Earliest known cases of chronic benzene poisoning were reported, where nine cases of "purpura hemorrhagica" were seen in young girls using rubber cement with high concentrations of benzene in a Swedish tire factory, four leading to death (Santesson, 1897). A similar case was reported in a man employed for several years in a dye factory, who died with spontaneous hemorrhaging and probable myocardial infarction (Lenoir and Claude, 1897). [Some believe that this latter case report is the first evidence of benzene being associated with leukemia, but the lack of clinical data describing the patient does not provide sufficient basis for this claim.]

1900s

Several case reports of acute industrial inhalational poisonings were described in the literature. Ironically, individuals who attempted to save coworkers collapsing in the work environment often experienced higher risk of mortality than the workers who were initially overcome by the vapors (Hamilton, 1929). Occupational settings for these

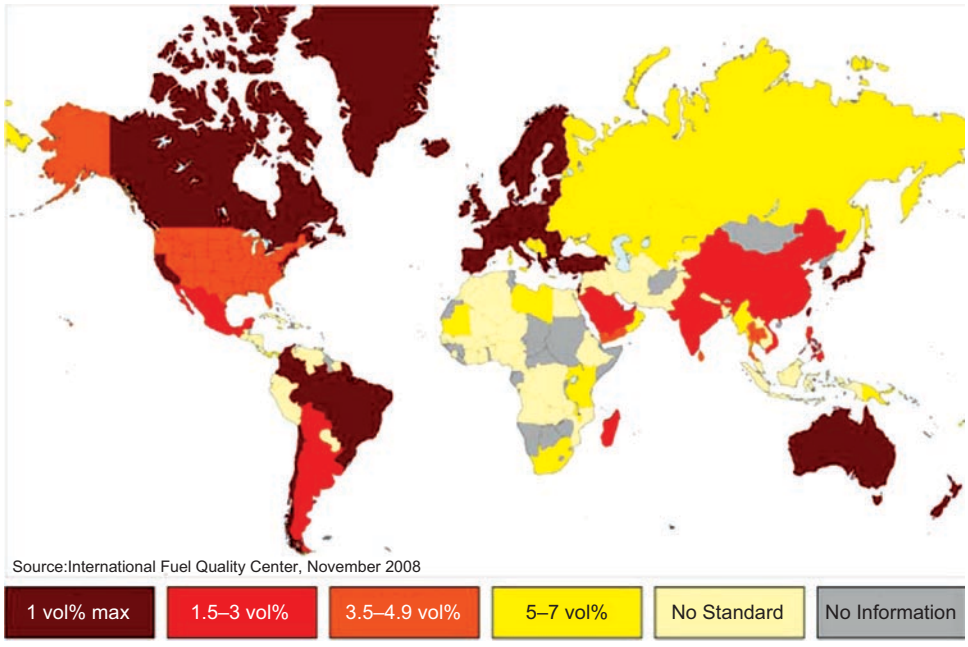

Figure 1. International limits on benzene concentration in gasoline. *Source*: International Fuel Quality Center, 2008.

Table 3. Occupations historically associated with benzene exposure.

- Printing
- Leatherwork/shoemaking
- Chemical manufacturing
- Petrochemicals (refining, distribution, service station operators)
- Scientific laboratories (particularly glassware cleaners)
- Rubber manufacturing
- Coal-based coke production (metallurgical and steel manufacturing)
- Plastics manufacturing

Source: ATSDR, 2000.

Table 4. Hematopoietic conditions associated with significant exposures to benzene.

- Hematotoxicity
  - Anemia
  - Leukopenia
  - Thrombocytopenia
- Aplastic anemia
- Acute myelogenous leukemia
- Myelodysplastic syndrome
- Immune dysfunction
  - Worker and animal data have shown diminished antibody levels and impaired cellular immunity

Source: ATSDR, 2007.

inhalational injuries were often connected to the manufacturing of explosives, with exposed workers found within the interior of chemical storage tanks that had supposedly been boiled out and cleaned of residual benzene (Hamilton, 1929). These deaths occurred long before obligatory procedures were developed for tank entry.

1910s

Twenty-one cases of acute benzene poisoning were reported in Germany in 1910 (Heffter, 1915). The first American cases of chronic benzene poisoning were described at a tin can factory, where three young girls were working with rubber solvent, two of whom died (Selling, 1910). Experiments by Selling in animals and his observations that benzene can cause a lowering of white cell counts led to the notion that benzene could be used as an effective treatment for leukemia (Koranyi, 1912). Hamilton reported on 14 cases of benzene poisoning, identifying a fatality rate of 50% (Hamilton, 1922). Benzene poisoning in American rubber workers was described, specifically in tire builders (Harrington, 1917). Three of the five cases presented were fatal.

The first cases of chronic benzene poisoning in Great Britain were noted in two men employed at a balloon manufacturing plant. Early measurements of occupational benzene exposures were described, ranging from 210 to 800 ppm, with peak levels over 1000 ppm (Legge, 1920).

1920s

Italian researchers reported seven cases of aplastic anemia in young women employed in raincoat factories where benzene-containing glues were used. Exposures at the facility were measured at one part per thousand, or 1000 ppm (Meda, 1922).

Workers in the German rubber manufacturing industry represented the first reports of chronic benzene poisoning (Brucken, 1923). A comprehensive study of American industries using benzene was conducted in 1926, where weekly volumes of benzene usage were seen to range from 50 to over 10,000 gallons (Greenburg, 1926). Measured air concentrations in these facilities showed mean concentrations of 70 to 1800 ppm benzene, with peak concentrations ranging from

110 to over 4000 ppm. The author concluded that although less toxic solvents should be substituted whenever possible, "…with proper care in construction, maintenance, and operation, the use of benzol [historic term, generally referring to coal tar-derived benzene] can be made sufficiently safe to warrant its employment" (Greenburg, 1926).

During this period, occupational health guidelines were written and they usually recommended that any worker who showed a drop of 25% in the white cell count or a total white cell count of less than 5000, 25% decrease in the red cell count, or a hemoglobin level less than 70% should be removed from exposure and sent to another area without benzene exposure (Williams and Paustenbach, 2003). The use of benzene as a treatment for leukemia was actively considered in the 1920s (Greenburg, 1926). [Benzene "poisoning" was often referred to in early discussions of the health effects of benzene, and generally referred to acute toxic chemical effects, such as nausea, visual changes, lack of coordination, unconsciousness, and death. As more became known in the late 1920s regarding intermediate and chronic exposures, aplastic anemia was considered the primary adverse effect associated with benzene "poisoning." Other nonmalignant hematologic effects have been reported at various levels of exposure (Table 1).]

In 1928, two workers at a pharmaceutical manufacturing plant developed hematologic disease attributed to chronic exposures to benzene, one of whom died of aplastic anemia, the other of acute lymphoblastic leukemia. The worker with leukemia had worked at the plant for 15 years; the last 5 of which were believed to be in a job where excessive benzene exposure was experienced. Although no measurements of exposure were provided, it was stated that his job was considered dangerous, and that none of his fellow workers were allowed to spend more than 1 month at a time in the job he performed. However, this man was allowed to remain in this high-exposure work station for 5 straight years. The latency period between his exposures and his diagnosis was not described; no bone marrow findings were reported. This case has generally been believed to be the first possible case of benzene-related leukemia, but the lack of clinical data and discussion of other chemical exposures or other risk factors in this case report makes it difficult to establish a certain connection to benzene (Delore and Borgomano, 1928).

A French automobile factory was noted to have four workers who developed "purpura hemorrhagica," two with fatal consequences. Workers were described as being on the job for less than 6 months (Hamilton, 1929).

1930s

A comprehensive review of the effects of acute and chronic benzene poisoning was provided by Hamilton in 1931 (Hamilton, 1931). The results of animal studies, as well as the clinical presentations of people who had experienced significant exposures to benzene in the workplace, were summarized. Blood, bone marrow, and pathologic findings at autopsy were presented, and Hamilton called for more research on the effects of benzene, stating that:

> "The greater part of the careful, detailed work on the action of benzene has been carried out on animals in the laboratory and for the most part with the employment of a technic [sic] which does not in any way reproduce what takes place in industrial poisoning of human beings. The victims of industrial poisoning whose bodies have come to autopsy are few in number, and their cases have not received as careful study, except in rare instances" (Hamilton, 1931).

Over the next 20 years, Hamilton would continue to document cases of acute benzene poisoning; these are chronicled in a book titled *Alice Hamilton: A Life in Letters* (Sicherman, 1984).

In the late 1930s, two cases of leukemia believed to be related to exposures to benzene were reported in Massachusetts (Mallory et al., 1939). One of these was in a 28-year-old leather worker who was exposed to benzene for 4 years, at levels that were described as causing "evidence of benzene intoxication." Six years later he developed an aggressive acute myeloid leukemia. The other case was that of a 12-year-old boy who was the son of a painter and played in his father's shop frequently; he was diagnosed with an immature lymphoblastic leukemia. No symptoms indicating excessive exposures leading up to their diseases were described.

A possible leukemia arising in a 38-year-old man was described in the same year (Erf et al., 1939). In 1918, this man worked with his brother "…in a studio in which benzene was used." That year, his brother developed aplastic anemia and died. Subsequently, the patient changed his occupation to avoid benzene exposure. Seventeen years later, he started using a rubber cement containing benzene for 14 hours a day. Less than a year later he was admitted to the hospital with weakness and bleeding gums. He left the hospital 5 weeks later, and died 2 months thereafter. Postmortem examination revealed findings consistent with myeloid leukemia. An additional case involved a chemist diagnosed with leukemia at another institution at age 23. He had worked for 18 months, using benzene to synthesize benzoic acid. He was admitted to the hospital and discharged after 2 weeks. A follow-up visit 2 years later found him to be in "perfect health" (Erf et al., 1939).

A study entitled "Benzene Poisoning in the Rotogravure Printing Industry in New York City" showed that all 332 printers studied were chronically overexposed to benzene, at air concentrations ranging from 11 to 1060 ppm. Evidence of poisoning was found in 130 workers, with 6 requiring hospitalization (Greenburg et al., 1939). As a result of the study, the use of benzene was discontinued, because air concentrations could not be consistently kept within safe levels, and chemicals with lesser toxicity were substituted.

1940s

Gafafer's *Manual of Industrial Hygiene*, prepared by the industrial hygiene division of the National Institute of Health, United States Public Health Service, related in 1943 that "…benzene, with its known harmful properties, has been

abandoned as a solvent by many industries." Maximum allowable concentrations of benzene in the workplace were then 100 ppm "on the basis of an eight-hour daily exposure" (Gafafer et al., 1943).

The next in the series of Hamilton's reviews of industrial toxicology noted that: "Acute benzene poisoning is of little importance under modern industrial management. The danger is well understood, and no longer are men sent unprotected into tank cars or vats" (Hamilton, 1945). She also noted that evidence seemed to be accumulating, "…that leukemia, myeloid or lymphatic, may be one of the forms benzene poisoning may take. Vigliani and Penati collected 10 cases, which had been reported by 1938, and Mallory and his colleagues have added 2" (Hamilton, 1945).

Marshall Clinton, a medical doctor under the direction of Philip Drinker, a Harvard industrial hygiene professor, prepared a report on benzene for the American Petroleum Institute (API). With regard to leukemia, it was stated that "… reasonably well documented instances of the development of leukemia as a result of chronic benzene exposure have been cited" (API, 1948). The possible occurrence of "latent bone-marrow injury due to benzene without any immediate alteration in blood findings" years after exposure had taken place was described, but conclusive proof for this possibility was stated to be lacking. The report mentioned that: "…the only absolutely safe concentration for benzene is zero." Later, the author of that report stated that he was defining zero as "the limit of analytical detection," which was about 50 ppm at that time (Clinton, 1994). This statement is not surprising since the report went on to recommend a lower occupational exposure limit than the 100 ppm level which was suggested by ACGIH, stating: "A limit of 50 ppm or less is strongly recommended, particularly where exposures are recurrent" (API, 1948). The 50 ppm occupational exposure limit that API proposed, although far in excess of modern standards, represented what was thought to be a tolerable exposure given the knowledge of benzene health effects at that time.

1950s

In the 1950s, over 100 cases of benzene poisoning were described in a European shoe factory. Area samples for benzene collected at three workstations had average values of 318, 433, and 470 ppm (Savilahti, 1956). Blood testing was performed in 147 workers, with 73% being found to have some abnormality. Low platelet counts were the most common finding (62%), followed by anemia (35%) and low white cell counts (32%). Thirty-one workers were found to have simultaneous effects on all three blood lines (platelet, white blood cell, and red blood cell counts). With removal from exposure, 120 workers recovered after three months, one died, 20 continued with minor symptoms, six remained on sick leave, and 1 was still hospitalized after one year (Savilahti, 1956).

1960s

In 1960, Dr. Drinker developed a second edition of his toxicological review for benzene at the request of the API and noted that "leukemia as a result of chronic benzene exposure has been reported." The maximum acceptable workplace concentration was 25 ppm over an 8-hour workday (API, 1960).

A summary of the evidence for radiation and various chemicals being associated with a higher incidence of leukemia was published by a research pathologist at the Brookhaven National Laboratory (Cronkite, 1961). In the author's opinion, at that time "…only benzol has been investigated sufficiently well to make it appear a serious contender for the part of a chemical leukemogen in man." Up to that point, many cases had been presented in the literature, and the "cause of death in some instances was ascribed to aplastic anemia, and in others, to leukemia," with reference to the autopsy series by Mallory (Mallory et al., 1939). Leukemia was described as an immediate consequence of chronic heavy exposures, rather than as a disease with a particular latency period. Animal studies attempting to show malignant health effects of benzene were described as "equivocal."

Researchers from Italy provided a review on benzene and leukemia, apparently in response to the comments made above by Cronkite, stating that "From this [Cronkite's paper] and other American papers on benzene poisoning it seems that the European and especially the Italian literature on benzene leukemias is poorly known" (Vigliani and Saita, 1964). Six cases of leukemia associated with chronic heavy benzene exposure were reviewed. In the two cases where workplace concentrations of benzene were available, they reported airborne concentrations "ten times greater than the maximum allowable values generally accepted" (e.g., at least 500 ppm). Three of the six cases had been diagnosed with aplastic anemia prior to their leukemias, and five of the six "…had low white-cell counts at the outset that were independent of any therapy." The authors also emphasized that they had never seen a case of "chronic myeloid or lymphatic leukemia in workers poisoned with benzene." With regard to previous claims of these diseases being caused by benzene exposure, "…we must emphasize our view that in some of these cases reported in the literature the occupational history was not convincing. The number of persons occasionally exposed to benzene or exposed to very low concentrations was so high that some cases of chronic leukemia could have occurred among them as among any other working population.… Great caution must be exercised before admitting the benzene etiology of chronic myeloid or lymphatic types of leukemia" (Vigliani and Saita, 1964). They also noted that: "We are fully aware of the fact that no final statement about the existence of a true "benzene leukemia" may be made, without a statistical analysis of the incidence of leukemia among workers exposed to benzene as compared with that among a control group of the same age, sex and living habits." A large fraction of shoe and leather workers were noted to be employed at home under uncontrolled conditions and the content of benzene in the solvents used by various home-based operations was characterized as uncertain at best (Vigliani and Saita, 1964).

Overall, the pre-1970s experience regarding the linkage between benzene and leukemia largely consisted of occasional case reports and small groups of patients, generally with known significant exposures to benzene. In many

situations, there was concurrent exposure to other solvents, as well as a history of smoking, which resulted in a lack of certainty regarding a cause and effect relationship.

1970s

Aksoy, a Turkish hematologist, has been credited with performing the first major epidemiologic study examining the effects of benzene on a specific occupational subgroup; that of shoe and leather workers. He and his colleagues acknowledged at that time that "…doubt still exists as to the causal relationship between benzene exposure and leukemia" (Aksoy et al., 1974), resulting from conflicting animal data and a lack of epidemiology data demonstrating an increased incidence of leukemia in exposed populations. The incidence of leukemia was calculated to be 13 cases per 100,000 in the population of 28,500 Turkish workers over a 7-year period of observation. Several cases of "preleukemia," the exact diagnosis of which was not defined by the authors, were added to the total incidence of leukemia. Their approach likely overestimated the true leukemia risk, as some proportion of these individuals would not be expected to progress to leukemia, and some might even recover if the hematological abnormalities noted were related to prolonged myelosuppression from benzene and not true myelodysplasia.

Earlier, Aksoy had summarized his observations of leukemia in four shoe workers, who "…were rather heavily exposed to benzene vapor for a period varying between six and fourteen years. Their places at work were unhygienic, and the air concentrations of benzene were between 150 and 210 ppm during working hours," with occasional readings up to 650 ppm (Aksoy, 1972). Three of the four workers had been previously diagnosed with aplastic anemia.

In 1974, a review of leukemia incidence in a population of petrochemical workers from eight Exxon affiliates in Europe was released, finding a slightly diminished rate of leukemia compared with the general populations in the countries studied. No individual exposure data were presented, and the author noted several difficulties with data collection and organization (Thorpe, 1974).

In their text on industrial toxicology, Hamilton and Hardy reflected that:

> "While there has been no doubt for many years that benzene can produce fatal aplastic anemia, the association between benzene exposure and leukemia has been a matter of more recent controversy.… Chronic myelogenous leukemia appears to be the most common type associated with benzene exposures, but acute myelogenous and acute and chronic lymphocytic varieties have been reported as well" (Hamilton and Hardy, 1974).

Vigliani and Forni provided an update on benzene health effects, stating that over the last 40 years, more than 100 cases of leukemia attributable to benzene had been described. However, the authors' use of the term "leukemia" was too general to be informative, as their definition included more than 20 distinctly different chronic effects on the blood-forming organs. As a result, it was difficult to specifically identify a causal link between benzene and any particular disease. He and his coauthor felt that "The most convincing cases of benzene leukemia are those occurring in factories where there were outbreaks of chronic benzene poisoning." He summarized his experience with rotogravure workers and shoe manufacturing where "All of the men had worked in factories where the concentration of benzene in the air of the working places was well above the threshold limit value." [At that time, the threshold limit value (TLV) was 25 ppm] (Vigliani and Forni, 1976). After the rotogravure industry stopped using benzene in favor of toluene as its main solvent, the authors noted: "…we have seen no new cases of aplastic anemia, nor of leukemia due to toluene exposure." Measured airborne concentrations of benzene in rotogravure plants were reported to be between 200 and 400 ppm, with peak concentrations up to 1500 ppm. Shoe factories where workers developed leukemia were found to have workplace concentrations between 25 and 600 ppm, with most measurements being between 200 and 500 ppm, with some solvents containing 100% benzene (Vigliani and Forni, 1976).

Epidemiologic studies on rubber workers in Ohio noted slightly reduced mortality from all causes, but detected significantly elevated numbers of cancers of the stomach, prostate, and blood-forming tissues, including leukemia (McMichael et al., 1974). The authors concluded that "…until it is demonstrated that specific exposures or jobs within the industry are associated with excess deaths, one can only suspect, rather than conclude with confidence, that working in certain jobs within the rubber industry entails an increased risk of dying from specific causes."

An expanded follow-up study involving multiple plant locations discovered an increased rate of "lymphatic leukemia" in workers exposed to organic solvents, one of which was benzene. Acknowledging that this finding was unusual, the authors stated that: "The sparse literature in this area indicates that, at least for cases of gross benzene poisoning, the leukemia tends to be of either the hemocytoblastic (or stem cell) kind or the myeloblastic kind. The association of lymphatic leukemia with exposure to organic chemicals appears to have no reported precedent" (McMichael et al., 1975). Six of the eight "lymphatic leukemia" deaths were of the chronic subtype. A third study of the rubber workers showed a slightly increased incidence of "all leukemias" with a standardized mortality ratio (SMR) of 130 (confidence limits not provided). However, when the cases were divided into "myeloid" and "lymphatic" categories, the authors found that mortality from lymphatic leukemia was strongly associated with working in a synthetic rubber plant, whereas myeloid forms did not demonstrate a significant association (McMichael et al., 1976). In this study, data on specific subtypes of leukemia were not reported. With regard to the McMichael et al. studies, however, we should note that the tire building process has historically involved a wide variety of chemical exposures at the various stages of production, which the authors recognized (McMichael et al., 1975).

Interestingly, a subsequent National Institute for Occupational Safety and Health (NIOSH) investigation of the synthetic rubber plant studied by McMichael et al. where they reported this very strong association with "lymphatic leukemia" was "unable to identify benzene-related exposures" (NIOSH, 1979). NIOSH also commented that animal studies still had not provided "…reliable information on [benzene's] capacity to produce an increased incidence of leukemias." They thought it "…quite conceivable that leukemias described in the earlier experiments in mice after benzene treatment have, in large part, developed spontaneously or by virus infection, rather than being caused by benzene" (NIOSH, 1979). They did conclude, though, that the accumulated clinical and epidemiologic evidence showed that benzene was a carcinogen and leukemogen and recommended that it be replaced with less harmful substitutes wherever possible.

The initial report on the incidence of leukemia in rubber workers in Ohio (the "Pliofilm" cohort) was published in 1977. This retrospective cohort study was unique compared to previously published cohort reports in that there was felt to be very minimal confounding exposures to other types of chemicals or dangerous solvents; that is, benzene was essentially the only chemical used at the facility (Infante et al., 1977; Paustenbach et al., 1992). Departments and jobs where direct exposure to benzene occurred were identified were based on a NIOSH-administered worker survey. NIOSH also provided detail regarding the Pliofilm manufacturing process, engineering controls, and most importantly, detailed personal and area air sampling data describing actual benzene exposures over time. After 75% of the worker population had undergone evaluation, the authors reported a 5-fold increased risk of all leukemias, and a 10-fold increased risk for myeloid and monocytic leukemias. No lymphocytic leukemias were noted (Infante et al., 1977).

In a comprehensive review of benzene health effects commissioned by the American Petroleum Institute, Goldstein concluded: "…there is reasonably good evidence that inhalation of benzene is associated with an increased incidence of acute myelogenous leukemia, and possibly other hematological neoplasms" (Goldstein, 1977).

In 1976, largely due to the Infante et al. report, NIOSH recommended that the benzene PEL be lowered to 1 ppm over a 2-hour sampling period in 1976, based on the overall conclusion that benzene was leukemogenic. The Department of Labor intended to reduce the standard, but was challenged in court and in July of 1980, the Supreme Court withdrew the 1 ppm standard, stating that further evidence of adverse health effects below 10 ppm was needed (Thomas, 1982). This decision had long-term significance, as the courts ruled that unless adverse effects were observed at the current standard, then it did not make sense to reduce the limits on exposure. Such an action would require substantial expenditures for compliance and enforcement, and society should expect to see a demonstrable improvement in worker health (Graham et al., 1991).

Aksoy reported in a follow-up study in 1978 that 6 out of 44 patients with pancytopenia resulting from chronic heavy benzene exposure developed leukemias after exposure periods ranging from 6 months to 6 years. Concentrations of benzene in the workplace were never measured to be less than 150 ppm, and patients were described as working long hours with little to no environmental controls (Aksoy and Erdem, 1978). In a letter to the *Lancet* that same year, Aksoy remarked that for "moderate or low levels" of benzene exposure, which he defined as 10–25 ppm, "…haematological abnormalities characteristic of chronic exposure to benzene were not usually seen" (Aksoy, 1978).

Fishbeck, reporting on the health effects of ten Dow Chemical employees historically exposed to benzene levels exceeding 25 ppm over many years, reported that all ten employees had an increase in their mean red blood cell volume (MCV) in testing performed in 1963. Some had minor reductions in hemoglobin levels, and no other significant bone marrow or peripheral blood effects were noted. It was concluded that "…the exposure experience has not caused a lasting deleterious effect on their hematopoietic systems or their overall health" (Fishbeck et al., 1978).

1980s

In 1980, Aksoy reported that he had observed several other diseases in the Istanbul workers that he thought might be associated with exposure to benzene. These included malignant lymphoma, (both Hodgkin's and non-Hodgkin's lymphoma), lung cancer, myeloid metaplasia, paroxysmal nocturnal hemoglobinuria (often associated with aplastic anemia), and multiple myeloma. The evidence provided by Aksoy came in the form of citing individual cases, both in Turkey and from other published case reports (Aksoy, 1980a).

A second article by Aksoy that year described a significant decline in the incidence of leukemia following the "phaseout" of benzene usage starting in 1969. (Aksoy, 1980b) He also emphasized that acute myeloid forms of leukemias were by far the most common type observed in benzene-exposed workers, "…followed by acute erythroleukemia and preleukemia." The rare occurrence of chronic forms of leukemia was described as "striking." In discussing other malignancies, he noted that all of the cases of lung cancer "…were heavy or moderate smokers," and that in two cases the form of lung cancer was oat cell carcinoma, a form of lung cancer heavily associated with cigarette smoking. He concluded, for the nonleukemic diseases he had noted in benzene-exposed people, that "Though these case reports [do] not prove a causal relationship between benzene exposure and the various malignancies, the frequent finding of this association suggests that benzene may not only cause leukemia but also be involved in other types of malignancy" (Aksoy, 1980b). The issues, however, of concurrent chemical exposures and alternative risk factors are important to note, and are clearly evident in the example of lung carcinoma put forth by Aksoy.

Nonmalignant effects of chronic benzene exposures were explored by petroleum industry researchers, who observed that occupational exposures (40 hours/week) above 50 ppm for extended periods could cause diminished numbers

of platelets, red cells, or white blood cells. They noted that "the clearest relationship between benzene exposure and leukemia was demonstrated in studies of benzene exposure prior to 1960 when high benzene concentrations (>200 ppm) in the air were likely," and stated that "a standard of 10 ppm time-weighted average (TWA) benzene in air is an acceptable value for protection from any hematological effect" (Brief et al., 1980). They went on to summarize the exposure experience in petroleum refineries, where they reported "…a low probability (<5%) of benzene levels exceeding 1 ppm TWA and a negligible chance of exceeding 5 ppm." Even so, they recommended that benzene should be replaced with less toxic solvents whenever possible, and that personal sampling of individual workers was the best method of verifying exposures. It was their belief that no test at that time was sufficiently reliable to track airborne concentrations to less than about 0.5 ppm of benzene; especially in environments with mixtures (Brief et al., 1980).

Najean, in a long-term follow-up study of 429 patients diagnosed with aplastic anemia, noted that "A possible or probable toxic etiology does not discriminate between favorable and unfavorable evolution" of aplastic anemia (Najean, 1981). In this series, 31% of the initial group were considered to have disease secondary to toxic drugs or industrial exposures; 27% of the surviving 137 patients had suspected disease secondary to toxic agents. Less than 1% of patients went on to develop leukemia as a complication of their disease (Najean, 1981).

Rinsky et al. provided an update on their Pliofilm cohort in 1981, after 98% of the vital data from the worker population had been discovered. Industrial hygiene data and a description of the workplace environment were provided, as well as job descriptions for the various classes of laborers. No additional cases of leukemia fulfilling their study criteria were reported; a total of seven cases were described in the worker population, all myelogenous or monocytic in cell type. The authors concluded that these findings confirmed their previous conclusion of increased leukemia risk in the cohort (Rinsky et al., 1981).

A review of petrochemical workers exposed to benzene concentrations from less than 0.1 to 25 ppm as an 8-hour TWA found no differences in red blood cell (RBC) count, white blood cell count, hemoglobin, or platelet count (Tsai et al., 1983).

Another review by Aksoy stated that "There is no doubt about the leukemogenic effect of benzene in man," citing data from his previous studies. He noted an additional possible risk factor of cholera vaccinations, which he felt could be acting as a promoter for leukemia in those persons exposed to benzene. Familial risk factors and differential human susceptibility were also discussed. The same nonleukemic disease states were again suspected of being linked to benzene exposures, though little new supporting data were provided (Aksoy, 1985).

A 1987 update of the Pliofilm study examined all workers with at least 1 ppm-day of exposure up to the end of calendar year 1965. Workers were stratified into four categories of cumulative exposure: up to 40, 40 to 200, 200 to 400, and greater than 400 ppm-years. The authors reported the new findings of an apparent excess of MM cases, but reported that the calculated mortality ratios for MM did not increase with increasing benzene exposures. The authors noted several gaps in the available data sets for various jobs, stating that these were filled by interpolation of existing data. Based on an observed positive trend of increased leukemia risk with benzene exposure, the authors recommended a lowering of the occupational exposure limits, which were then 10 ppm (as an 8-hour time-weighted average) (Rinsky et al., 1987).

In 1988, a review of the evidence linking benzene to leukemia was performed, concluding that the available epidemiologic data supported the notion that chronic heavy exposures to benzene were associated with an increased risk of developing acute myelogenous leukemia, even though the evidence came from a single "relatively small study by Rinsky" (Austin et al., 1988). The rest of the accumulated epidemiologic data were believed by the authors to be very weak in demonstrating a possible causative relationship between benzene and leukemia, even at historically very high levels of exposure. "However, the available data are too sparse, or suffer other limitations, to substantiate the idea that this causal association applies at low levels (i.e., 1–10 ppm) of benzene." A critique of previously published risk assessments was provided, along with the authors' own risk assessment for benzene (Austin et al., 1988).

Goldstein provided a lengthy review of benzene toxicity in 1988. In describing benzene's effect on the bone marrow, he opined: "As anticipated for an effect of this nature, there is an apparent threshold, i.e., at some benzene dose no overt decrease in blood count is observed." Furthermore, "A decrease in peripheral blood counts has not been observed in any species at exposure doses below the current U.S. Occupational Health Standard of 10 ppm TWA" (Goldstein, 1988). In discussing the Italian and Turkish experience with benzene, he noted that measurements of ambient benzene levels exceeded 200 ppm, and that replacement of the glues in the shoe and leather workers with solvents not containing benzene "…put an end to this outbreak of overt hematological consequences due to benzene exposure" (Goldstein, 1988). He also explored the nonleukemic associations described by Aksoy, relating that "The causal relationships have not yet been established," and "…in no case does the evidence appear to be sufficiently clear-cut to overwhelmingly demonstrate a causal relationship between benzene and one or more human lymphatic tumors" (Goldstein, 1988).

Goldstein discussed a 1985 study of benzene exposure in the United States petroleum industry by Runion and Scott (Runion and Scott, 1985), where "…87% of exposures were below 1 ppm and 98% below 10 ppm TWA." In describing the difficulties in the surveillance of a population exposed to benzene, Goldstein stated that each component of a complete blood count had a wide range of normal values, and that interpreting findings just barely different from "normal" was unclear. In addition, routine surveillance of even a small work force was "likely to find at least one blood count below

the statistically normal range." Also, changes over time could also be difficult to address: "A major cause of such a change over time can simply be laboratory variability, particularly as a small change of such a nature is not of clinical pertinence and thus unlikely to be of concern to a routine clinical laboratory" (Goldstein, 1988).

In a separate analysis, Goldstein worked with Kipen and Cody to examine the blood studies from the Pliofilm cohort from 1940 to 1975 and determined that "…benzene exposure for the cohort during the 1940s was significantly higher than in subsequent years." However, although they observed significant effects on the peripheral blood findings at these high levels of exposure in the 1940s, Goldstein et al. also found "…that surveillance of blood counts to monitor populations of workers exposed to benzene may not readily detect cohort effects of exposures in the range of the current standard of 1 ppm, as during the 1950s and beyond these workers had exposures likely exceeding these values without apparent aggregate depressions in their blood counts" (Kipen et al., 1989). Kipen et al. concluded that: "these data suggest substantial limitations of hematologic examination of populations to detect abnormalities in populations currently exposed to benzene" (Kipen et al., 1989).

1990s

In a Monsanto study of the peripheral blood effects from chronic low level exposures to benzene, 200 workers with exposures to benzene in air ranging from 0.01 to 1.40 ppm as an 8-hour TWA were evaluated. The "no effect" level for producing blood effects in humans at the time of publication was judged to be 25 ppm. Using a multiple regression approach to control for the effects of smoking and other possible confounding variables, the authors found no effects for benzene exposure on any of the main peripheral blood parameters (red and white cell counts, hemoglobin, platelet count, and MCV) (Collins, 1991).

Rushton performed a mortality analysis of refinery and distribution center workers employed for at least one year from 1950 to 1975, an update of two prior reports on the same population (Rushton, 1993). Consistent with earlier findings, overall mortality was significantly less in the worker population, felt to be related to a healthy worker effect. Total observed deaths from leukemia in both worker cohorts were similar to expected numbers. However, workers with medium to high exposures were found to have twice the risk of developing leukemia compared to workers with low exposures. Earlier findings suggesting an increased risk of myelofibrosis were not observed in the follow-up study.

Paxton and colleagues performed a comprehensive review of additional data released and published by NIOSH involving the Pliofilm cohort (Paxton, 1994). Several observations were made:

- The absence of additional cases of multiple myeloma in the 1987 update "weakened to nonsignificance" the prior published association with benzene exposure.
- No increased incidence of solid tumors was observed, going against animal studies that suggested this might be an additional toxic endpoint in humans.
- The assembled exposure and disease data were felt to be "…consistent with a threshold model for leukemogenesis by benzene" (Paxton et al., 1994).
- Leukemia deaths in the entire cohort occurred exclusively in workers who started employment prior to 1950, suggesting that later worker exposures may have differed substantially from earlier years.
- A more detailed review of exposure data concluded that estimates of leukemia risk reported by Rinsky et al. overestimated actual risk by an order of magnitude, and that exposure estimates derived by Paustenbach et al. (1992) agreed with those of Crump and Allen (1984) attempting to identify the minimum lifetime dose that increased the leukemia risk.

Over the next few years, there continued to be analyses of the likely exposure of the Pliofilm cohort and, ultimately, the US Environmental Protection Agency (EPA) and the American Conference of Industrial Hygienists (ACGIH) TLV committee fundamentally gave equal consideration to the various exposure estimates and derived a "weight of evidence" approach to identify acceptable levels of exposure.

A study involving 19 separate cohorts totaling 208,000 petroleum workers in the USA and UK was analyzed for the incidence of individual leukemia subtypes. The authors did not find an increased risk for any leukemia subtype, including AML. They hypothesized that this was due to benzene exposure in the petroleum industry being "…substantially lower than needed to reach the observed threshold." This threshold, based on data from the Pliofilm cohort, was believed to be at least 200 ppm-years by the authors, and possibly as high as 400–500 ppm-years (Wong and Raabe, 1995).

In 1996, Ward et al. reexamined the blood screening data from the Pliofilm cohort in the 1940–1975 time period, using Rinsky's estimates of exposure from 1987. The authors acknowledged the difficulty of their task, since little exposure data were available in the 1940–1946 time period, and neither laboratory documentation describing any methods used to analyze blood samples nor changes in the laboratory routines and instrumentation over time were found. The authors determined that "in a group of workers with substantial benzene exposure," that there was evidence for an exposure-response relationship for benzene and effects on white blood cell count (WBC), with a lesser effect on red blood cells. [The authors estimated that maximum daily benzene doses in the worker population were 34 ppm; however, they did not consider either the exposure estimates of this cohort conducted by Crump and Allen (1984) or Paustenbach et al. (1992).] No other changes in blood testing results were thought to be of significance [Smoking and recent infection do not appear to have been considered] (Ward et al., 1996).

Yin et al. provided an expanded update for a large Chinese worker population initially studied in the late

1980s, reporting on about 75,000 benzene-exposed workers and a control population of around 36,000 workers with no benzene exposure. The authors found an increased risk of myelogenous leukemia in the worker population, particularly AML, but also CML to a lesser degree (AML risk provided the only statistically significant leukemia subtype relationship). They also reported elevated increased risks for developing acute lymphocytic leukemia (ALL) and non-Hodgkin's lymphoma, but did not find an association with Hodgkin's disease, multiple myeloma or chronic lymphocytic leukemia (CLL) (Yin et al., 1996).

Hayes et al. extended the analysis of the Yin et al. cohort to include estimates of worker exposures, concluding that workers with relatively low-level chronic exposures to benzene (average workplace exposures less than 10 ppm and cumulative exposures less than 40 ppm-years) were at significant risk for developing hematologic malignancy. With increasing levels of exposure, the authors detected a marginal tendency for increased risk for nonlymphocytic leukemia (ANLL) and for the combined category of ANLL and myelodysplastic syndrome (MDS) (Hayes et al., 1997).

Because their reports have had a measurable impact on the perceived health effects of human exposures to benzene at fairly low doses, it is worthwhile to note a few aspects of the Yin and Hayes et al. studies (conducted in cooperation with the National Cancer Institute).

1. There were no corrections or considerations for multiple or alternative exposures in the worker population. According to Wong, 95% of the exposed Chinese workers were also exposed to other chemicals, and only 5% described themselves as being exposed only to benzene (Wong, 1999).
2. The exposed population was considerably older, on average, than the unexposed population with respect to age at first employment. Twenty-five percent of the benzene-exposed group was older than 30 at first employment, compared with only 11% of the unexposed group (Yin et al., 1996; Hayes et al., 1997).
3. There were significantly more women in the exposed compared with the unexposed group. If, as some have opined, women were more susceptible to the effects of benzene (Brown et al., 1998; Duarte-Davidson et al., 2001), this sex discrepancy could magnify the effects of benzene in the two test populations.
4. Wong observed that prior to the National Cancer Institute (NCI) involvement in the Chinese worker studies, the investigators did not attempt to measure exposure in the cohort as a whole, but only reported benzene measurements in the leukemia cases (Wong, 1999). Overall, Wong felt that worker exposures were seriously underestimated by NCI and the Chinese Academy of Preventive Medicine, and that these estimates had a profound impact on the dose-response data reported in the Yin, Hayes, and later publications involving this cohort (Hayes et al., 2000).
5. No disease category or combination of disease categories reported by Hayes et al. demonstrated a consistent dose-response trend for cumulative benzene exposure and increasing risk of disease (Hayes et al., 1997).
6. The implicit assumption made by Hayes that MDS represents an early stage of AML is debatable. Combining these two diseases to achieve more statistical significance may not be valid, particularly if MDS patients in the cohort did not uniformly go on to develop leukemic transformation (Hayes et al., 1997).
7. Both study populations in Yin et al. (1996) were noted to have much lower all-cause mortality numbers when compared to the general population of the region. The reasons for significant mortality deficits in the study populations were unclear, but the authors suggested that this could have been related to diagnostic criteria for malignancy in the regions being different from their study criteria. Data for local mortality were restricted to the 1973–1975 time period, and rates were not available for all 12 cities being represented in the study (Yin et al., 1996; Hayes et al., 1997).

Because of the concerns raised in the Hayes study, a series of additional studies were initiated in China to help deal with the shortcomings in their original work. Some of the results were presented at the International Conference on Benzene held in 2009 (Bird et al., 2010).

In 1996, Rothman et al. evaluated a subset of patients from the Yin and Hayes studies. He looked at 44 workers with relatively high exposures to benzene (average of 31 ppm as an 8-hour TWA) and then compared them to 44 age- and gender-matched controls, evaluating the two groups in terms of a variety of hematologic measurements (Rothman et al., 1996). He found that these workers had reduced total white blood cells, platelets, red blood cells, and hematocrit values compared to control subjects. For workers who were never exposed above 31 ppm on 5 days of sampling (with a median 8-hour TWA exposure of 7.6 ppm), only the absolute lymphocyte count (ALC) was statistically significantly different compared to controls.

Rothman et al. concluded that the ALC was the most sensitive indicator of benzene-induced toxicity to the bone marrow in the population studied, particularly at the lower levels of exposure (less than 31 ppm). It was noted that: "Individual benzene air levels in these factories were much higher than we had expected to find, based on historical monitoring data." As a result of improved occupational standards and workplace practices over the prior few decades, the authors opined that: "exposure patterns in the three factories evaluated in this study are not representative of general exposure patterns in China today" (Rothman et al., 1996).

A cohort study of 19,000 service station workers in Nordic countries exposed to low levels of airborne benzene did not find an increased risk for leukemia, nor for acute myelogenous leukemia specifically. Average exposures were estimated to be less than 1 mg/m3 (approximately 0.3 ppm) (Lynge, 1997).

In 1999, Khuder conducted a study of 105 petroleum workers exposed to low levels of benzene over an 18-year

period. He suggested that "CBC values" could serve as a useful tool to monitor workers. In 3855 total complete blood count (CBC) records, the means, medians, and the majority of the CBC results were within the normal ranges provided by the laboratories. None of the individual CBC parameters studied had a mean for any year of the period studied that was outside of the normal reference range, and none of the individual CBC parameters was correlated with the degree of benzene exposure. The average TWA exposure for the workers studied was 0.81 ppm. It was concluded that "whether chronic, low-level benzene exposure can affect the RBC count, hemoglobin, MCV and platelet count values cannot be stated conclusively in the study presented here. However, our findings are consistent with the existence of a threshold for hematological insult.… It is possible that higher levels of exposure are needed in order to show any effect on the WBC or RBC counts" (Khuder et al., 1999).

Goldstein, in a letter to the editor to the publishing journal, *Journal of Occupational and Environmental Medicine*, took issue with the Khuder et al.'s conclusion of the value of CBC results in monitoring low-level exposures to benzene (Goldstein and Cody, 2000). In particular, he pointed out that "…the finding of small but statistically significant effects within the normal range over a 17 year period may simply be due to aging of the cohort. It is generally accepted that in men, red cell parameters decline with age beginning at approximately age 30." The study also had the curious finding of a decrease in the MCV, contrary to what has been normally noted in animals and humans subjected to benzene exposure. The authors did not employ a control group for comparison, and Goldstein and Cody noted that none of the individual CBC components showed any relationship to benzene exposure, making the value of the testing, particularly for low-level exposures, seem questionable.

A small nested case-control study on Canadian petroleum distribution workers who experienced long-term, low-level exposures to benzene was reported in a 1996 paper by researchers at Exxon (Schnatter et al., 1996). The authors stated that in workers with daily exposures (8-hour TWA) to benzene ranging from 0.01 to 6.2 ppm that there was no observed increased risk for developing leukemia with increasing cumulative exposures. Reflecting on their study and three previous epidemiologic studies with lifetime exposures under 100 ppm-years (Bond et al., 1986; Paxton et al., 1994; Wong, 1987) the risks for all types of leukemia were "…approximately evenly distributed around 1.0" (Schnatter et al., 1996).

A similar study of petroleum distribution workers by Rushton and Romaniuk concluded that there was no association between benzene exposure and lymphocytic leukemias, acute or chronic. There was the suggestion of an association with myeloid forms of leukemia, but the findings did not display a consistent dose-response, with zero cases reported in the highest category of exposure (≥45 ppm-years). The authors observed that over 80% of the workers had less than 5 ppm-years of benzene exposure, making estimates for risks above 10 ppm-years highly imprecise (Rushton and Romaniuk, 1997).

2000 to the present

Scientific advances in toxicogenomics and molecular biology have enabled an increasingly detailed perspective regarding proposed mechanisms of benzene-related disease, "precursor" stages to malignancy, and suggested factors (genetic, environmental, dietary, etc.) that could result in an increased predisposition for malignant hematopoietic disease. Although scientific disciplines have made use of an array of greatly improved tools to study the health effects of benzene over the last 10 years, we unfortunately are still searching for a clear understanding of the mechanisms underlying leukemic transformation related to benzene exposure (Mondrala and Eastmond, 2010; Laskin et al., 2000; Kalf, 2000; Irons, 2000; Larson, 2000; French and Saulnier, 2000). The specific lifetime cumulative doses of benzene over time leading to a heightened risk for disease also remain uncertain, although studies seem to have narrowed the likely threshold dose to somewhere between 40 and 400 ppm-years; a surprisingly wide range in light of nearly 100 years of study.

Reflecting this uncertainty, Ross noted in a comprehensive toxicologic review of benzene toxicity that "there is no conclusive evidence for a single metabolite or combination of metabolites as being responsible for benzene toxicity." Benzene was accepted as being an inducer of aplastic anemia, myelodysplasia, and acute myeloid leukemia following chronic exposures, but other diseases of the bone marrow were not identified as having an association with benzene (Ross, 2000; Gross et al., 2010; Irons et al., 2010; Ross and Zhou, 2010).

The issue of disease latency for benzene exposure and its association with future malignant disease has been a topic of discussion for a number of years. For example, Finkelstein, in a review of original data from the Pliofilm cohort, found that the greatest risk for developing leukemia was related to benzene exposures sustained in the 10 years prior to diagnosis, with exposures more than 15 years from diagnosis not being significantly different from matched unexposed controls (Finkelstein, 2000). This observation was later reinforced in the Rinsky update of this cohort reported in 2002 (Rinsky et al., 2002).

Glass also commented on the question of latency, concluding from her review of the *Health Watch* data that "benzene-induced leukemia can be restricted to the period up to 15 years prior to diagnosis" (Glass et al., 2004). More recently, Richardson performed another review of mortality data from the Pliofilm study, determining that the association between benzene exposure and leukemia mortality was strongest in the 10 years immediately following exposure, was weaker in the 10–20-year period, and that there was "…no evidence of association 20 or more years after exposure" (Richardson, 2008).

In the last 10 years, several refinery studies have been published, addressing the question of increased mortality from leukemia and other hematologic malignancies. In 2001, a comprehensive cohort mortality study of over 3000 employees from a petroleum refinery over the period from 1959 to 1997 did not find any increased mortality from leukemia or

any of its subtypes in all workers, or in the subgroups of maintenance workers or process workers. Duration of employment was used as a surrogate for worker exposures, as insufficient exposure data were available to construct quantitative exposure indices (Wong et al., 2001).

In 2003, a cohort mortality study of over 25,000 Canadian petroleum workers hired between 1964 and 1994 did not find any evidence of increased mortality from leukemia, lymphoma, or other lymphohematopoietic malignancies. Again, length of employment was used to estimate lifetime exposures, and "similar exposure group" (SEG) codes were used to categorize workers by location, department, job description, and dates of employment. The SEG codes contained estimates of likely exposure constructed by an industrial hygienist using available pertinent data (Lewis et al., 2003).

The Australian Health Watch study of petroleum industry employees has provided two reports in this decade. In 2001, it was reported that there was an increased incidence of multiple myeloma, as well as a statistically significant increase in the incidence of "all leukemias combined." If these results had been confirmed, because they were associated with fairly low doses, they would have been important. The authors noted, however, that "…the combining of all leukaemias into a single 'leukemia' entity is somewhat arbitrary, since their differing occurrence and natural histories suggest that they are probably different diseases" (Gun et al., 2000). Leukemia mortality in the cohort was slightly but not significantly elevated, with an overall leukemia mortality ratio of 1.16 (confidence interval [CI] = 0.66–1.18). Numbers of the individual leukemia subtypes were not provided (Gun et al., 2000). Total hydrocarbon exposure was estimated from employee job codes, ranked by a committee of industrial hygienists into seven categories of exposure; uncoded jobs were assigned an intermediate "default" category of exposure.

In the latest 2005 *Health Watch* update, the previously reported leukemia excess in the cohort disappeared (Gun et al., 2005). Neither total leukemia incidence, nor total leukemia mortality were appreciably elevated (1.07 standardized incidence ratio [SIR] and 0.99 SMR, respectively). Interestingly, chronic forms of leukemia were observed more frequently than acute forms, at variance with previous studies that have evaluated chronic exposures to benzene. This finding is not unexpected given the lack of statistically significant differences observed in the first study. Furthermore, neither duration of employment nor magnitude of benzene exposure appeared to be related to leukemia incidence. The authors acknowledged that "…only acute ANLL is likely to be causally related to benzene exposure," while noting that there was no excess of ANLL in the cohort compared to the general population. Low-dose exposures leading to disease were also not seen, as there were no cases of ANLL seen in the three lowest occupational exposure categories described by the authors (Gun et al., 2005).

After the publication of the *Health Watch* 2001 update, Glass et al. (2003) published the results of a nested case-control study, finding that leukemia risk was increased at cumulative lifetime benzene doses of just 2 ppm-years or more. They stated that their data did not provide any evidence of an exposure threshold for developing hematopoietic malignancy, and did not find any association with NHL or MM (Glass et al., 2003). In a letter to the editor, Schnatter suggested that there was an unusually low rate of leukemia in the control population, and that the relative risk numbers reported by the authors were uncharacteristically high when compared with similar case-control studies involving benzene, even "higher than those found in more highly exposed cohorts used in risk assessments" (Schnatter, 2004).

Goldstein also pointed out that a substantial number of the leukemia cases reported by Glass were of the chronic lymphatic leukemia subtype, "a disease that has not been shown conclusively to be caused by benzene" (Goldstein, 2004). Specifically, Glass reported 17 chronic leukemias, and 11 cases of AML (Glass et al., 2003). Goldstein suggested that surveillance bias, or testing the case population of exposed workers more than the unexposed control population, could have led to preferential case discovery, and could explain the "…unusually low levels of benzene exposure associated with leukemia" (Goldstein, 2004).

Despite the lack of association for leukemia with benzene exposure in the 2005 *Health Watch Report*, Glass et al. (2006) found that low-dose benzene exposure was related to the incidence of other forms of leukemia. In a reanalysis of their 2003 study, they acknowledged the problems with their original reference group and combined their two lowest exposure groups to serve as a comparison for their "heavy" exposure group. This new comparison changed their odds ratio from 98 in the original study to 51.9, which then dropped to 7.8 when they accounted for exposures related to "high exposure events," or industrial accidents/spills that were judged to have been relevant for individual job types (Glass et al., 2006). Leukemia subtype incidence was also calculated for various exposure subgroups, combining the three lowest exposure groups and the two highest exposure groups into "low" and "high" categories due to small numbers of cases. Glass et al. reported a single statistically significant finding; an increased risk of ANLL in workers with exposures >8 ppm-years. They noted difficulty in comparing their findings to other epidemiologic studies involving benzene exposure, which had primarily been based on mortality, not incidence (Glass et al., 2006). Interestingly, the latest *Health Watch Report* (the parent population for the Glass et al. nested case-control study) found no cases of AML diagnosed in the lowest three (of seven) job exposure categories, ordered by total hydrocarbon exposures. Leukemia incidence was also not statistically different from the general population with an SIR of 1.06 (CI 0.53–1.90) (Gun et al., 2005).

A small study of shoe factory workers in Italy found increasing leukemia mortality risk with increasing cumulative benzene exposure (Costantini et al., 2003). Their results, however, are difficult to assess, as exposures to benzene were noted by the authors to be reduced to zero after 1965 and more than half of the deaths from "hematolymphopoietic cancer" occurred after 1985, more than 20 years after there was no further benzene exposures at the plant. Leukemia subtype

analysis showed that there was only one case of "acute leukemia" and one case of "acute myelogenous leukemia" in these post-1985 cases, and eight cases of non-ANLL subtypes (including three cases of multiple myeloma and two cases of NHL). Thus, the frequency of diseases traditionally associated with heavy chronic exposures to benzene was drastically reduced after cessation of benzene exposure, and the bundling by the authors of diseases into a combined "blood malignancy" category is of unclear value in understanding the contribution of benzene to mortality in these workers.

In 2002, Rinsky et al. provided another update on the disease incidence in the Pliofilm cohort (Rinsky et al., 2002). Five additional benzene-exposed workers were found to have died from leukemia, but a careful examination of the cohort revealed that the relative risk of leukemia from chronic benzene exposures clearly diminished over time. Whereas earlier studies of these workers suggested an increased risk for MM, the 2002 update largely reversed these findings. There were four additional cases of multiple myeloma described: three of which were judged to be in workers without exposure to benzene; the last was exposed for only a month, with a cumulative benzene exposure of just 0.10 ppm-years. Latency issues were also problematic in this update, as 6 of the 15 deaths from leukemia occurred more than 30 years after the worker's first exposure to benzene, a result that is generally considered to be well outside the expected latency period for benzene and leukemia. Thus, for 40% of the Pliofilm leukemia cases, benzene exposures at the plant were unlikely to have played any significant role in their disease, particularly for workers with excessive disease latency who were employed less than a year at the facility.

An updated mortality study of petrochemical workers at two California refineries found that there were no significant increases in mortality from leukemia overall, or from any individual subtype. Multiple myeloma, however, was significantly increased, but only in workers who started employment before 1949 (Satin et al., 2002).

In 2002, occupational exposures to benzene in gas and electric utility workers in France were evaluated, under the assumption that exposures to petroleum solvents would entail exposure to benzene as a component of those solvents (Guenel, 2002). They found that the risk of leukemia was increased "…only among workers with first exposure to benzene before 1960," but even this subgroup did not achieve statistical significance. There were very few cases in this study, and the only association that did achieve statistical significance was the finding of an increased rate of "all acute leukemias," combining myeloid and lymphocytic subtypes, at the highest level of exposure in the cohort. The authors stated that "no association with a particular leukemia cell type was apparent." Contrary to the findings of most other epidemiologic studies involving benzene, they found that leukemia seemed to be more associated with benzene exposures "…after allowing for a 10–20 years latency period, i.e., with distant past exposures."

Peripheral blood hematologic effects from benzene exposures in Chinese workers were studied, finding that depressions in red and white blood cell counts seemed to correlate with levels of benzene exposure (Qu et al., 2002). However, contrary to the findings of Rothman et al. (1996), the authors reported that neutrophil counts were more sensitive to benzene effects than lymphocyte counts, with little effect from exposure duration. Mean neutrophil counts also displayed an opposite trend with benzene exposure intensity, increasing in number from the lowest exposure group to the next two groupings of cumulative exposure, then decreasing in the highest mean exposure group. This study used a relatively small control group (51 unexposed subjects and 130 exposed workers), and exposures to other chemicals, as well as prior smoking and occupational histories were not well described.

A study of hematologic parameters in petrochemical workers with benzene exposure was conducted, looking at six separate complete blood count measurements. The authors found that there was no increased incidence of any blood component abnormality among exposed employees. Mean exposures were reported to be 0.60 ppm in the 1977–1988 time frame, and 0.14 ppm in the 1988–2002 time frame. The study was limited by not having individual exposure data, nor did it evaluate possible confounding variables, such as personal lifestyle or other chemical exposures (Tsai et al., 2004).

In another study of 250 Chinese workers published in the same year, it was found that exposures to less than 1 ppm of benzene in air could give rise to reductions in several white blood cell parameters and platelets (Lan et al., 2004). However, there were several potential problems with this study:

- There were substantially more smokers, both numerically and proportionately, in the control group compared with the study groups. This difference was most notable in the lowest benzene exposure group (28% smokers in control group, 18% smokers in exposure group).
- There were no data provided on past smoking behavior in the study participants. The effects of cigarette smoking can linger for years, and the degree of former usage in subjects who may have recently quit could be important.

> "…a significant residual smoking effect on white blood cell count after quitting smoking was observed in our follow-up of 6.5 years" (Sunyer et al., 1996).
>
> "It is possible that the association of smoking with the leukocyte count persists because ex-smokers have bronchial inflammation that declines gradually with time since quitting" (Petitti and Kipp, 1986).

- Control subjects were noted to have the highest incidence of "recent infection" of any of the study groups. Consistent with this, the standard deviation of the total white blood count in control subjects was reported to be substantially greater than that reported for the low and high exposure groups. These observations could indicate that a few control subjects were ill, leading to elevations

in their WBC counts and a larger *SD* for the control group, which might have impacted the study findings.

- Pregnancy status and use of oral contraceptives, in a study population that was predominantly female (about 2/3 of subjects) were not controlled for, which may have led to systematic bias from failing to consider these potentially confounding variables (Fisch and Freedman, 1975; Dodsworth et al., 1981).

Of note, comparison of the low and intermediate benzene exposure groups revealed that a substantial proportion of the hematologic parameters studied (total WBC, granulocytes, CD8 T cells, natural killer (NK) T cells, monocyte counts, and lymphocyte counts) increased or stayed the same with a 5-fold mean increase in benzene exposure, results that countered the overall trend described by the authors.

In 2004, a cohort mortality study of 2266 chemical workers exposed to benzene since 1935, a mild increase in total leukemia was noted (SMR = 1.14) as well as a slight increase in ANLL (SMR = 1.11). The magnitude of risk appeared to increase with higher cumulative exposure to benzene, but did not seem to be related to intensity of exposure. Exposures below approximately 30 ppm-years were not seen to result in an increased risk for ANLL or total leukemia (Bloemen et al., 2004).

A detailed review of case-control and cohort studies of benzene-exposed workers was performed by Schnatter et al. (2005), characterizing these according to industry focus, presence and adequacy of exposure assessment, presence of leukemia subtype determination, and quality of controlling for potential confounding variables. The authors concluded that there was a consistent finding of increased risk for AML with benzene exposure across the studies they reviewed, a relationship that seemed to be more significant with increasing study quality. This relationship did not hold for any other leukemia subtype. Instead, sporadic and inconsistent associations were described for other forms of leukemia, although the authors noted that sparse data, particularly for ALL, precluded more definitive conclusions.

A broad review of occupational exposures and their relationship to hematologic cancers was provided by researchers in France in 2005. Radiation exposure and "high daily exposure to benzene (more than 10 ppm)" were described as being strongly associated with AML. "Cohort studies of workers in the petroleum, gas and electricity industries have not shown any significant excess risk of other types of leukemia, and in particular of chronic myeloid leukemia." The authors also concluded that there was insufficient evidence to claim a causal association for benzene exposure and either NHL or multiple myeloma (Descatha et al., 2005).

In 2005, Sorahan reported on a large cohort of over 5500 benzene-exposed workers in England and Wales prior to 1967. Acute nonlymphocytic leukemia (ANLL; often used interchangeably with AML, but is a bit more inclusive a term) was found with an increased frequency that was not statistically significant; all other forms of leukemia were not found in excess. Though the study had a relatively small number of cases, the authors concluded: "…this study does not support claims that exposure to benzene affects risks for lymphohaematopoietic malignancies other than ANLL" (Sorahan, 2005). Exposure data for individual cases were not available.

A small cohort of 311 Dutch nylon workers exposed to rather high cumulative doses of benzene (average of 159 ppm-years) from 1951 to 1968 was studied up to 2001 to evaluate the risk of leukemia. The authors concluded there was "No excess leukemia despite substantial exposure to benzene" (Swaen et al., 2005). They further opined that despite the small sample size (only 311 workers were followed), their findings supported the notion that leukemia risk from benzene likely had a threshold of exposure that needed to be surpassed prior to generating an increased risk of developing leukemia. They reported an SMR of 85.6, with a very wide CI of 1.1 to 433.

In an update of North American synthetic rubber workers, researchers found a slightly increased incidence of total leukemia (SMR = 116), mostly in long-term hourly workers. The study included employees from any of eight synthetic rubber plants who worked for at least one year prior to 1992, and used work histories to classify individual into various occupational subgroups. Workers were exposed to a wide variety of chemicals, but no exposure data were presented, and the excess of leukemia seemed to be concentrated in workers hired in the 1950s. Leukemia subtype information was absent in 28% of the cases. The authors stated it was difficult to make definitive statements about risks for individual leukemia subtypes due to the small numbers of cases, lack of information on nonoccupational variables (such as smoking, diet, weight), and relatively small overall increased risk (Sathiakumar et al., 2005).

A review of occupational benzene exposures at a chemical manufacturing facility by Williams and Paustenbach looked at 3700 air samples of benzene across a variety of job categories and plant locations (Williams and Paustenbach, 2005). These represented personal worker sampling, area TWA measurements, and short-term area measurements. The authors concluded that chemical operators in the facility sustained daily TWA benzene exposures of about 2.0 ppm from 1976 to 1981 and 1.0 ppm from 1982 to 1987. The results supported a long held belief that in the major corporation, since about 1955, airborne concentrations of benzene have tended to decrease over time, consistent with the changes in either the ACGIH TLVs or OSHA PELs (as originally proposed by Crump and Allen in 1984) (Williams and Paustenbach, 2005). No health effects were evaluated.

In 2007, Natelson provided a review of benzene and acute myeloid leukemia from a clinical perspective. He studied benzene dose and latency issues, discussed mechanisms of disease, chromosomal studies, and certain occupational cohorts. He concluded that in developed countries, due to improved industrial hygiene and reduced exposures to workers, AML was not likely to occur as a result of benzene exposure in modern petrochemical facilities (Natelson, 2007b).

Tsai et al. (2007) performed a cohort mortality study of 10,621 employees in the petroleum refining industry who were

employed between 1948 and 2003. No statistically significant increase of overall leukemia or any leukemia subtype was discovered. In workers with 20 or more years of employment, there were seven cases of ANLL observed, compared with six expected, resulting in an SMR of 1.20. The authors opined that their results were consistent with the notion that benzene exposures in the past at the facility were too low to result in an increase in ANLL or AML (Tsai et al., 2007).

Which hematopoietic diseases are associated with benzene?

Benzene exposure and acute myelogenous leukemia (AML)

Acute myelogenous leukemia, acute myeloid leukemia, and acute nonlymphocytic leukemia are terms frequently used in the literature to describe a group of hematopoietic diseases of acute onset that are distinct from those with lymphocytic origins. Most frequently, the malignant cell type is a myeloblast, or predifferentiated monocyte or myelocyte. In about 5–10% of cases, however, the condition involves erythroblasts (predifferentiated red blood cells) or megakaryoblasts (cells that eventually differentiate to produce platelets) (List et al., 2004). These cell types are contained within the French-American-British (FAB) classification as M6 and M7 leukemias. There were 13,300 new cases of AML expected in the United States in 2008, with over nine out of ten cases occurring in adults (American Cancer Society [ACS], 2008a). Of all the claims about the chronic hazards of exposure to benzene, the weight of evidence from the epidemiology literature is that AML is the only leukemia clearly shown to be elevated. Many government health agencies have weighed in on the relationship between benzene and future risk of developing AML.

> "Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute myelogenous leukemia (AML)" (ATDSR, 2007).

> "Epidemiological studies of benzene-exposed workers have demonstrated a causal relationship between benzene exposure and the production of myelogenous leukaemia. A relationship between benzene exposure and the production of lymphoma and multiple myeloma remains to be clarified" (WHO, 1993).

> "The strongest epidemiological evidence that benzene causes cancer is from several cohort studies in various industries and geographical locations, which found that occupational exposure to benzene increased the risk of mortality from leukemia (mainly acute myelogenous leukemia)" (NTP, 2005).

> "There is sufficient scientific evidence from the numerous human epidemiological studies to assume a causal relationship between benzene exposure and acute non-lymphatic leukaemia" [Multiple myeloma and NHL were specifically excluded from this relationship] (ECB, 2007).

The NCI study reported a minimally statistically significant increased risk of ANLL with a relative risk (RR) of 3.0 (95% CI 1.0–8.9). However, the data did not demonstrate a consistent increased risk of malignancy with increasing exposure duration or cumulative exposure. Combining ANLL and MDS cases into a single disease category resulted in a stronger association with benzene exposure (RR of 4.1, 95% CI 1.4–11.6) (Hayes et al., 1997).

The Pliofilm cohort provided a broad range of worker exposures to benzene and, unlike many other industrial cohorts, had few other confounding chemical exposures. The initial published study for the Pliofilm cohort occurred in 1977, and reported a 10-fold risk of dying from "…myelogenous and monocytic leukemia" (Infante et al., 1977). In 1987, Rinsky et al. reported a standardized mortality ratio of 3.37 for all leukemias in the entire population, with workers having less than 40 ppm-years of exposure not observed to have any measurably increased risk. Workers at higher levels of exposure did demonstrate an increased mortality trend for leukemia, with the highest exposure group (more than 400 ppm-years) being 66 times more likely to die from leukemia than control subjects. Rinsky's follow-up study, reported in 2002, showed that the leukemia risk diminished over time, falling to an aggregate SMR of 2.56 for all leukemias in the population (Rinsky, 2002). Of the 17 leukemias reported in the history of the Pliofilm study, 14 were myeloid and only one was lymphoid. Two cases were unspecified.

McCraw et al. described a retrospective mortality study of white males at an oil refinery in Illinois, finding a statistically significant excess of deaths due to leukemia, chiefly AML (McCraw et al., 1985). A subsequent case-control study showed that cases were not exposed to more benzene compared to controls, and the reasons for the excess leukemia could not be identified (Austin et al., 1986).

Sathiakumar et al. described a positive association between oil and gas field work and AML, with a trend of increasing risk with increasing duration of employment (Sathiakumar et al., 1995). Kirkeleit et al. examined hematologic disease incidence and mortality statistics for upstream petroleum workers in Norway from 1981 to 2003, reporting a significantly increased risk of developing AML (RR = 2.89, 95% CI: 1.25–6.67), particularly for workers employed earlier in the study period (Kirkeleit et al., 2008).

In contrast to these studies, many other occupational studies of chemical workers and petroleum industry workers in the United States and Canada (Bloemen, 2004; Wong and Raabe, 1995; Wong and Raabe, 2000; Tsai et al., 2004; Swaen et al., 2005; Satin et al., 2002; Lewis et al., 2003) have not shown statistically significant increases in the rates of any lymphohematopoietic malignancies, including all leukemias and AML looked at as separate categories of disease. These findings might be partially explained by the level of worker

exposures in these industries likely being considerably less than those experienced by the Pliofilm cohort, worker populations studied in China, and the upstream petroleum industry.

Regulatory agencies and various scientific bodies are in agreement that significant exposure to benzene (in excess of 40 ppm-years) results in an increased risk for myeloid forms of leukemia, chiefly AML. It is difficult to define the exact dose-response relationship for humans, since there are clearly differences in interindividual susceptibility. The World Health Organization (WHO) appears to agree with the early Rinsky findings, that exposure to 1 ppm as a time-weighted average exposure to benzene over a 40-year working career (40 ppm-years) has not been associated "with any increased deaths from leukemia" (WHO, 1993).

There is an ongoing debate regarding the level of cumulative exposure to benzene that might represent a disease threshold for developing AML (Rushton and Romaniuk, 1997; Schnatter et al., 1996; Williams et al., 2008; Johnson et al., 2009). Some scientists believe that there are data suggesting that some risk of various effects on the blood, including AML, exists at very low doses and they point to the Lan et al. (2004) study, which reported peripheral blood effects at workplace benzene concentrations of less than 1 ppm (assuming lifetime exposure for 8 hours/day, 5 days/week). If benzene at these low doses impacts peripheral blood cell counts, then one might conclude that benzene could be having an effect on the bone marrow.

Although evaluating peripheral blood progenitor cells may be the least invasive method of evaluating more immature components of the blood, it cannot be assumed to reflect what is occurring at the level of the bone marrow in an occupationally exposed individual, particularly at very low doses. In other words, demonstration of impaired function in a peripheral blood cell cannot be assumed to reflect cellular damage or abnormalities in other body compartments, nor has it been shown whether these possible changes have prognostic significance for any acute or chronic disease process (Stockstad, 2004).

The Glass nested case-control study of Australian petrochemical workers (Glass et al., 2003) also reported that very low level exposures could lead to leukemia, far below what was previously believed. As we noted earlier, however, this study does not provide enough information to establish a solid foundation regarding benzene risks at low doses of exposure. For example, a substantial fraction of their cases were chronic leukemias, both CLL and CML, diseases that have historically had less compelling associations with benzene exposure (and are classified in different categories of disease by the WHO) (Glass et al., 2003; Swerdlow et al., 2008). Addressing "all leukemias" as a single entity is a problematic epidemiological approach when seeking to understand the malignant potential of benzene, due to the significant differences in these various hematopoietic diseases. Attempts to characterize subgroups of leukemia (such as ANLL) in their study were hampered by low case numbers, leading to risk estimates with very poor precision, and cases of leukemia were supported by histologic confirmation of disease in only half of the cases cited. The very low rates of leukemia in the control population used also are suspect, as pointed out by Schnatter (Schnatter, 2004).

Some researchers have suggested that the metabolism of benzene may differ at low concentrations of exposure compared to higher levels. That is, some researchers have suggested that different metabolites are formed at different rates at different doses. Kim et al., for example, reported that individuals exposed to airborne benzene concentrations of less than 0.1 ppm metabolized benzene about nine times more efficiently than heavily exposed workers. They concluded that the health risks of low and very low exposures may be underestimated by toxicokinetic models currently in use, particularly for exposures less than 3 ppm (Kim et al., 2006). Rappaport has recently expressed the belief that there are, in fact, multiple enzymatic pathways for benzene metabolism: a low concentration enzyme, or "high-affinity" enzyme, operant at low levels of exposure, and a high concentration enzyme, or "low-affinity" enzyme. The "high-affinity" enzyme he believes to be more likely to generate toxic metabolites compared with the "low-affinity" enzyme (Rappaport et al., 2010).

Various genetic polymorphisms of genes involved in benzene metabolism have been proposed as biomarkers to identify members of the population who may be at increased (or decreased) risk of developing adverse biologic effects following benzene exposure (Ross and Zhou, 2010). Understanding the various permutations of these genetic polymorphisms in a given population, and how they might affect individual susceptibility, however, is an extremely complex task, but may well deliver considerable benefits for our understanding of the intricacies of benzene-related disease susceptibility. An excellent review of this topic has been provided recently by Dougherty et al. (Dougherty et al., 2008).

Back in the early 1980s, in an attempt to break the stalemate that had occurred between the various stakeholders (e.g., regulatory agencies, the regulated community, nongovernmental organizations [NGOs], and academics) involved in drafting legislation regarding carcinogens, the term "practical threshold" was offered. This term seems to be particularly appropriate for benzene. For purposes of this discussion, a practical threshold represents a risk that is too small to be measured in an epidemiology study or toxicology study. In short, one cannot rule out that a particularly susceptible person in a very large population might not develop a particular disease, but that the risks are so low that they cannot be accurately quantified. In many cases, there may actually be no increased risk at a particular level of exposure, but science is unable to determine an answer one way or the other. This concept was recently discussed by the Canadian Science Advisory Panel, which was asked to evaluate the risk of mesothelioma associated with exposure to low concentrations of chrysotile asbestos (Health Canada, 2009). They noted that it would be very difficult to show that there were or were not health risks associated with doses that seemed to produce risks of less than 1 in 10,000 in human populations.

A substantial fraction of toxicologists support the notion that there is a safe dose for virtually all substances, even genotoxic carcinogens (Scott, 2008; Waddell, 2006; Hooker et al., 2004; Weisburger, 2001), which aligns with the views of the ACGIH and the OSHA, the two bodies providing the predominant leadership in the United States regarding tolerable or acceptable exposures of worker's to airborne chemicals.

Studies of human DNA repair mechanisms have shown that the various repair pathways are impressively efficient at identifying and repairing mutations (Hartwig, 2010; Wogan et al., 2004). These mechanisms are responsible for identifying and repairing millions of DNA mutations every day in each of us. Virtually all of these mutations occur in the context of the biochemistry of everyday life (Ames et al., 2000). Most persons are exposed to benzene and other carcinogens on a daily basis as a result of smoking, environmental tobacco smoke, gasoline, emissions from vehicles, and industrial emissions. Beyond that, a certain proportion of the reactive metabolites associated with exposure to industrial chemicals are produced naturally in the body or result from normal dietary sources (e.g., phenol, hydroquinone, etc.) (Medinsky et al., 1995; MacDonald et al., 1994; Ames et al., 2000). For example, a glass of "citrus punch" may contain up to 3–5 μg of benzene (US FDA, 2007), and many components of our diet are known to contribute to our body burdens for benzene and other chemicals of concern (EFSA, 2005; US FDA, 2007; Binner et al., 2007). The total daily intake of inhaled benzene for the average nonsmoking person in Western society is about 200 μg per day (Wallace, 1996a, 1996b). We believe, based upon an appreciation of background levels of environmental and dietary exposures, that there exists a daily and lifetime dose of benzene that is sufficiently small so as to not increase the risk of adverse effects for virtually everyone in our society, what would generally be termed a "safe" dose. This dose represents a level of exposure that would not represent a level of concern to warrant regulatory or other protective action (Travis and Arms, 1987; Wilson et al., 1987; Capleton and Levy, 2005).

A very interesting perspective on the evolving concept of disease thresholds was recently shared at the recent Benzene 2009 conference in Munich. Dr. Zarbl cited the example of the changing definition of "no residue" requirements for various pesticides and other chemicals by regulatory authorities resulting from improvements in analytical capabilities to measure smaller and smaller chemical quantities over the years (Zarbl, 2010). He felt that society could well be on the verge of a similar shift in the area of environmental toxicology, this time fueled by major advances in toxicogenomics. Instead of calculating or estimating allowable levels of chemicals by relying upon "no observed adverse effect levels" (NOAELs) in animal and/or human studies, the concept of a NOTEL was suggested, or a "no observed transcriptional effect level." This approach might eliminate the urge to rely upon various low-dose cancer models for estimating the risk at very, very low doses (Zarbl, 2010).

The issue of childhood leukemias and their possible relationship to environmental benzene exposure was also recently explored. Following a comprehensive review of case-control and cohort studies, it was concluded that there is insufficient epidemiologic support for such a relationship, and that the literature to date has been characterized by a lack of exposure data, and the frequent failure to separate out cases of childhood AML from ALL. A wide variety of confounding variables were also discussed by the authors (Pyatt and Hays, 2010).

Wong et al. (2010a) reported on the findings of a hospital-based case-control study for the development of AML in Shanghai, China, as part of the Shanghai Health Study. Environmental and occupational risk factors, including benzene exposures, were evaluated for their potential risk contribution for workers developing AML or AML subtypes as classified by WHO. The authors provided the results of univariate and multivariate analyses based on worker exposures to specific chemicals and provided risk calculations for various occupational and industrial worker categories. For benzene, the authors stated:

> "Our study confirmed that benzene exposure was significantly associated with an increased risk of AML-total (OR = 1.43, 95% CI = 1.05–1.93) ... analysis indicated that the group affected by benzene exposure most was AML [recurrent cytogenetic abnormalities] (RCA) (OR = 1.61, 95% CI = 1.00–2.61). The largest subgroup within the category of AML-RCA was [acute promyelocytic leukemia] (APL) (n = 124). A borderline significant risk was found between benzene exposure and APL (OR = 1.95, 95% CI = 1.00–2.61) ... benzene exposure was significantly associated with the specific subtype AML with t(8;21)(q22;q22) with an OR of 4.26 (95%CI = 1.01–17.96). The risk of AML with t(8;21)(q22;q22) appeared to be most strongly associated with recent first exposure in or after 2000 (OR = 11.97, CI = 1.44–99.64)" (Wong et al., 2010a).

The question of whether there exists a threshold for benzene exposure related to AML, identifying the precise dose for such a threshold, and identifying particularly susceptible subgroups in the population (who surely have a different threshold dose), are topics that will likely continue to spur active debate. The use of novel platform technologies and the synthesis of knowledge from diverse scientific disciplines will no doubt provide the substance for even livelier discussions in the years to come.

Benzene exposure and non-Hodgkin's lymphoma (NHL)

The causes of non-Hodgkin's lymphoma in the general population remain mostly unclear. Over the last few decades, NHL has shown an average 3–4% increase in incidence each year, and in 2008, over 66,000 cases were expected in the United States (American Cancer Society, 2008c). As with AML, more than nine out of every ten cases occur in adults. The reasons for the striking increase in NHL are not well understood, with only melanoma, prostate cancer, and lung cancer in women showing higher rates of increased cancer incidence in recent years (American Cancer Society, 2008c).

Likely factors contributing to the increase include the aging of the American population, an increasing prevalence of human immunodeficiency virus (HIV) (Smith et al., 2004) [NHL is 100 times more prevalent in HIV-infected populations], certain autoimmune disorders (Smedby et al., 2008) and possibly some occupational exposures (Boffetta et al., 2007; Fritschi et al., 2005; Dreiher and Kordysh, 2006). Despite numerous studies involving a wide variety of proposed agents, however, "at present, no conclusive evidence of causal relations between occupations and increased NHL risk exists" (Boffetta et al., 2007). Certainly, the net impact of the risk factors noted above are not believed to adequately explain the doubling of disease incidence rates over the past 30 years (Table 5) (Greer et al., 2004; Evans and Hancock, 2003). Disease classification changes, as we shall later explore, have led to additional cases of NHL previously tallied in other categories (multiple myeloma, chronic lymphocytic leukemia, for example). A recent review by Bosetti et al., however, suggests that the incidence and mortality of NHL have leveled off in most developed countries worldwide (Bosetti et al., 2008).

When attempting to review the epidemiology literature regarding the relationship between exposure to benzene and an increased risk of NHL, one must be aware that it is nearly impossible to compare medical diagnoses of lymphoma from the 1950s through 1990s with those from the last decade (e.g., 2000 to 2010). This results mainly from the classification strategies for lymphoma having undergone a tremendous amount of reorganization in the last 30–40 years. The initial wave of change was started by Rappaport in 1966 who advocated a sorting of lymphomas that relied upon pathologists describing the morphology of entire lymph nodes involved with malignancy (Rappaport, 1966). Simply put, malignancies were classified into the broad categories of "nodular" and "diffuse," with further characterization based upon the cellular characteristics of the tumor. Revision of the Rappaport classification was subsequently recommended by Lukes and Collins (Lukes and Collins, 1974, 1975). Borrowing techniques from the relatively new field of immunology, these researchers related malignant lymphomas to aberrations in the development of B and T lymphocytes. They further suggested that superior therapies would be more likely to come about with a nomenclature that appreciated the origins and cellular characteristics of lymphomas, rather than relying so heavily on the gross appearance of the tumors (Lukes and Collins, 1975).

Despite the scientific battles over whose nomenclature was superior, both sides agreed that the previous methods of reporting lymphomas were inferior, and generally confusing to anyone attempting to make sense of these complex disease processes.

> "The histologic classification which employs the terms 'reticulum cell sarcoma,' 'lymphosarcoma,' and 'giant follicular (nodular) lymphoma' has long been known to correlate poorly with prognosis. Striking discrepancies in the clinical course of patients with 'reticulum cell sarcoma' have been particularly puzzling to clinicians for years" (Jones et al., 1973).
>
> "The terms 'reticulum cell sarcoma' and 'lymphosarcoma' in addition have been applied in an extraordinarily variable fashion and achieved a meaningless status, by preventing effective comparison of results from different centers" (Lukes and Collins, 1974).

It is anticipated that future epidemiology studies will more carefully incorporate this information into their analyses.

The striking advances made in immunology and histopathology have allowed much more explicit and prognostically useful categorizing of patients with lymphoma and leukemia. Therapeutic options have created better patient outcomes, with the result being that the previously most devastating and aggressive tumors now often demonstrate the best chances for having a complete remission. For example, in children under the age of five, the 5-year survival for ALL in the 1996–2004 time frame was 91.2%, a disease that 40 years ago was an almost automatic death sentence (Leukemia and Lymphoma Society, 2009c). Because of the often inaccurate perceptions regarding hematopoietic disease combined with "meaningless" nomenclatures that have been used over the

Table 5. Factors that have been reported to be associated with an increased risk of developing non-Hodgkin's lymphoma in susceptible individuals.

- Infectious agents
  - Epstein-Barr virus
  - HTLV types 1 and 2
  - *Helicobacter pylori*
  - Hepatitis C virus
  - Human herpesvirus 8 (Kaposi's sarcoma)
  - Human herpesvirus 6
- Male gender
- Advanced age
- Family history of NHL
- History of cancer, cancer treatments, use of immunosuppressive agents
- Medications
  - Phenytoin
  - Methotrexate
- Occupational exposures to:
  - Herbicides
  - Pesticides
  - Wood dust
  - Epoxy resins and glues
  - Solvents
- Workers in certain industries; studies have evaluated:
  - Farming
  - Forestry
  - Painting
  - Carpentry
  - Tannery/leather working/shoe manufacturing
- Other
  - Use of hair dyes
  - Excessive exposure to ultraviolet rays
  - Nutritional factors
  - History of blood transfusions

HTLV = human T-lymphotropic virus; NHL = non-Hodgkin's lymphoma.
Sources: American Cancer Society, 2009; NCI, 2007; ATSDR, 2007; Vose et al., 2002; Alexander et al., 2007.

years to describe lymphomas, attempting to correlate data between various time periods can be impractical and often impossible.

Currently, the medical community is in yet another transition in the evolving landscape of lymphoma classification. The Revised European-American classification system for lymphoid neoplasms (REAL), first presented in 1993 by an international lymphoma study group, organized lymphomas on the basis of morphology and further subdivided on the basis of genetic and immunologic parameters (Skarin et al., 1997). This approach was reminiscent of the Lukes and Collins modifications to the original Rappaport system in 1966 (Lukes and Collins, 1975). The REAL classification has since been updated by the World Health Organization in 2001, and the classification of leukemias and lymphomas will forever be a "work in progress" as our knowledge of the basic science improves and new treatment advances lead to further understanding of the pathophysiology of the lymphomas (Jaffe et al., 2001). More recently, the 2008 revision of the WHO classification of lymphoid neoplasms has elevated "precursor lymphoid neoplasms" to a separate category of disease and recognizes the overlap characteristics of these tumors by naming each individual entry as a "lymphoblastic leukemia/lymphoma" with particular cytogenetic characteristics (Swerdlow et al., 2008). By segregating tumors by degree of differentiation, cellular identity (B cell, T cell, NK cell, etc.), location of disease, and clinical characteristics, there are now 74 separate subtypes of non-Hodgkin's lymphoid neoplasms, a number that is certain to grow even further in the future as disease processes are even more discretely characterized (Swerdlow et al., 2008).

At the international conference on benzene recently held in Munich, Vardiman described the WHO classification system for tumors of the hematopoietic and lymphoid tissues (with an emphasis on the myeloid neoplasms) (Vardiman, 2010). He noted: "in general, the classification stratifies neoplasms according to their lineage (myeloid, lymphoid, histiocystic/dendritic) and distinguishes neoplasms of precursor cells from those comprised of functionally mature cells." A review of his paper rapidly convinces any skeptic that it is not useful to conduct any future study of the relationship between exposure to benzene and various tumor types without a very careful description of the classification.

Although most international regulatory authorities have agreed that chronic exposures to significant airborne concentrations of benzene increase the risk of developing AML, there is no such consensus for NHL, despite animal studies that have shown the occurrence of thymic and nonthymic lymphomas in mice at relatively high chronic airborne concentrations of benzene (300 ppm 6 hours/day, 5 days/week over the lifetime of the animals) (Cronkite et al., 1984, 1985; Farris et al., 1993). Studies have also evaluated the effects of various levels of exposure to benzene and/or benzene metabolites upon peripheral blood lymphocytes in both animal and human studies, with varying conclusions. Yager et al., for example, performed in vitro studies with hydroquinone, benzoquinone, phenol, and catechol, finding a particularly noticeable effect with hydroquinone in causing micronuclei formation, an indication of chromosomal damage (Yager et al., 1990). Farris and colleagues exposed mice to varying concentrations of benzene (1, 5, 10, 100, and 200 ppm) by inhalation for up to 8 weeks (6 hours of exposure/day, 5 days/week) and measured the effects on various lymphocyte populations. A dramatic decrease in splenic, thymic, and femoral lymphocytes was seen at the 100 and 200 ppm levels of exposure, with no significant change compared to controls at lower airborne concentration levels (Farris et al., 1997).

In a study of ten workers exposed to a 1200-gallon spill of benzene during the loading of a ship, substantially increased levels of urinary phenol were detected in the exposed workers. Three months after the accident, peripheral blood was obtained from the exposed group and compared to 11 control workers who had not experienced the spill. Cells were examined for all types of chromosomal damage and sister-chromatid exchange. Similar amounts of damage were found in both the exposed and control groups, suggesting that there was no evidence of any long-term damage in the group of exposed workers (Clare et al., 1984). There have been many other similar in vivo and in vitro studies that have been performed, but these analyses typically have suffered from one or more deficiencies:

- For occupational studies, a lack of clarity as to historical exposure levels that workers were exposed to
- Concomitant exposures to other potentially harmful chemicals that are often neither identified nor considered as possible confounding agents for the endpoints being studied
- Improper control groups
- Inability to correlate simple hematologic parameters with definable risk for developing cancer endpoint
- For animal studies, difficulty in comparing high-dose exposures to likelihood of disease at lower concentrations in humans

A number of laboratories continue to study benzene and they are generally working to mitigate the above-mentioned issues.

Claims of an occupational link between benzene exposure and lymphoma were expressed in a 1979 publication that used a combination of death certificate and census data, along with assumptions regarding benzene exposure in certain occupational subgroups (Vianna and Polan, 1979). Workers with likely exposures to benzene were found to be at increased risk for certain types of lymphoma, though no actual benzene exposure data were reported. Some of the chemical exposures experienced by workers but not considered by Vianna and Polan in their analysis were

Arsenic
Asbestos

Fertilizers
Lead
Mercury
Pesticides
Fungicides
Epoxy resins
Aniline
Hydrogen cyanide
Phosgene
Nickel
Uranium
Phthalates
(Stellman and Daum, 1973).

The findings of Vianna and Polan were challenged by Enterline in a Letter to the Editor of the publishing journal, who found inconsistencies in their calculations and doubted that there was actually any increased mortality for the diseases noted (Enterline, 1979).

Confounding exposures are also relevant for the NCI studies describing Chinese workers with hematopoietic disease (Yin et al., 1996; Hayes et al., 1997). Other chemical exposures were not controlled for, and in fact, the two occupational categories with the greatest relative risk reported for non-Hodgkin's lymphoma were "Chemical workers," where one would presumably be exposed to a wide variety of different potentially toxic substances; "Coatings," another occupation where many types of agents are likely to have been employed; and "Other/mixed occupations" where the types of exposures were not described. These three subcategories of workers represented 14 of the 16 cases of NHL described in the study, with the other two cases coming from rubber workers and shoe workers, respectively (Yin et al., 1996).

Yin et al. described their methods for case validation as follows:

> "All histopathologic and bone marrow aspirate slides and peripheral blood smears were reviewed systematically, by expert hematopathologists affiliated with the Mayo Clinic, NCI and Peking Union Hospital using structured abstract forms to objectively characterize hematopoiesis" (Yin et al., 1996).

The proportion of cases, however, where researchers had bone marrow slides and peripheral blood smears available for evaluation was minimal. In describing their protocol for case confirmation, Yin et al. referred to Travis et al. (1994), who described the same cohort of almost 75,000 Chinese workers. That study used a review of printed pathology reports and medical records to "confirm" 16 cases of "malignant lymphoma or related malignancy" (the definition of "related malignancy" was not provided) and reviewed histopathologic material (i.e., slides, blood smears, or original biopsy material) for only 4 cases of "malignant lymphoma." Hodgkin's and non-Hodgkin's lymphoma were not separately described, making it difficult to know if any cases of NHL were verified by tissue analysis at all (Travis et al., 1994).

As we have noted above, the classification methods for lymphoma here in the United States over the past 30–40 years have changed dramatically, and it is unclear when different pathologists from different regions of the world would have adopted the varying sets of disease criteria. The failure to do a more comprehensive verification of lymphoma and leukemia cases makes it difficult to place much confidence in the conclusions reached by these researchers, which have varied from more recent occupational studies conducted in China (Wang et al., 2006; Wong et al. 2010a; Wong et al. 2010b).

A review by Wong (1999) evaluated the methodologies employed in the NCI/CAPM studies. Exposure assessment was felt to be particularly problematic.

> "… the control workers were exposed to neither benzene nor any other occupational carcinogens, whereas the exposed workers were exposed to not only benzene but also possibly to other occupational carcinogens. Thus, by design, a comparison between the benzene-exposed workers and control workers would not be limited to the effects of benzene, but would also reflect the effects of other occupational carcinogens. Therefore, the study was not designed properly to address the effects of benzene only. Yet, the authors were ready to attribute any increased risk of diseases found in the study to benzene exposure" (Wong, 1999).

Wong reported that exposure estimates were "consistently lower than the actual exposure data." Although NCI had admitted earlier on in their involvement the problems associated with their exposure calculations, "…the inadequacy of the estimates was never mentioned or discussed in any subsequent analyses or in the [Hayes et al., 1997] report. Instead, the exposure of the workers was hailed as 'well characterized'" (Wong, 1999).

Wong concluded his analysis with the following note:

> "Unfortunately, there were many inherent problems in the data, as well as serious limitations in the exposure estimates. Because of these unresolved problems and limitations, many of the results in the CAPM–NCI study are unreliable. Therefore, the conclusions of the study, particularly those involving exposure estimates, are not justified" (Wong, 1999).

Lamm et al. (2005) reviewed benzene exposure and NHL, specifically excluding studies sponsored by the petroleum industry to avoid possible researcher bias and included 18 studies with 21 different study groups in their analysis. Overall, they noted that these studies had an aggregate of 404 observed cases of NHL compared with an expected number of 390 in the populations that were looked at, yielding an odds ratio of 1.04 (95% CI: 0.94–1.14). When the authors removed studies that failed to adequately address

the question of multiple exposures, they were left with 359 observed cases and 373.2 expected, with a corresponding odds ratio of 0.96 (0.86–1.06). The authors observed that the latest report, the Rinsky 2002 study, on the Ohio Pliofilm worker population (a cohort singled out for having detailed exposure data, few alternative exposures, and strong epidemiologic standards) provided further support for their conclusions. In all, there were five cases of NHL reported and slightly more than five expected, generating an odds ratio almost identical to that of their meta-analysis, which was calculated to be slightly less than unity. In short, no increased risk was observed.

Smith et al. reviewed case-control and cohort studies that examined populations with probable exposures to benzene and assessed their relationship to the endpoints of NHL morbidity or mortality (Smith et al., 2007). The authors concluded that "…overall, the evidence supports an association between occupational benzene exposure and NHL" (Smith et al., 2007), citing relative risks and odds ratios reported in the various studies. After examining in greater detail the quality of the underlying data and original conclusions made by the individual study authors summarized by Smith et al., we believe the conclusions of these studies were selectively, and often inaccurately, conveyed. We provide several examples as follows.

Bernard et al. was a prospective case-control study of all cases of "lymphoma and lymphocytic leukemia within a population of 1.5 million people in England between 1979 and 1981" (Bernard et al., 1984). In their study, the authors indicated that the "Number of cases are small and confidence intervals wide" (Bernard et al., 1984), with the lower and upper 95% confidence interval limits of petroleum workers diagnosed with NHL varying by almost two orders of magnitude, while also failing to be statistically significant. Not noted by Smith et al. was the additional finding that use of "petroleum products" by men and women was also associated with a very low risk ratio of 0.59 (0.25–1.39). Thus the combination of very few cases in this study, along with the failure to demonstrate any chemical-specific risk related to benzene by the authors, makes the value of the Bernard paper questionable in terms of supporting the notion that benzene exposure is linked to an increased risk for NHL.

The Blair study, cited by Smith et al., was a population-based case-control study designed to evaluate cancer risks from agricultural exposures in Iowa and Minnesota. The study suffered from a very small number of cases involving benzene exposure. The highest relative risks that Smith et al. reported from those provided by the authors were follicular lymphoma with a RR of 1.9, based upon five cases, and "diffuse" lymphoma with a RR of 1.8 based on four cases. Smith et al. mentioned that workers associated with "petroleum refining" were observed to have a higher risk of developing NHL with a RR of 1.6, but neglected to observe a host of other industries with far greater calculated risks, not known to have regular exposure to solvents. These industry worker subgroups are summarized in the following table:

Industry	RR
Agricultural products	2.3
Apparel	2.4
Barbers	2.7
Forestry	6.2
Furniture sales	4.9
Camps and trailer parks	5.5
Labor unions	2.3
Masonry	2.6
Metalworking	3
Physician offices	3.4
Labor unions	2.3
Retail bakeries	4.4
Stone products	2.6

In fact, it was just as risky to be an insurance agent or a theatrical producer as it was to be a member of the petroleum refining profession, according to Blair et al. They concluded their study with the following:

> "In summary, this evaluation does not indicate that industrial exposures are a major contributor to the etiology of NHL" (Blair et al., 1993).

Fabbro-Peray was a questionnaire-based study that made assumptions as to benzene exposure occurring in certain occupations, without any actual measurements of benzene exposure. The data describe a very small number of benzene-exposed cases and controls, with 22 self-reported benzene-exposed cases and 23 controls.

Interestingly, although the "benzene-exposed" workers were believed to come from the chemical, rubber production, printing, shoemaking, and painting industries, exposures to paints, rubber solvents, petroleum products, and waste oil were not individually found to be associated with an increased risk for developing NHL. Only coal tar exposure was identified by the authors as having a substantially increased risk, which was not statistically significant.

Employment as a "radio operator" was associated with a more than 50% greater risk for developing NHL compared with professions deemed to be at risk for benzene exposure (3.3 versus 2.0). These authors concluded:

> "… we cannot forget that the OR values have been estimated from a very small proportion of subjects, leading to large confidence intervals, and thus, imprecise estimations. That is true for history of hematological malignancy…and especially benzene exposure" (Fabbro-Peray, 2001).

Franceschi et al. was a regional study of 208 cases of NHL and 401 controls in the northeastern part of Italy, performed between 1985 and 1988. Smith et al. mischaracterized the findings reported by these authors, implying that the marginally increased RR of 1.14 was associated with benzene exposure, when it actually referred to exposures to "benzene and solvents" more generally. The marginally elevated risk reported for petroleum workers did not show an expected

relationship with length of employment, reflected in the quote below:

> "There was a hint that employment in chemical and petrochemical industries may increase the probability of the onset of NHL, in agreement with a few previous reports. The elevation of risk, however, did not tend to be higher in those individuals who had been employed in such industries for longer periods" (Franceschi et al., 1989).

They also emphasized that:

> "Moreover, the absence of strong findings in the present investigation should be stressed. In fact, despite the large number of potential risk factors investigated, only one plausible and significant association (with positive history of chronic infectious diseases) emerged, thus confirming the difficulties in studying epidemiologically the aetiology of lymphoreticular neoplasia" (Franceschi et al., 1989).

Far from implicating benzene or any other chemical, they found that:

> "Specific exposures to chemical or physical agents suspected of being related to lymphoma risk were even rarer and none of them apparently resulted in a significant elevation of risk" (Franceschi et al., 1989).

Fritschi et al. reported the incidence of NHL from a cancer registry in New South Wales from January 2000 to August 2001. As Smith et al. correctly report, there was no increased risk for developing NHL discovered by the researchers. What Smith et al. did not point out was that in the subgroup of subjects exposed to "substantial" quantities of benzene solvents, the odds ratio was calculated to be 0.31, the lowest finding of any of the 11 individual chemical exposure subgroups described by Fritschi et al. in their publication (Fritschi et al., 2005).

A study of patients with malignant lymphomas by Hardell and colleagues reported on individuals diagnosed between 1974 and 1978, with exposures that occurred well before many major occupational and regulatory changes applying to the use of benzene-containing solvents. To determine cases of NHL, the authors stated that they relied upon the original Lukes-Collins system, which was modified to "retrospectively reclassify" patients with lymphoma at the Swedish hospital involved in the study from 1959 to 1975 (Hardell et al., 1981).

In the cases described, there was only one patient with heavy exposures to benzene. The relative risk of 4.5 (1.9–11.4) quoted by Smith et al. represented a total of ten cases, matched with eight controls. Of the ten cases, seven were exposed to TCE, one to styrene, one to tetrachloroethylene, and one to benzene. There were no mixed exposures with benzene, and there were zero control patients examined with benzene exposure.

Thus, 90% of the cases leading to the RR of 4.5 reported by Smith et al. had nothing to do with benzene. Moreover, the control population used by Hardell et al. were uniformly either exposed to TCE or styrene, agents that would have the potential to confound the reported results.

Smith et al. also reported on a later Hardell et al. study that described 105 patients with NHL who were admitted to a Swedish hospital between 1974 and 1978 (Hardell et al., 1994) This was a questionnaire-based analysis with self-reported exposures and no measured chemical exposures, as with the 1981 study. Cases linked to benzene exposure amounted to only three in number, with only a single control patient.

The lack of statistical power and lack of precision is reflected in the upper and lower bound limits for the confidence interval of risk in benzene-exposed patients, which vary by almost three orders of magnitude. The other values cited by Smith et al. refer to "organic solvent" exposures, encompassing a wide variety of chemicals, which may or may not contain small amounts of benzene. The findings reported by Hardell et al. for heavy exposures to organic solvents, incidentally, were dwarfed by their calculations for patients who sustained any exposure to phenoxyacetic acids (OR of 5.2 [1.6–17]) as well as all reported exposures to chlorophenols (OR of 4.8 [2.7–8.8]).

The Hardell et al. (1994) data, then, regarding benzene and NHL, suffer from woefully small numbers, both for numbers of cases and controls, and when addressing risks pertaining to organic solvents, suffer from a lack of specificity relating to the possible actions of benzene on the patient population in question.

Kato et al. summarized the incident cases of NHL from 1995 to 1998 in upstate New York, identified through the New York State Cancer Registry. This questionnaire-based study looked at a very small number of benzene-exposed cases and controls (seven and five, respectively) and found a small increased risk for cases having occupational exposure to benzene. However, the authors were very clear with their interpretation of the data:

> "Overall, history of exposure to organic solvents was not associated with the risk of NHL. A statistically significant increase in risk associated with occupational exposure was observed only for the subjects whose first exposure occurred before 1970" (Kato et al., 2005).

It should also be noted that Smith et al.'s citation of a statistically significant odds ratio of 1.40 for NHL does not relate to solvents associated with benzene, but for paint thinners and turpentine, which are known to predominantly contain toluene and/or xylene.

Mao et al. reported on data obtained from the Canadian National Cancer Surveillance System (NECSS) from 1994 to 1997. Based on a small number of workers exposed to benzene, the authors found a small statistically insignificant increased risk for developing NHL in men, and a small statistically insignificant decreased risk in women (OR 1.2 [0.8–1.9] and OR 0.6 [0.2–1.8], respectively) (Mao et al., 2000). These risks were not even commented on by Mao et al. in their review, focusing their comments instead on benzidine, mineral oil, and pesticides.

Miligi et al. conducted a population-based study looking at newly diagnosed cases of NHL in 11 areas of Italy from 1991 to 1993. The authors grouped participants into four levels of perceived exposure according to their probable net exposure to a wide variety of chemicals, as determined from responses to a questionnaire. After failing to find "…increased NHL risk from "very low/low" intensity exposure to any class or specific solvent" (Miligi et al., 2006), the authors bundled together the two lower and two higher exposure groups, reporting a "protective" odds ratio for very low and low exposures to benzene of 0.6, and for "medium and high" exposures, they obtained an odds ratio of 1.6, which included one on the lower bound of the confidence interval. It is also interesting to note that the ORs reported for both xylene and toluene exposures were greater than those reported for benzene at both combined exposure levels reported. More importantly, when the authors segregated out exposures, and only looked at subjects with "medium to high" exposures to benzene alone (eliminating mixed exposures), their OR dropped to 1.2 and became statistically insignificant (0.7–2.2). The authors offered caution about how to interpret their findings:

"In our data, there was a high degree of correlation among exposures to benzene, xylene, and toluene. For this reason, caution must be exercised when interpreting the evidence for any one of these 3 solvents" (Miligi et al., 2006).

Ott et al. studied a cohort of 29,139 men employed by Union Carbide in manufacturing facilities and a research center in the 1940–1978 time period. Smith et al. correctly point out that the OR for workers likely exposed to benzene compared to workers without benzene exposure was 1.0 for developing NHL. However, the rest of the citations from the 1989 Ott et al. study are improperly characterized and not adequately explained by Smith et al. in their review.

First, an OR of 1.6 for workers with more than five years of exposure to benzene and developing NHL was cited. This odds ratio, described by Ott et al. in Table IV of their 1989 article, pertains to multiple myeloma, not NHL. Next, an OR of 3.2 for "benzene-exposed foremen and maintenance/construction workers" was cited for developing NHL compared to those workers without benzene exposure. However, Ott et al.'s calculations have nothing to do with historical exposure to benzene. Instead, the authors are describing odds ratios for workers ever having been employed in various work areas, with no mention of the specific chemicals that they might have been exposed to. Also, the category of workers described by Ott et al. with an OR of 3.2 is only the subgroup of "foremen and others," and not the broader group of maintenance and construction workers, as seems to be implied by Smith et al. There are nine separate occupational subgroups under the heading of maintenance and construction workers, each with their own calculated OR. This same problem occurs with Smith's citation of the OR for "instrument men." This OR has nothing to do with benzene exposure, but relates to a specific job classification. Specific chemical risks are not the subject of discussion (Ott et al., 1989).

Scherr et al. reviewed all cases of NHL diagnosed from 1980 to 1982 at any one of nine participating hospitals in the Boston area. They found that the relative risk for NHL was highest in those employed in the agriculture, forestry, and fishing industries. Decreased risk was found for those working in the chemical, pharmaceutical, and painting industries, and these decreased risks were found to be statistically significant.

With regard to benzene exposure, they noted:

> "We did not observe an increased risk for those exposed to benzene. The association between lymphoma and benzene had been reported by Vianna and Polan, where they compared the proportional mortality rate for lymphoma among those who had worked in an industry that used benzene with the rate for those who worked in all other industries. A review of the article showed that the strength of their association was strongly supported by the increased risk of disease presumably due to benzene for woodworkers and farmers. Subsequently, it has been suggested that the increased risk among woodworkers and farmers may be due to other agents. A later study also found no increased risk with exposure to benzene" (Scherr et al., 1992).

Schnatter et al. looked at all cases of fatal lymphohematopoietic cancer in an occupational cohort. They noted that half of the cases of NHL reported occurred in clerks or technicians, individuals with little to no opportunity for exposure to benzene. The only risk value cited by Smith et al. is specifically addressed by Schnatter et al., describing this RR of 5.85 as being imprecise, based on only two cases with low level "peak" exposures to benzene of 0.5 to 1.0 ppm. There was only one case of NHL that experienced a maximum benzene exposure of over 1 ppm, associated with a RR of 0.54.

Schnatter et al. concluded: "…this study did not show a relation between lymphohematopoietic cancer and long term, low level exposures to benzene" (Schnatter et al., 1996).

Wilcosky et al. reported on a cohort study of rubber workers in Akron, Ohio, who were examined from 1964 to 1974, a period when higher levels of benzene exposure were experienced, in a profession known to use solvents with benzene present in measurable concentrations. These authors examined a number of cancer endpoints, finding that:

> "Benzene, a suspected carcinogen, was not significantly associated with any of the cancers [including NHL]" (Wilcosky et al., 1984).

Instead of reporting confidence intervals, the authors instead indicated where reported values had a P value of $<.05$. This was not the case for any of the relative risk numbers cited by Smith et al. from the Wilcosky study.

The underlying studies and data supporting Smith et al.'s conclusion of an association between benzene exposure and NHL appear to be quite weak, and in many cases, improperly cited by Smith and his colleagues. We believe the case for benzene as a causative agent for NHL remains far from clear.

However, Wong and colleagues (2010b) reported on a case-control study designed to examine risk factors for the development of NHL in Shanghai, China, as part of the Shanghai Health Study. The authors indicated that their findings confirmed several risk factors that had been previously reported in other studies, discovered new potential risk factors for NHL, and for the first time provided detailed data on individual NHL subtypes using the updated WHO classification system. With regard to benzene exposures and the development of NHL, the authors stated:

> "In our study, exposure to benzene was not associated with an increased risk of NHL [neoplasms]-total (OR = 1.06, 95% CI = 0.74–1.51), based on 50 exposed cases and 95 exposed controls… which was consistent with the literature. Furthermore, no increased risk was found for most subtypes of B-cell neoplasms, or T/NK-cell neoplasms… However, seven cases of [follicular lymphoma] and two corresponding controls were exposed to benzene, resulting in a significant OR of 7.00. The 95% CI (1.45–33.70) was rather wide, primarily because the OR was based on only two exposed controls" (Wong et al., 2010b).

When multivariate analysis was performed, benzene was not found to be associated with NHL overall, although benzene emerged as the only significant variable associated with the development of follicular lymphoma. They advised, however, that:

> "The finding of an association between benzene and [follicular lymphoma]… should be interpreted with caution as the OR was based on only two exposed controls" (Wong et al., 2010b).

Benzene exposure and chronic lymphocytic leukemia (CLL)

Chronic lymphocytic leukemia has historically represented the most common subtype of leukemia, with about 15,000 estimated new cases diagnosed in 2008 (ACS, 2008b). It is almost exclusively a disease of adults, with the average age at the time of diagnosis being 70. Many patients with the disease, however, are asymptomatic, becoming diagnosed only as a result of routine blood screening tests. Thus, it is likely that the true population incidence may be much higher than that reported by cancer statistics.

Unlike ANLL, CLL has not been strongly associated with any environmental or occupational exposures, including benzene exposure (Greer et al., 2004):

> "Exposure to high-dose radiation or benzene is not a risk factor for CLL" (Leukemia and Lymphoma Society, 2009a).

> "There is no link to radiation, cancer-causing chemicals, or viruses" (NIH, 2009b).

The American Cancer Society recognizes the following risk factors as being relevant for CLL:

- Family history of CLL
  Parents, siblings, or children of patients with CLL have a two to four times increased risk of developing the disease
- Gender
  CLL is slightly more common in men, for unknown reasons
- Ethnicity
  CLL is more common in Europe and North America than in Asia. The basis for this appears to be genetic rather than environmental, as Asian immigrants have not been discovered to increase their risk of CLL to that of their host country.
- Certain chemical exposures
  Some studies have suggested that exposures to Agent Orange may increase the risk of developing CLL. Other studies have claimed an association between farming and pesticide exposures, but results have been inconsistent.

The ACS concluded their discussion by stating:

> "There are no other proven risk factors for CLL. The risk of getting CLL does not seem to be affected by smoking, diet, exposure to radiation, or infections" (ACS, 2008b).

We should note that CLL and small lymphocytic lymphoma (SLL) are being increasingly considered to be separate instances of the same disease process; the distinction being whether the disease is believed to be based in the bone marrow (CLL) or in lymph nodes (SLL), tissues outside the bone marrow. For example, in 2001, the World Health Organization classified CLL and SLL into a single disease category in recognition of their similar cellular appearance, as well as their immunologic characteristics (both diseases express similar cell surface antigens) (Jaffe et al., 2001). This recognition is important when reviewing historical publications looking at the epidemiology of these diseases; as well as more recent studies that have tended to group the diseases together (Dores et al., 2007). The most recent WHO revision for classification of leukemias and lymphomas lists CLL and SLL as the same entity (Swerdlow, 2008). The elimination of CLL as a distinct disease category and its bundling with SLL naturally will have a great impact on future epidemiologic studies, and will complicate the interpretation of historical studies that have treated SLL and CLL as separate malignancies. Whether this will improve our ability to more definitively describe the possible relationship between benzene exposures and this now unified disease process remains to be seen.

Despite general agreement by national and international cancer authorities that there are no known environmental risk factors for developing CLL (other than that some believe

exposure to Agent Orange may be a factor), some have opined that exposures to benzene can cause CLL in humans.

In 1997, Savitz and Andrews published a review of the scientific literature, including 18 community-based and 16 industry-based studies linking benzene to "lymphatic and hematopoietic cancers." Their review found "sporadic reports" linking benzene to NHL and multiple myeloma, but the authors determined that the weight of the evidence suggested that benzene should be considered to cause all forms of leukemia, not simply AML:

> "The epidemiologic evidence linking benzene to leukemia in the aggregate, as well as acute and chronic lymphocytic and myeloid leukemia, is no less persuasive than that for AML alone" (Savitz and Andrews, 1997).

Savitz and Andrews admitted, however, that exposure data for benzene in the studies they reviewed were generally lacking, and the risk for confounding chemicals in most of the work environments would make it difficult to make conclusive statements regarding benzene and disease causation.

> "Failure to isolate benzene from closely associated agents that may themselves cause lymphatic and hematopoietic cancers could introduce confounding. In the rubber industry, for example, associations between benzene and lymphocytic leukemia were reported [Checkoway et al., 1984], yet other solvents were as or more strongly associated with lymphocytic leukemia, making isolation of the etiologic agent difficult. Environments associated with the petrochemical industry, shoe manufacturing, and painting contain a wide array of organic solvents and other potentially carcinogenic chemicals which were not fully addressed in the published reports" (Savitz and Andrews, 1997).

An earlier study by Savitz on leukemia mortality and its relationship to occupation reported no increased incidence of AML in occupations traditionally associated with regular exposure to benzene-containing products, such as printing machine operators, "cleaners and laborers," metal-, plastic-, and woodworking machine operators, painters, vehicle mechanics, and motor vehicle operators (Loomis and Savitz, 1991). Instead, the occupation with the highest risk reported for AML was "mathematical and computer scientists." The authors noted in this study that:

> "This study shares other limitations of occupational investigations based on death certificates. These weaknesses…include the inadequacy of usual occupation as an indicator of lifetime work and exposure histories and the absence of information on the timing or duration of the listed occupation."
>
> "The lack of information on exposure to specific etiologic agents and the potentially confounding exposures are more significant disadvantages" (Loomis and Savitz, 1991).

In the 1997 analysis, Savitz and Andrews cited a number of papers that they believed were supportive of the idea that benzene caused not only AML but all leukemias. The remainder of the text in this portion of our review summarizes a few of the findings reported by various authors.

Checkoway et al. (1984) performed an analysis of the possible effects of a variety of solvents used by rubber industry workers. Benzene, however, was not singled out as being particularly notable in terms of its perceived risk.

> "The associations with lymphocytic leukemia risk observed for a number of solvents, most notably carbon tetrachloride and carbon disulfide, were stronger than those detected for benzene" (Checkoway et al., 1984).

To be specific, out of the 24 solvents tested, 12 were reported by the authors to be more associated with the development of subsequent leukemia than benzene, including:

- Acetone
- Methanol
- Ethyl acetate
- Toluene
- Xylenes
- Hexane

The fact that these solvents, none of which are known to have a significant relationship to leukemia, were observed to be more highly associated with leukemia than benzene would appear to reduce the value of the Checkoway findings of increased risk for benzene.

Checkoway summarized the impact of benzene on more modern worker populations as follows:

> "While benzene exposure still occurs in the industry, it is present primarily as a contaminant of other substances, and exposures are much lower than in the 1920's; thus, it is quite likely that only small proportions of the cohorts studied were ever exposed to benzene in concentrations known to induce leukemia" (Checkoway, 1984).

Girard and Revol (1970) provided data on the incidence of various leukemia subtypes in a region of France where the population was felt to be exposed to benzene. It seems unlikely, however, that a community-based study almost 40 years ago would have been able to quantify the degree of benzene exposure that individual residents experienced, particularly since airborne concentrations are normally at the ppb level; too low to be measured reliably in the early 1970s. It is also unclear from the title of the article ("La Frequence d'une Exposition Benzenique au Cours des Hemopathies Graves," roughly translated as "Incidence of Exposure to Benzene in Severe Hemopathies") that leukemia was even

the focus of the study (Girard et al., 1970). It would be helpful to review a translated version of this study to understand how it was constructed, how the data were obtained and leukemia diagnoses confirmed, and how pertinent the findings are with respect to relating benzene exposure to leukemia subtypes (the only two categories listed by the authors are "CLL" and "acute leukemia") (Girard et al., 1970).

Linet et al. (1987) compared two different methods for coding job titles in collecting and reporting data from the National Occupational Hazard Survey. However, Linet et al. did not claim to find an increased incidence of CLL in the population studied; in fact, the reverse was stated.

> "It is interesting to note that the relative odds [for exposure] in general, were less than one when the analysis was confined to subject responders and, in fact, all of the relative odds, with the exception of asbestos in the Hoar et al. analysis, and carbon tetrachloride in the analysis based upon the NOHS data, were less than or equal to one" (Linet et al., 1987).

The authors then concluded that:

> "No statistically significant associations were found for CLL with occupations or industries of employment, or suspected exposures suggested by previous studies" (Linet et al., 1987).

Malone et al. was a population-based case-control study of CLL, where questionnaires were used by trained interviewers to obtain data on a wide variety of possible chemical exposures and other risk factors. The results for benzene associations were not striking.

> "Specific examination of reported exposure to benzene, which has long been suspected of being related to chronic lymphocytic leukemia, revealed a relative risk estimate of 1.1 (95 % CI 0.6–2.0)" (Malone et al., 1989).

The authors thought the reason they found little association with benzene and CLL may have been "…because of a lack of exposure to coal tar-based solvents within the study population, which may be young enough to have avoided massive exposure to coal tar-based solvents" (Malone et al., 1989). The numbers of cases thought to be associated with benzene exposure were not reported.

Others have suggested that CLL may be linked to benzene exposure. In a chapter from a European Environmental Agency Report (2001), Infante cited the Savitz and Andrews (1997) paper noted above as an epidemiologic study that "expanded the carcinogenicity of benzene to all major forms of leukaemia." He also pointed to the McMichael et al. (1975) study of rubber workers as further evidence of a link between benzene and CLL (Infante, 2001).

The McMichael publications examined mortality in a cohort of 6678 rubber workers over a 9-year period (1964–1972) and compared the observed deaths to those expected from the 1968 US age-specific death rates. McMichael et al. did not present exposure data from the workers included in the study, but instead assigned the "several hundred departments" and the more than 1000 different types of jobs into a reduced set of 70 occupational titles that the authors designed. With the help of some collaborating industrial hygienists, they created three solvent-exposure occupational groups defined as heavy, medium, and light exposure for 19 of the 70 new job categories. The 51 remaining categories were designated as "no solvent-exposure." McMichael et al. did not provide any description as to how benzene might have been used within the plants over the years, or when alternative solvents were put into service. Although the authors noted an elevated risk for "lymphatic" leukemias, they appeared to be aware that their findings were controversial, stating: "The association of lymphatic leukemia with exposure to organic chemicals appears to have no reported precedent" (McMichael et al., 1975).

Over the past 30 years, the findings of an increased risk for developing lymphocytic leukemias in rubber workers have not been replicated. The following characteristics of the McMichael et al. (1975) study make it difficult to rely upon as a basis for claiming a relationship between an increased risk of CLL and exposure to benzene:

- Exposures to multiple chemicals were described, but no characterization of these other exposures was provided
- Nonoccupational risk factors
  Smoking and other risk factors were not addressed
- Small population size and number of cases led to imprecise results
- Lack of benzene exposure data for individual workers or in the work environments
- Authors relied upon death certificate data without histologic confirmation of disease
  McMichael et al. included as "cases" any worker whose death certificate *mentioned* leukemia, regardless of its relationship to the cause of death
- Although the authors described a program of comprehensive research at four different major rubber companies, data on leukemia mortality were presented from only a single company

An investigation into a suspected excess number of CLL cases in a region of Queensland, Australia, was recently performed by Queensland Health, at the request of the regional government (Queensland Health, 2007). Looking at the incidence of CLL during the years from 1996 to 2004, the researchers found 22 cases, compared with an expected number of 14. After performing a comprehensive review of the reported cases, reviewing and summarizing the published literature with regard to the causation of CLL, and evaluating environmental emissions, occupational exposures and other alternative risk factors, these government researchers reported the following:

1. Six cases were found to have a first-degree relative known to have CLL; a markedly higher ratio than has been reported historically, suggesting an increased genetic component to the population studied.

2. No common occupational link was identified as occurring with greater frequency in cases compared to a normal background population.
3. No links were found between the chemical emissions studied and the incidence of CLL in the region.
4. The increased incidence initially reported was difficult to interpret, because the geographic boundaries and period of interest were defined after the cases were discovered. The authors felt that "Even given this, the difference could reasonably be the result of random variation in time and place" (Queensland Health, 2007).

The report concluded that: "The causes of CLL are unknown. Apart from increasing age and genetic variation in risk, there are no known risk factors. No environmental risk factor has been found to predict risk of CLL" (Queensland Health, 2007).

The WHO has been careful to distinguish myelogenous, rather than the broader category of "all leukemias," as being associated with benzene exposure (WHO, 1993). The ATSDR has been more specific, stating that: "Exposure to benzene has been associated with development of a particular type of leukemia called acute myeloid leukemia (AML)" (ATSDR, 2007). The only mention of CLL in their review was to relate the results of several industry cohort studies, where the incidence of CLL was not elevated in any of the categories of net benzene exposure in workers.

IARC has been similarly focused in its wording, stating: "The relationship between benzene exposure and the development of acute myelogenous leukaemia has been established in epidemiological studies" (IARC, 1982).

The case, then, for relating benzene exposure to CLL appears to be poorly developed. Well-designed studies that have included worker exposure data and that have considered alternative risk factors and chemical exposures will be necessary before it can be concluded that CLL can be reasonably associated with present or historical exposures to benzene in the workplace.

Benzene exposure and multiple myeloma (MM)

At various times over the past 15 years, there have been questions raised about whether occupational exposure to benzene might increase the risks for developing MM. Multiple myeloma is a disease of B lymphocytes that have matured into plasma cells and is typically very difficult to treat. Plasma cells are normal components of our immune system that create antibodies against invading agents. The transformation of normal plasma cell creation into the uncontrolled process of multiple myeloma, however, leads to the destruction of bone marrow, crowds out the process of normal blood component maturation, and often leads to frequent infections due to impaired immune capabilities (Rajkumar et al., 2005; Kyle and Rajukmar 2004; American Cancer Society, 2009). Multiple myeloma, like CLL, is a disease of adults, with an average age of onset of 70. Almost 20,000 new cases of MM were expected to be diagnosed in the United States in 2008, and typically represents about 10% of hematologic malignancies (American Cancer Society, 2009; Rajkumar et al., 2005). On the question of benzene and its relationship to multiple myeloma, health and regulatory authorities appear to generally be in agreement that the data do not support a link.

> "A higher risk of multiple myeloma was once thought to be associated with exposure to benzene. However, later studies have failed to confirm this" (EPA, 1998).

> "Reports linking exposure to benzene with other malignancies [other than AML] were considered to be inadequate for evaluation" (IARC, 1982).

Recent reviews of multiple myeloma have also emphasized the lack of clarity in the etiology and mechanisms of developing multiple myeloma.

> "Despite evidence for some clustering of MM and MGUS within families, the roles of genetic background and environment remain unclear" (Bergsagel, 2005).

> "The aetiology of myeloma remains essentially unknown, although recent studies suggest links to agricultural exposures and lifestyle factors, such as low socioeconomic status and obesity" (Joshua, 2005).

Multiple myeloma is characterized by the following risk factors:

- Age: Growing older increases the chance of developing multiple myeloma. Most people with myeloma are diagnosed after age 65. The disease is rare in people under 40.
- Race: The risk is highest among African Americans and lowest among Asian Americans. The reasons for the observed difference between racial groups is not known.
- Personal history of monoclonal gammopathy of undetermined significance (MGUS): MGUS is a condition in which abnormal plasma cells manufacture a variety of certain proteins. The current theory is that all cases of multiple myeloma are preceded by a period of MGUS that may last as long as 16-20 years (Landgren et al., 2009; Weiss et al., 2009).

> "Scientists are studying other possible risk factors for multiple myeloma. Radiation, pesticides, hair dye, certain viruses, obesity, and diet are under study. But it is not clear that these factors are involved in the development of the disease" (NCI, 2006).

Historically, there have been several case reports and a single major study that have suggested a link between benzene exposure and multiple myeloma. These are discussed below.

Aksoy in 1984 reported on seven patients with multiple myeloma, four of whom were workers with exposure to benzene. These four patients came from four different industries over a 10-year time period (Aksoy, 1984).

Decoufle summarized mortality statistics from a small petroleum refinery that was converted to a chemical manufacturing facility. A population of 259 workers who had worked some time at the company between 1947 and 1960 were followed through the end of 1977. Although the total deaths due to malignancy were lower than expected, the incidence of hematopoietic cancers was higher than expected. In the cohort, one worker died from multiple myeloma, and a second was diagnosed with multiple myeloma, succumbing ultimately to AML (Decoufle et al., 1983).

A careful reading of both the Aksoy (1984) and Decoufle et al. (1983) papers clearly indicates that the workers were exposed to other chemicals, but they are not described qualitatively or quantitatively. In addition, the complete occupational histories, understanding of familial medical history, and other possible risk factors are not provided; nor is there an indication as to the level of benzene exposure that might have been experienced by the workers who developed disease.

The Pliofilm cohort, mentioned earlier as the best population for evaluating the specific health risks of benzene exposure, has perhaps been the most widely cited study that reported an association between benzene and multiple myeloma. Rinsky et al. (1987) reported four cases of multiple myeloma ten years after their initial description of the cohort, although one of the cases worked just four days at the plant, making a benzene-induced disease seem rather unlikely. Overall, the risk of developing multiple myeloma did not seem to be related to cumulative exposure, with three of the four cases occurring in the lowest category of exposure reported by Rinsky et al. Total ppm-years for the cases were 0.11, 7.75, 19.5, and over 652 (Rinsky et al., 1987).

A follow-up report on the Pliofilm cohort 15 years later, however, appeared to dismiss the possibility of an association between benzene exposures and multiple myeloma (at least in this cohort) (Rinsky et al., 2002). Four new cases were described, but three of these cases were judged to be in workers unexposed to benzene in the workplace. The last worker had only a month of exposure, as well as an unusually long latency period of 38 years. These factors in combination make it virtually implausible that benzene played a role in these illnesses (Rinsky et al., 2002).

The Agency for Toxic Substances and Disease Registry (ATSDR) has summarized their impressions on benzene and multiple myeloma as follows:

> "The risk of mortality from multiple myeloma was increased in one of the early assessments of the Pliofilm cohort. The implication of this finding is unclear because the risk declined to non-significant levels in subsequent follow-up studies, and was not supported by the findings of other cohort mortality studies. Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma. A meta-analysis of case/control studies [Wong et al., 1997] found no significant association between occupational exposure to benzene and benzene containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents, petroleum, or petroleum products" (ATSDR, 2007).

Bergsagel et al. (1999) performed a comprehensive review of the literature with respect to benzene and multiple myeloma, summarizing data from a wide variety of industries in Europe, North America, and Australia and determined the plausibility of an association. They concluded that although high levels of benzene exposure were associated with an increased risk for AML,

> "There is no scientific evidence to support a causal relationship between exposure to benzene or other petroleum products and the risk of developing multiple myeloma" (Bergsagel et al., 1999).

Infante reviewed all "benzene cohort studies" reporting on the endpoint of multiple myeloma, and selected a subset of these for inclusion in a meta-analysis, coming to the opposite conclusion that there appeared to be "…a significant excess in the relative risk of MM in relation to benzene exposure" (Infante, 2006). His treatment of the underlying data for his analysis, however, is subject to question.

- Rinsky 2002 noted five cases of multiple myeloma from the exposed worker population. Two of these workers had very short periods of employment (4 days and 1 month), with net occupational exposures of 0.11 and 0.10 ppm-years, respectively. Attributing a causative role for benzene in these two workers with transient exposures appears unjustified, in our opinion.
- The latency periods reported for all five cases were remarkably long, compared to reported latencies for other benzene-related hematopoietic malignancies (Finkelstein, 2000; Glass, 2004). These ranged from 22.5 years to 38 years, with a mean latency of 27.4 years; time periods much greater than accepted latencies for benzene and AML (Finkelstein, 2000; Glass, 2004). Collins et al. also noted a prolonged latency of disease in their cases, with all 10 deaths occurring 20 or more years after first exposure (Collins et al., 2003). Justification for including these cases, and commenting upon a possible mechanism by which benzene might substantially extend its latency for causing hematopoietic disease in the case of multiple myeloma was not provided by Infante.
- Although he provided commentary on both the 1981 and 2002 Rinsky Pliofilm studies, Infante decided to perform his risk calculations on the study with 20 fewer years of accumulated data for his arguments regarding disease association. Using this earlier, less complete study substantially increased his reported risk numbers.

- Infante assumed that populations across the various studies in his meta-analysis were comparable and could be reasonably combined. However, exposures sustained by the various worker populations differed tremendously from one another. For example, half of the cases of MM reported by Collins et al. occurred in worker with less than 6 ppm-years of net benzene exposure (25% were less than 1 ppm-year of exposure). Fu et al. estimated that exposures for the Florence facility may have ranged from 25 to 600 ppm, in accordance with those reported by Aksoy (Fu et al., 1996).

A less confident statement as to the relationship between benzene and multiple myeloma was made by Goldstein 16 years earlier. His review commented on the 1987 follow-up by Rinsky of the Pliofilm cohort, and appears to have provided the source for many of the biologic plausibility arguments emphasized by Infante in his later review. Goldstein concluded that: "Overall, these findings are not sufficient to make an unequivocal statement that benzene is a cause of multiple myeloma" (Goldstein, 1990).

Apparently in response to Goldstein, Bezabeh et al. reviewed population-based and hospital-based case-control studies looking at the relationship between MM and benzene exposure. Odds ratios were approximately 1.0 from these studies, and although some individual studies of petroleum combustion products seemed to indicate an increased risk of disease, studies focusing on exposures to benzene, solvents containing benzene, and cigarette smoking all failed to show a positive relationship. The authors concluded that:

> "The current published case-control literature on benzene exposure is not ambivalent and does not indicate that benzene exposure is a risk factor for multiple myeloma" (Bezabeh et al., 1996).

Two recent papers have suggested a relationship between benzene exposure and the development of multiple myeloma, Kirkeleit et al., (2008) and Costantini et al., (2008). However,in light of the following shortcomings, we considered them unreliable.

As discussed previously, Kirkeleit et al. (2008) reported on the mortality statistics of upstream petroleum workers in Norway during two separate time periods between 1981 to 2003: "First exposure 1981-1985" and "First exposure 1986-2003." In the absence of actual quantitative exposure data for the worker population that was studied, the authors "…assume[d] that the 'upstream operators offshore,' who have had the most extensive contact with crude oil and natural gas, was highest and most homogeneously exposed to benzene." (Kirkeleit et al., 2008). Upstream operators in the earlier time period were found to have an increased risk of multiple myeloma, with nine observed cases and a reported relative risk of 2.85 compared to the general population. However, in the later time period, no cases of multiple myeloma were reported. The authors explained that this change might have occurred as a result of improved benzene regulations and exposure protection, but that explanation tends to dilute their argument that ongoing exposures to crude oil and petrochemicals are still creating excess risk of disease for petrochemical workers. In other words, if workers are still being exposed, but at lower levels, we would still expect to see some observable disease. In addition, the authors admit that detailed information on occupation, job tasks and potential exposure was lacking in the groups that they studied, which could have contributed to exposure misclassification. The authors admit that:

> "Previous studies that assessed the association between benzene exposure and hematologic neoplasms in cohorts of petroleum workers were limited by the lack of good exposure estimates including information on the variability of benzene exposure. This is also a major limitation of our study." (Kirkeleit et al., 2008).

Costantini et al. (2008) conducted a population based case-control study in Italy and used questionnaire data to assess exposures to solvents and other environmental agents. In this study, the authors reported odds ratios for select agents in 586 cases of leukemia and 263 cases of MM. The risk for the development of MM in the "benzene exposed" workers was not statistically significant, suffered from very small numbers, and showed very low precision (confidence intervals very wide in most cases). In particular, in workers with over 15 years of benzene exposure, five cases and three control subjects were reported with odds ratio of 4.1 (CI= 0.8-20). Interestingly, the authors also reported very similar associations for multiple myeloma with both xylene and toluene in the 15+ year exposure groups (odds ratios of 3.1 [CI=0.6 to 17.0] and 3.1 [CI=0.6 to 17.2], respectively). These reported findings, which are inconsistent with the literature on xylene and toluene health effects, provide further doubt as to the validity of the Costantini et al. data. More importantly, the authors failed to find any association between exposure to any solvent (including benzene) and the incidence of AML. This fact alone makes their claims of association between benzene exposure and other hematopoietic diseases much less plausible. The authors believe their failure to find AML in the population was "explained by the strict regulation of benzene in Italy nearly three decades prior two study initiation" (Costantini et al., 2008). If this reasoning is correct, then strict regulation of benzene should also remove the risk of other hematopoietic malignancies; a fact they fail to acknowledge.

In a recent study of 279 patients with multiple myeloma, compared to 782 control patients, variants of genes involved in the metabolism of exogenous chemicals were explored to determine a possible association with altered metabolism of various chemicals and the risk for developing MM. NAD(P)H:quinone oxidoreductase (NQO1) was selected as a focus for the metabolism of benzene, whereas genes involved in the metabolism of polyaromatic hydrocarbons (PAHs), dioxins, pesticides, and other agents were selected to evaluate MM risk with altered metabolism for these other chemicals.

Variants in NQO1 were not found to be associated with an increased risk for MM, providing suggestive evidence that variable abilities to metabolize benzene were not found to be associated with an increased risk for disease (Gold et al., 2009).

With the recent classification of multiple myeloma by the WHO as a "Mature B-cell neoplasm," the discussion regarding MM and benzene exposure will likely become absorbed into the discussion of NHL and benzene exposure. After considering the available studies, similar to our expressed opinions regarding NHL, we do not feel that there is sufficient evidence to establish a convincing relationship between multiple myeloma and benzene.

Benzene exposure and the myelodysplastic syndromes (MDS)

The myelodysplastic syndromes present a heterogeneous set of neoplastic diseases that are characterized by cytopenias in the peripheral blood, dysplasia in the bone marrow and progressive failure of hematopoiesis in one or more of the myeloid cell lineages (Bennett, 1982; Jaffe et al., 2001; List, 2004; Swerdlow, 2008). MDS are thought to originate in hematopoietic stem cells and a diagnosis of MDS offers an increased risk for the development of certain types of AML (Aul, 1998). Although progression of MDS to AML can occur as a natural course of the disease, the diagnostic distinction between MDS and AML is simply determined by the threshold percentage of myeloblasts observed in the peripheral blood and bone marrow: greater than 20% myeloblasts meets the criteria for a diagnosis of AML and less than 20% myeloblasts meets the criteria for a diagnosis of MDS (Jaffe et al., 2001; Swerdlow, 2008). The MDS subtypes are morphologically categorized by their varying degree of blast cells, lineage dysplasia, and presence of ringed sideroblasts (e.g., ringed sideroblasts are erythroid precursor cells with an accumulation of iron in the mitochondria). As with WHO's standardized criteria for diagnosis of leukemia subtypes and NHL, advances in tumor biology and diagnostic tools used to identify histopathological and genetic features have allowed the WHO to set forth uniform criteria for the diagnosis of MDS subtypes in the WHO tumor classification scheme published in 2001 and 2008 (Jaffe et al., 2001; Swerdlow, 2008).

Historically, epidemiologic reports studying the risks of occupational chemical exposures for developing hematopoietic disease have frequently not provided details on MDS subtypes. This lack of more specific MDS data has made it extremely difficult to associate exposures to benzene and other chemicals with specific subtypes. The changing definition of the various subtypes over the past 30 years has complicated matters even further. For example, MDS was often referred to as "preleukemia," "preleukemic state," or "subacute leukemia," terms that may encourage confusion with other hematopoietic diseases (Block, 1953; Dreyfus, 1976; Jaffe et al., 2001; Swerdlow, 2008). As such, the historic reports often included either a description of MDS along with a diagnosis of AML or MDS was categorized with other chronic myeloproliferative diseases (e.g., myelofibrosis) (Jaffe et al., 2001; Swerdlow, 2008).

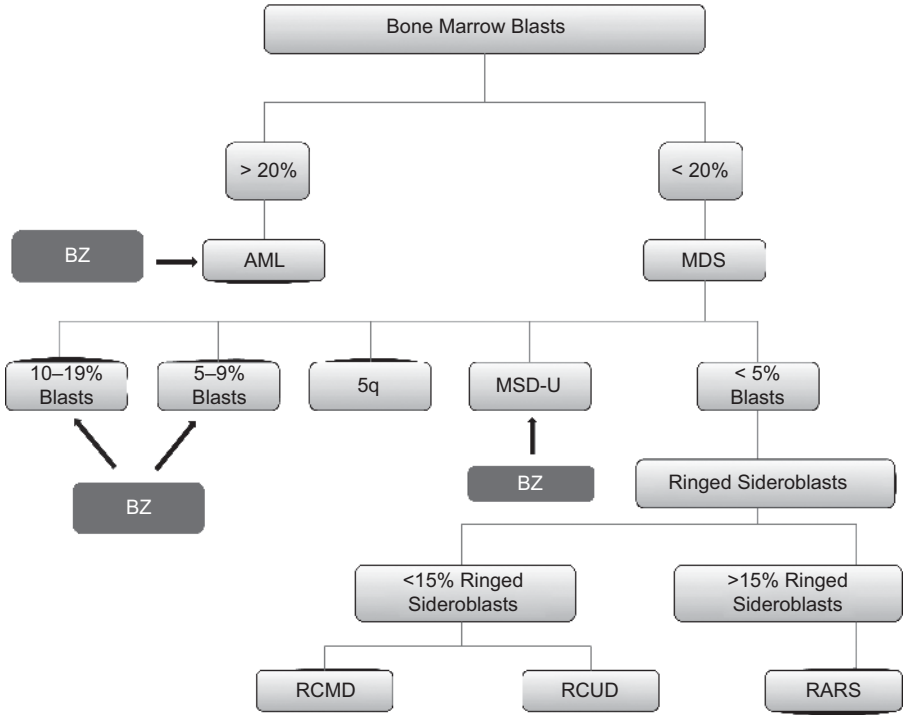

Figure 2. A schematic flow chart adapted from Bennett (2005) illustrates MDS subtypes as defined by the 2008 WHO. A (Bz) notation represents that an association between the MDS subtype and benzene exposure has been reported.

In 1976, the FAB cooperative proposed a classification scheme for a distinct group of conditions wherein the bone marrow exhibited hypercellularity but, unlike AML, fewer leukemic blast cells were present in the bone marrow. The FAB classification for this group was the "dysmyelopoietic syndromes" and two distinct subtypes were identified: refractory anemia with excess blasts (RAEB) and chronic myelomonocytic leukemia (CMML). In RAEB, the erythroid component predominated in the bone marrow and the erythroid precursor cells exhibited hyperplasia and dysplastic changes with or without the presence of ringed sideroblasts. In CMML, the granulocytic and monocytic components predominated in the bone marrow and the peripheral blood monocytes were elevated and displayed atypical morphology (Bennett, 1976). In 1982, the FAB began referring to the syndromes as "myelodysplastic" and proposed a new classification scheme following their review of the morphologic features of 80 separate cases, some of which exhibited difficult diagnostic clarity. In the 1982 report by Bennett and colleagues, they described the morphologic features of the bone marrow in cases of MDS, and these features were used to define five distinct subtypes. These subtypes included refractory anemia (RA), RA with ringed sideroblasts (RARS), RAEB, RAEB in transformation (RAEB-t), and CMML (Bennett, 1982).

In 1997, the WHO appointed a new committee that revised and updated the FAB classification scheme. In the process of revision, the committee took into account blast cell percentage, dysplastic features, prognostic features, and therapeutic outcomes (Bennett, 2005). The new criteria were published in 2001 by WHO and several distinct changes in the 1982 FAB classification were noted:

1. RAEB-t was eliminated as a MDS subtype and patients with bone marrow containing greater than 20% blast cells were considered to have AML.
2. The categories of RAEB-1 and RAEB-2 were created with blast cell percentage used as a distinguishing feature (5–9% and 10–19%, respectively).
3. CMML was eliminated as a MDS subtype and a new category was created, Myelodysplastic/Myeloproliferative Diseases, which now included CMML.
4. Three additional MDS subtypes were created which encompassed cytopenias with multilineage dysplasias (RCMD), MDS with no classifiable features (MDS-u), and the 5q minus syndrome (distinct from 5q– chromosomal abnormality) (Bennett, 2005; Jaffe et al., 2001).

The latest revision of the MDS classification scheme was published by WHO in 2008 and again the majority of MDS subtypes were distinguished by the observed percent of bone marrow blast cells, presence or absence of ringed sideroblasts, and evidence of dysplasia (Bennett, 2005). The 2008 WHO criteria is currently used for the diagnosis of hematopoietic diseases, although the classification of MDS continues to evolve and will no doubt be refined as we learn more about the biology and etiology of this disease. In the WHO 2008 classification scheme for MDS, refractory cytopenia with unilineage dysplasia (RCUD) and refractory anemia with ringed sideroblasts (RARS) represent MDS subtypes that are most commonly associated with erythroid dysplasia (Swerdlow, 2008) (Figure 2). RCUD includes refractory anemia, refractory neutropenia, and refractory thrompocytopenia as defined by a single cytopenia in the peripheral blood and a single lineage dysplasia in the bone marrow. Bone marrow findings for RCUD also include less than 5% myeloblasts and less than 15% of the erythroid precursors are ringed sideroblasts (Figure 2). RARS is defined by anemia in the peripheral blood and erythroid dysplasia in the bone marrow. Additional bone marrow findings for the classification of RARS include greater than 15% of the erythoid precursor cells are ringed sideroblasts and the observation of less than 5% myeloblasts (Bennett, 2005). Both RCUD and RARS have a low incidence of transformation to AML (Swerdlow, 2008). Refractory cytopenias with multilineage dysplasia are represented by one or more cytopenias in the peripheral blood and dysplasia in at least two myeloid lineages in the bone marrow. Levels of blast cells in the bone marrow are less than 5%. Both RAEB-1 and RAEB-2 are characterized by cytopenia(s) in the peripheral blood and unilineage or multilineage dysplasia in the bone marrow; the criteria for bone marrow blast cell number remain the same as in WHO, 2001 (Swerdlow, 2008).

It is noteworthy that MDS with an isolated 5q deletion are classified as an independent MDS subtype under WHO 2001 and WHO 2008 criteria. This subtype is unique in that cells exhibits a single del(5q) cytogenetic abnormality and the patient may present with increased numbers of platelets (thrombocytosis) instead of thrombocytopenia in the peripheral blood. Additional features characteristic of MDS del(5q) include anemia with or without other cytopenias in the peripheral blood and blast cells observed at less than 5% in the bone marrow (Jaffe et al., 2001; Swerdlow, 2008). Myelodysplastic syndrome-unclassifiable (MDS-u) represent clear cases of bone marrow dysplasia but they do not fit under any other MDS subtype classification. Two indications for classification in the MDS-u category:

1. A MDS case with lineage dysplasia in one or more of the myeloid lineages that is accompanied by pancytopenia, and RCUD or RCMD subtype that exhibits less than 1% blast cells in the peripheral blood.
2. A MDS case represented by bone marrow dysplasia in one or more lineage with fewer than 1% blast cells in the peripheral blood and less than 5% blasts in the bone marrow that is accompanied by a cytogenetic abnormality (Swerdlow, 2008).

As mentioned previously, the WHO classification schema for clonal hematopoietic neoplasms are a "work in progress" and, as our knowledge increases, MDS will be further defined (Swerdlow, 2008). As such, our interpretation of the association between benzene and the development of specific MDS subtypes most likely will evolve as well (Figure 2). Our current understanding is described below.

Benzene and preleukemia

Although some historic epidemiologic reports have described benzene-induced AML as a transition through a "preleukemic" state, MDS has rarely been reported as a toxicological endpoint. For example, Aksoy reported that "preleukemia" was a common observation in benzene-exposed workers in Turkey (Aksoy, 1980b). Some of the older reports of benzene-induced hematotoxic effects refer to pancytopenia occurring in the presence of "paradoxical hyperplasia" of the bone marrow, which is reminiscent of MDS. The blood dyscrasias that have been associated with high levels of benzene not only include cytopenias, pancytopenia, anemia, and aplastic anemia but also "preleukemic" dysplastic changes that are now generally consistent with MDS. In contrast, approximately 10% of the MDS cases diagnosed today are hypoplastic, making the differential diagnosis between MDS and aplastic anemia difficult (Swerdlow, 2008). That said, benzene-induced aplastic anemia as described in earlier studies may not meet currently accepted criteria for the proper diagnosis of aplastic anemia (i.e., pancytopenia and hypocellularity of the bone marrow). Moreover, some of the early cases of benzene-induced aplastic anemia were diagnosed primarily by pancytopenia in peripheral blood or by bone marrow findings at autopsy (Gross, 2010). By the early 1960s, bone marrow biopsies paved the way for the study of marrow architecture to assist in making a correct hematologic diagnosis (Mc Farland and Dameshek, 1958; Parapia, 2007).

Benzene and MDS in general

In more recent epidemiologic literature, the bone marrow morphology in benzene-exposed cases has been described but resolution for MDS subtypes associated with benzene exposure is lacking. For example, Ruis and colleagues (1994) described bone marrow morphology in 192 workers exposed to high concentrations of benzene at a steel plant in Brazil. The authors noted that the majority of bone marrow samples they examined were hypoplastic with a large decrease in number of granulocytic precursors documented. The authors reported erythroid dysplasia, atypia in all three myeloid cell lineages, and frequent stromal changes. Eosinophilia was also documented (Ruis et al., 1994). Travis et al. (1994) defined a series of seven MSD cases within a cohort of 74,828 benzene-exposed workers from across China. MDS subtyping was performed using the Proposed Classification Schema for MDS published by Bennett in 1982. The authors described MDS subtypes in four out of the seven MDS cases. They documented one case of RA and one case of RAEB in transformation. The other two MDS cases were defined as "not otherwise specified" (NOS). Travis also included one case of CMML, which would not be considered under the umbrella of MDS by WHO 2001 or WHO 2008 criteria for diagnosis (Travis et al., 1994). Linet et al. (1996) published findings from this same cohort and again, associated seven cases of MDS with benzene exposure. The authors provided an overall description of the bone marrow morphology in benzene-exposed workers diagnosed with MDS that included evidence of hypocellular bone marrow and dyserythropoietic changes (Linet, 2996).

Irons et al. (2005) reported on the bone marrow morphology in 23 Chinese workers exposed to high concentrations of benzene. The authors applied WHO 2001 criteria for diagnosis and reported that the bone marrow pathology of the workers was characteristic of hypoplasia, stromal degeneration, dyserythropoiesis, multilineage dysplasia, and severe dysplasia in eosinophilic precursor cells (Irons et al., 2005). Even with the improvement in diagnostic tools, the morphologic findings by Irons and colleagues are similar to that described by Ruis and colleagues in the Brazilian workers in 1994. However, Irons also observed hematophagocytosis and clonal expansion of T cells; observations that the authors suggest are a result of immune activation. In addition, Irons found no cytogenetic abnormalities in any of the analyzed samples. Irons concluded that these findings do not support the hypothesis that the disease process of benzene-induced AML shares a common mechanism with the development of therapy-related AML following exposure to alkylating agent or radiation (Irons et al., 2005). The authors acknowledged that quantitative information on the development of specific MDS subtypes in benzene-exposed individuals is currently lacking and future studies will no doubt provide insight into the disease specificity (Irons et al., 2005). The suggestion by Irons and colleagues that benzene-induced morphologic bone marrow changes were immune mediated was recently supported in a study performed by Song et al. in which 19 out of 22 patients with benzene-induced hematotoxicity responded to treatment with immunosuppressive therapy with cyclosporin A (Song et al., 2010).

Benzene and RCUD

As previously noted, the 2008 WHO classification scheme combined RA with refractory neutropenia and refractory thrombocytopenia into a single disease subtype, RCUD (Swerdlow, 2008). According to the 2008 WHO, RCUD is mainly diagnosed in older individuals and makes up approximately 10–20% of all MDS cases reported. RA is diagnosed most frequently, whereas refractory neutropenia and thrombocytopenia are rarely diagnosed (Swerdlow, 2010). It is not clear whether RCUD can develop in benzene-exposure individuals, as only a few cases have been reported in the literature, although single cytopenias, most notably anemia, leukopenia, and thrombocytopenia, have been reported following benzene exposure (ATSDR, 2007). Using the 1982 FAB criteria for diagnosis of MDS, Travis and colleagues reported one case of RA from a cohort of 74,828 benzene-exposed workers (Travis et al., 1994). Irons et al. incorporated the 2008 WHO classification system in a recent report of 29 cases of MDS that were occupationally exposed to high levels of benzene and the authors referred to these cases as "benzene signal cases." The authors noted that out the 29 benzene-exposed signal cases, two cases of RA, and two cases of RCUD were diagnosed. However, the authors determined that odds ratios for the development of these RA

and RCUD subtypes were 0.67 (95% CI 0.13–3.3) and 1.0 (95% CI 0.18–5.4), respectively (Irons et al., 2010).

Benzene and RARS

RARS has been a recognized disease entity for over a half a century and the unifying diagnostic feature of RARS has been the presence of ringed sideroblasts in the mitochondria of erythroid precursor cells, which are typically identified by iron staining of the bone marrow cells (Natelson, 2007a; Aul, 1998; Bennett, 2005; Catenacci, 2005). RARS occurs primarily in older individuals and accounts for approximately 3–11% of MDS cases (Swerdlow, 2008). As for benzene, the cumulative literature indicates that exposure to benzene is consistently not associated with the development of RARS (Bennett, 2005). In a recent publication, Natelson summarized the bone marrow morphology from several primary studies on benzene-exposed workers, and pointed out that there was a lack of documentation for ringed sideroblasts (Natelson, 2007a). For example, analysis of the bone marrow by iron stain from some of the AML and MDS cases identified from the cohort of 74,828 of benzene-exposed workers showed no indication of ringed sideroblasts (Linet, 1996). In Aksoy's study of Turkish shoe workers, he described the bone marrow abnormalities associated with benzene exposure for the AML cases identified from the shoe worker cohort and he reported that none of the observations was consistent with RARS (Aksoy, 1980b). Independently, Irons et al. (2010) and Ruiz et al. (1994) provided descriptive reports of bone marrow abnormalities in workers occupationally exposed to benzene and in both authors' description ringed sideroblasts were not noted (Ruiz et al., 1994; Irons et al., 2005). In addition, a 1992 study conducted by Garand and colleagues evaluated whether any of the 84 patients who were diagnosed with RARS recalled specific exposure to benzene. One out of the 84 RARS patients surveyed confirmed previous exposure to benzene, although radiation therapy was also reported in this patient (Garand et al., 1992).

Benzene and RCMD

The category of RCMD is a fairly new MDS subtype and was only incorporated into the MDS classification scheme in 2001 WHO (Jaffe et al., 2001). RCMD accounts for approximately 30% of diagnosed MDS cases (Swerdlow, 2008). Additional demographic characteristics of RCMD include a preponderance for the development of the disease in older males (Jaffe et al., 2001; Swerdlow, 2008). Due to the recent introduction of this subtype into the classification schema, reports on the development of RCMD following occupational exposure to benzene are limited. Irons and colleagues (2010) identified 17 cases of RCMD in the benzene signal cases outlined in their recent publication. But it is important to note that in this same study, 441 out of 611 MDS cases that were diagnosed over the 4-year study period were classified as RCMD, which resulted in 72% of the total MDS cases reported (Irons et al., 2010). Further, the authors reported the odds ratio associated with the development of RCMD subtype following occupational exposure to benzene was 0.58 (95% CI 0.2–1.4) (Irons et al., 2010).

Benzene and MDS-u

The unclassifiable category of MDS subtypes is another new category added to the 2001 WHO classification scheme for MDS and basically this category was recreated to define MDS cases that essentially lack features appropriate for classification in any other subtype (Jaffe et al., 2001; Swerdlow, 2008). Not surprisingly, the incidence for the development of MDS-u is unknown and there are only a few reports of the development of MDS-u following occupational exposure to benzene (Swerdlow, 2008; Irons et al., 2010, Travis et al., 1994). In 1994, Travis and colleagues reported two cases of MDS—not otherwise specified (NOS) in their cohort of 74,828 benzene-exposed workers (Travis et al., 1994). However, reclassification of these two MDS-NOS using current criteria for MDS subtypes may result in different conclusions as to disease risk. In a later study, Irons and colleagues found an increased number of MDS-u cases ($n=7$) within their 29 benzene signal cases as defined in their 2010 study, and they reported that the odds ratio for the development of this disease subtype was 11.1 (95% CI 1.34–92.4) (Irons et al., 2010).

Benzene and 5q minus syndrome

As previously discussed, MDS with an isolated deletion of chromosome 5q32 (5q– syndrome) is a unique MDS subtype with a single clonal chromosome aberration and distinct set of clinical features that include presentation in older individuals, preferentially affecting women, a median survival rate of greater than 10 years, and less than 10% of the cases actually transforming to AML (Swerdlow, 2008). 5q minus syndrome should be distinguished from therapy-related MDS and AML, which can occur following therapy with alkylating agents, evolve quickly to AML, and often exhibit cytogenetic abnormalities involving deletions in chromosomes 5 and 7 and/or complex cytogenetic abnormalities involving the two chromosomes; these are two distinctly different diseases with distinctly different disease outcomes (Jaffe et al., 2001; Swerdlow, 2008). However, exposures to benzene and other solvents have been associated with cytogenetic abnormalities involving chromosome 5 and 7 (Brandt, 1992; Rigolin, 1998; Natelson, 2007b; Pederson-Bjergaard, 1981; Fagioli, 1992; Cuneo, 1992).

Benzene and RAEB

It is important to note that any one of the MDS subtypes described above can evolve or transform into RAEB and that RAEB has been characterized as an MDS subtype in the MDS classification scheme since its inception in 1976 (Bennett, 1976). Without attention to morphology, cases of MDS are diagnosed as RAEB-1 if the blast cells observed in the bone marrow are between 5% and 9% and RAEB-2 is assigned to cases where the bone marrow blast cells fall between 10% and 19% (Jaffe et al., 2001; Swerdlow, 2008). It is also important to note that as of 2001, the criteria for the diagnosis of RAEB has changed in terms of blast cell number. Under 2001 criteria, the RAEB-t cases with

20–29% blast cells were considered to be AML (Bennett, 2005). This 2001 change in definition for MDS and AML has implications in the benzene literature, as there are several reports of the development of RAEB following occupational exposure to benzene. For example, Travis and colleagues (1994) noted one case of RAEB in their benzene-exposed cohort and Strom and colleagues documented an increased risk in the development of RAEB and RAEB-t following exposure to solvents, including benzene. Both of these reports used the 1982 FAB criteria for the classification of MDS subtypes (Travis et al., 1994; Strom 2005). Using the 2008 criteria, Irons et al. (2010) noted two cases of RAEB in the described benzene-exposed signal group, although he reported that in his study, the odds ratio for the development of RAEB subtype was 1.4 (95% CI 0.19–11.1) (Irons et al., 2010).

As with NHL nomenclature discussed previously, it is difficult, if not inappropriate, to directly compare historic epidemiologic reports of "preleukemia" and even "RAEB-t" with the current MDS classification scheme.

Toxicology studies and models for benzene toxicity

Over the years, benzene has been implicated as a cause or contributor to a wide variety of conditions, both acute and chronic, malignant and nonmalignant. Despite the best efforts of researchers across the globe, the exact mechanism of how benzene may lead to harmful effects in humans is still not well understood. There are several reasons for this. First, there is still no good animal model that has proved reliable for simulating the development of human leukemia after heavy and prolonged benzene exposure.

> "Animal responses to benzene exposure are variable and may depend on factors such as species, strain, duration of exposure, and whether exposure is intermittent or continuous. Wide variations have also been observed in normal hematological parameters, complicating statistical evaluation" (ATSDR, 2007).

> "Lack of genetic concordance between experimental hematopoietic neoplasms and human leukemias is a key limitation to the use of many animal models to leukemia hazard assessment" (McCormick et al., 2004).

The animal studies that are most frequently cited regarding the inhalational carcinogenicity of benzene have been performed in rats, where tumors of the Zymbal gland (an auditory sebaceous gland not found in humans) and oral cavity have been most commonly observed. In mouse studies, a broader range of lesions have been observed, including hematopoietic malignancies. The reasons for not being able to develop an effective and dose-dependent animal model for benzene-induced leukemia are not well understood, but may be influenced by the differing metabolisms and enzymatic detoxification systems relied upon by different mammalian species (Henderson, 1996). The varying organization and control of different mammalian genomes may also play a significant role.

Second, there is a lack of agreement as to how benzene or its metabolites cause the injury that leads to leukemic and preleukemic conditions. Four main mechanisms have been suggested, as described by Whysner et al. (2004):

1. DNA adduct formation or cross-linking by oxidized metabolites of benzene, such as hydroquinone, phenol, or benzoquinone
2. Direct oxidative damage to DNA strands by these intermediates
3. Physical damage to progenitor cell protein microstructures, which then directly affects the ability of these cells to replicate and divide
4. Direct inhibition by benzene and/or its metabolites on enzyme function, particularly a class of enzymes called topisomerases that operate in the coiling and uncoiling of DNA and assist in the transcription and replication process of cells

Transgenic mouse models have been used by some researchers in an attempt to shorten the latency of mutational events leading to hematopoietic diseases such as leukemia and lymphoma. Studies of benzene in drinking water, for example, have shown that benzene suppressed the proliferation of hematopoietic progenitor cells in transgenic mice and altered gene expression in key signaling pathways (Nwosu

Table 6. Risk factors for leukemia.

- Genetic predisposition
  - Down syndrome
  - Fanconi's anemia
  - Bloom syndrome
  - Blackfan-Diamond syndrome
  - Ataxia telangiectasia
  - Polymorphisms of genes active in metabolism and detoxification
  - Polymorphisms of genes responsible for DNA repair enzymes
  - Family history of leukemia
- Treatment with chemotherapy agents, particularly alkylating agents and topoisomerase inhibitors
- History of prior hematologic disorders
  - Myelodysplastic syndrome
  - Myeloproliferative diseases
- Exposure to radiation, either therapeutic or environmental
  - Diagnostic radiation is typically of much smaller magnitude, but excessive exposure to certain types of procedures may impart increased risk
- Occupational exposures
  - Chronic exposure to benzene exceeding federally approved safety limits (for AML)
- Environmental exposures
  - Certain viral agents
  - Cigarette smoke, primary and secondary
  - Dietary factors

Sources: American Cancer Society, 2009; National Cancer Institute, 2008; McCormick and Kavet, 2004; Leukemia and Lymphoma Society, 2009b.

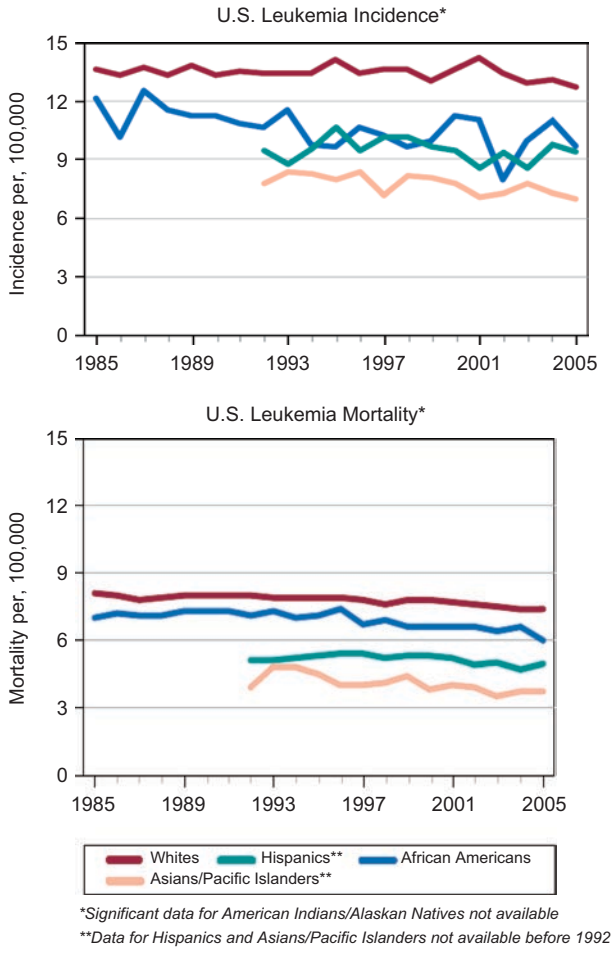

Figure 3. Age-adjusted total leukemia incidence and mortality in the United States compiled by Surveillance, Epidemiology and End Results (SEER). *Source*: NCI, 2005.

et al., 2004). The relevance to inhalational exposures is unclear.

Other researchers have employed alternative strategies to accelerate or increase the likelihood of malignant transformation in the animal populations studied. Trp-53 is a tumor suppressor gene product that inhibits malignant transformation (Siddique et al., 2006). Mice with a Trp-53 deficiency are less able to induce apoptosis following genotoxic damage. Trp-53–deficient animals thus show an increased genomic instability, and will experience a heightened rate of malignant transformation after DNA damage caused by various genotoxic chemicals. Kawasaki et al. recently examined Trp-53–deficient mice, and were able to induce hematopoietic cancers in a dose-dependent fashion in up to 100% of animals, demonstrating the importance of the Trp-53 function in preventing malignant transformation (Kawasaki et al., 2009). Researchers are optimistic that similar "knockout" studies with mice deficient in various metabolic, DNA repair, and other key functions will continue to expand our knowledge of a variety of disease pathways, including the events leading to malignant transformation following benzene exposure (Meek, 2010).

Dr. Zhang summarized the various possible mechanisms of benzene hematotoxicity at the 2009 Munich Benzene symposium as follows (Zhang, 2010):

- Generation of reactive oxygen species
- Direct chromosomal damage
- Inhibition of topoisomerase II
- Immune dysfunction
- DNA methylation
- Accumulation of toxic metabolites in the liver

Benzene has been identified by several researchers as an excellent candidate to exploit discovery strategies made possible by advances in toxicogenomics and toxicogenetics (McHale et al., 2009; Bollati et al., 2009). These approaches will allow more comprehensive and accurate risk assessments for any chemical with sufficiently characterized biomarkers and other "bioindicators of disease" (Edwards et al., 2008). The new "systems biology" approach is a major focus of the National Institute of General Medical Sciences (NIGMS; one of the National Institutes of Health), which currently supports ten National Centers for Systems Biology (NIH, 2009a). This trend of integrating experimental, computational, toxicologic, and clinical disciplines to study and better understand fundamental biologic processes in cells, tissues, and whole organisms represents a paradigm shift that will undoubtedly deliver enormous insight and ultimately better treatments for a wide variety of diseases.

Trends of benzene-related disease

Leukemia is an uncommon disease, with an annual incidence of approximately 16 cases per 100,000 in the male population (NCI, 2005) and the great majority of cases occur in people with no identifiable risk factors.

> "Weak or questionable exposure data may cause considerable confusion in the interpretation of studies, mainly if no personal measurements are available. Currently known risk factors account for about 15%–20% of the incident cases of leukaemia worldwide. Thus, the majority of leukaemia cases still remains unexplained" (Zeeb, 1998).

A compilation of risk factors for leukemia is provided in Table 6.

Although diseases such as mesothelioma have been related to contemporaneous asbestos exposure levels and regulatory guidelines (Price et al., 2004), no such relationship has been demonstrated in the case of benzene and leukemia, when looked at either by total leukemia incidence, or when subdivided into the disease most closely linked with benzene exposure, AML. Surveillance, Epidemiology, and End Results (SEER) data from the National Cancer Institute compiled in 2008 (Figure 3) show that age-adjusted total leukemia incidence in the United States has remained remarkably constant from 1975 to 2005, with a range of only 11.8 persons

per 100,000 to 13.5 persons per 100,000. These data seem to argue against any influence of exposure to benzene due to its presence in gasoline or vehicle exhaust, whose compositions have changed markedly over the past 30 years.

The ratio of affected males to affected females has also remained remarkably constant at around 1.7 to 1. AML data during the same time period ranged from 3.4 to 4.0 cases per 100,000, with a male:female ratio of 1.53 to 1 (NCI, 2005). As we have mentioned earlier, the accepted latency period for benzene exposure to developing malignant transformation is less than 20 years from the date of first exposure, with the strongest association being found for disease occurring within 10 years following exposure. No doubt, this figure is not precise since the latency should be based on the date of first "significant exposure" (perhaps defined as 1–10 ppm or greater on an 8-hour TWA basis). One would expect that with more stringent regulatory standards and improvements in industrial hygiene, we would be able to observe a drop in disease incidence in the last several decades as a result of reduced occupational exposures. The lack of a decrease in AML and total leukemia incidence in the 1975–2005 time period could be explained by occupational exposures to benzene already having fallen below "threshold" levels for benzene that at one time would have increased one's risk for disease.

Although studies of occupations with historically significant benzene exposures have provided evidence of the association of benzene with human disease, it should be recognized that at low exposures, these same associations are not well supported. In particular, a comprehensive meta-analysis of workers in the petrochemical industry involving over 200,000 workers found no increased risks for mortality due to any of the four major leukemia subtypes, even though low airborne concentrations surely existed in this industry (Raabe, 1996). Specifically, for the most part, workers experienced mean exposures during the average workday of less than 1 ppm, and had lifetime exposures less than 45 ppm-years. This is reinforced by recent studies of various Exxon refineries (Gaffney et al., 2009; Panko et al., 2009).

An industry-wide survey of petroleum worker exposures from over 14,000 samples found a mean exposure of 0.22 ppm (Runion, 1988) even during an era when benzene was not as well controlled or strictly regulated as today. Similar levels of exposure were reported for workers in the chemical industry, who likewise have been found to have no significant increased risk for leukemia (Bloemen, 2004; Bond, 1986; Ott, 1978). Exposure, then, is the critical feature distinguishing the levels of risk faced by the workers involved in these different industries. Where heavy, frequent exposure has been found, and occupational standards have either not existed or been ignored (Aksoy et al., 1972, 1974), significant risks of malignant and nonmalignant diseases have been found. Dermal exposures also factored into the total absorbed dose, but this route of exposure probably constituted less than 10–20% of the overall dose (Williams et al., 2005). Conversely, occupations with nominal or "trace" levels of benzene exposure would be expected to have a negligible or no increased risk of disease.

A few years ago, a detailed literature review summarized occupational exposures to benzene where quantitative data were available in North American studies across a wide variety of industries (Wijngaarden et al., 2003). The analysis concluded that:

> "It is surprising that in spite of the focus on benzene since the 1980s and the longtime recognition of its hematological effects, so little information was available on levels and determinants of exposure for many industries in the United States and Canada with potential exposure" (Wijngaarden et al., 2003).

There are no scientific studies showing that workers in occupations with "trace benzene" exposure are at increased risk of benzene related disease. For industries that do not possess industrial hygiene data, exposure simulations that mimic the working environments and occupational practices for certain classes of workers can provide a compelling basis for characterizing exposures and calculating historical and future health risks (Madl et al., 2002; Williams et al., 2007, 2008; Hollins et al., 2009).

Impact of Short Term or Peak Exposures

Environmental exposures are fairly constant during the day compared with occupational exposures, which often have two components: a low background concentration and a number of intermittent events which often involve so-called "peak exposures." For example, in the majority of studies in petroleum workers, it has been reported that industrial hygiene samples were collected during a dozen or so specific tasks within each workday. These tasks were often less than 15 minutes in duration and included activities such as sampling from a pipeline or changing in-line filter cartridge; both tasks are known to present some degree of elevated exposure unless respirators are properly worn (Gaffney et al., 2009).

The first mention of concern about the possible significance of peak exposures may have occurred in 1983, when Dr. Richard Irons addressed the International Conference on Benzene sponsored by the Collegium Ramazzini (Irons, 1983). Certainly, it was among one of the first times that benzene and "peak exposure" were the focus of the discussion. Dr. Irons noted at that time that "…our experimental evidence would highlight a concern that has been focused recently on transient exposure in the occupational environment rather than continuous low level exposure." He also stated: "In closing, I would like to mention, vis-à-vis the experimental regimen that we've used, intermittent exposure appears to be much more potent at producing bone marrow effects than is continuous exposure, and it may be that protection of the worker in an occupational setting requires prevention of peak exposures rather than the progressive lowering of the TWA in the absence of regulating or limiting transient exposure situations" (Irons, 1983). Our reading of this now quite dated paper is that the intermittent exposure regimen he tested had nothing to do with 15 min peak exposures versus 8 hr TWA,

but rather, he was concerned about the the increased toxicity of daily doses significantly above the occupational exposure limits. However, for many years, his statement has been cited as evidence for potential increased benzene toxicity following intermittent peak exposures, even when the peaks were as low as 10 ppm for up to 15 minutes per day.

Twenty years ago, Paxman and Rappaport (1990) tackled this issue regarding the hazard of peak exposures directly when they decided to evaluate the scientific basis for OSHA promulgating an STEL for benzene. By this time, physiologically based pharmacokinetic (PBPK) models had advanced to a point where they could do a reasonable job at predicting target tissue concentrations for a number of reactive metabolites for chemicals such as methylene chloride, styrene, and benzene, among others. These authors specifically evaluated the papers upon which OSHA had decided that a STEL could be justified (Divine and Barron, 1986; Picciano, 1979; Irons, 1983; Tice et al., 1989; Tort et al., 1982). Paxman and Rappaport (1990) concluded that "in summary, we contend that, to justify the setting of a short-term exposure limit (STEL) for benzene, OSHA incorrectly used the epidemiologic and toxicologic data." Later, they noted that "the animal data do indicate the possible importance of regularly spaced exposures of several hours duration, but this is not relevant to the issue of short-term exposures." Here the authors focused on exposure periods of 15-30 minutes or less, since they were the time periods of most interest in the ACGIH guidelines and the OSHA regulations.

Recently, Knutsen et al. (2008) extended the prior work of Paxman and Rappaport (1990) and that of other researchers. They developed a kinetic model that indicated that benzene and metabolite concentrations in the bone marrow resulting from widely varying exposure profiles were very similar when the cumulative workday exposures (dose) were limited to 8 ppm-hrs, which is equivalent to the current OSHA PEL at 1 ppm as an 8-hour TWA. For the scenarios studied, only minor changes in metabolite levels with or without CYP2E1 induction were observed at 8 ppm-hr. These results are consistent with the physiologic damping effect reported by previous investigators (Paxman and Rappaport, 1990). These authors used simple toxicokinetic methods to conclude that the health hazard or potential for increased toxicity resulting from a dose of eight ppm-hours delivered over 15 minutes was not appreciably different than the same dose delivered over eight hours. While the peak concentration is 32-fold greater in the former exposure scenario, both Paxman and Rappaport, as well as Knutsen et al. (2008) concluded that the peak benzene concentration in the liver should be within the capacity of the cytochrome P450 system to maintain first-order metabolism. Thus, the various tissue doses, expressed as "areas under the curve" (AUCs), the preferred dose metric for a chemical like benzene, would be the same for both exposure scenarios (Andersen et al., 1987).

As noted by various researchers, for an agent like benzene, in which the average blood concentration of the reactive chemical over time above a particular level in the target organ is the most likely cause of toxicity, the daily AUC (per workday) should be the best dose metric for predicting adverse effects, provided that peak blood concentrations remain below levels in which significant in vitro toxic effects are induced. It is acknowledged that duration of exposure at concentrations around 25-30 ppm can be biologically significant since several researchers have reported increased risks of frank benzene hematotoxicity and leukemia in workers with chronic workday 8 hr/day exposures to benzene averaging about 30 ppm or higher (Aksoy and Erden, 1978; Aksoy et al., 1974; Schnatter et al., 1996; Qu et al., 2002, 2003; Yin et al., 1987).

Even though additional compartments and metabolic pathways were added to the Knutsen et al. model, their conclusions were similar to those offered by Bois and Paxman (1992). That is, it appears that the area under the tissue concentration-time curve remains the same as long as the daily cumulative exposure is less than about 40-80 ppm-hr. This exposure would be equivalent, at 80 ppm-hr, to a employee being exposed to 40 ppm for 15 minutes up to 8 times per day, assuming there was no other exposure to benzene. It is not surprising that physiologic damping occurs with benzene due to the effect of distribution and metabolism of occupational exposures near the current OSHA PEL of 1 ppm (AUC of 8 ppm-hrs) and approaching daily doses of 80 ppm-hrs. A similar physiological phenomenon would be expected for those chemicals with moderate biological half-life and moderate lipid solubility. We would also agree with Bois and Paxman's conclusion that: "…the evidence was insufficient to implicate the rate of exposure as a causal factor in the chronic health effects of benzene" (Bois and Paxman, 1992). We acknowledge, however, that recent work by Rappaport et al. (2010) suggests that a different benzene metabolite appears to be formed at very low doses, and that this moiety may eventually be shown to be important in the understanding the overall toxicity posed by benzene.

Environmental exposures to benzene

Many studies have been conducted on human exposure to benzene in our environment. Benzene is present in the air of our cities due to the combustion of gasoline and diesel fuel from our automobiles and trucks, as well as from the burning of fuel in our homes and exposure to cigarettes (either directly or secondhand). It has been estimated that the average nonsmoker in the United States takes in around 200 micrograms of benzene per day in the course of performing their normal daily activities (Wallace, 1996a, 1996b). More recently, it has been suggested that environmental exposures are typically below 50 ug/m3 (Capleton and Levy, 2005).

The single greatest societal source of nonoccupational benzene exposure remains cigarette smoking, with smokers taking in approximately 40–80 μg with each cigarette. Specifically, a one-pack-per-day smoker receives a benzene "dose" of 0.8–1.6 mg (one-thousandth of a gram), or about ten times the daily intake of a nonsmoker (Wallace, 1996a, 1996b). It has been estimated that more than half of our net societal human exposure to benzene occurs in the form of

cigarette smoking (Wallace, 1996a, 1996b; Capleton and Levy, 2005). Combining efforts to continue reducing smoking with efforts to reduce hydrocarbon emissions from combustion sources would appear to be the most effective ways to reduce the risk of benzene exposure to our citizenry at large. Further research on the association between cigarette smoking and leukemia appears to indicate that the FAB subtype M2 has been most tightly linked to disease risk, with subtypes M4 and M5 implicated to a lesser degree (Pogoda et al., 2002; Sandler et al.,1993). A more recent case-control study on smoking as a risk factor for MDS and AML reported a "weak association between smoking and MDS, and for an association between smoking and abnormalities involving chromosomes 5, 7, and 8 in MDS and AML. No apparent overall effect of smoking on the risk for AML was observed" (Bjork et al., 2009).

The human health impact from benzene exposures at "environmental" concentrations has also been explored.

> "Overall, the evidence from human studies suggests that any risk of leukaemia to adults at general population continuous exposure concentrations of 3.8 to 42 μg/m^3, which have been derived from available United Kingdom exposure data—that is, it is at concentrations three orders of magnitude less than the occupational lowest observed effect level—is likely to be exceedingly small and probably not detectable with current methods" (Duarte-Davidson et al., 2001).

Studies of traffic density in metropolitan areas have been performed, with some claiming an association between high traffic density and the incidence of childhood cancers, particularly leukemia (Savitz et al., 1989; Nordlinder et al., 1997; Knox et al., 1997; Harrison et al., 1999; Pearson et al., 2000). These reports, however, are difficult to interpret, since levels of benzene and other potentially harmful chemicals are not actually measured, and the childhood leukemias are often lumped together into a single disease category. One study that did provide a breakdown on the incidence of various leukemias in the studied population (Crosignani, 2004) reported that acute lymphocytic leukemia, a disease not generally associated with benzene exposure, was responsible for 79% of the leukemias. Furthermore, it reported that "the low number of exposed cases with other leukemia subtypes (5 cases) precluded further analysis by histotype" (Crosignani, 2004).

Other studies have failed to confirm an association between traffic density and incidence of childhood cancers. In a comprehensive California study looking at cancers in children under five years of age over a 10-year period, the authors concluded that: "…no increased cancer risk among offspring of mothers living in high traffic density areas for all cancer sites or leukemia." In fact, the highest-exposed subgroup had an odds ratio for all cancers, and for leukemia in particular, of less than one (Reynolds, 2004). In addition, a Danish study that related benzene concentrations, nitrogen dioxide levels, and traffic density to the incidence of childhood diseases failed to find any relationship to leukemia (Raaschou-Nielsen, 2001). Similar findings were reported by Langholz (2002) and Reynolds (2002). For an overview of various environmental and genetic risk factors that have been evaluated for childhood leukemias over the years, we recommend the review by Buffler et al. (2005).

Conclusion

In summary, there is general agreement that benzene exposure, in high concentrations over many years, leads to an elevated risk for AML. Although there have been reports of disease associations with other forms of leukemia, as well as with lymphomas and multiple myeloma, the overall evidence to date has been spotty and does not indicate a consistent causal relationship. At lower doses of benzene, there may be a physiologic response, but studies performed to date have not shown a convincing relationship between these responses and an increased risk of developing acute or chronic diseases. It has been historically acknowledged in toxicology and medicine that a biological response, in and of itself, is not necessarily indicative of an increase health risk. Indeed, there is an entire body of science that has evaluated the hermetic response to low-level exposures, where the end result is a lesser incidence of adverse effects (Calabrese, 2008).

It is clear that most significant exposures to benzene in the United States occurred in the workplace prior to 1950. Workers, as well as the public, have been exposed to lesser concentrations of benzene since that time due to a better understanding of the hazards as well as increasingly strict regulatory standards (especially post-1980). The increased appreciation of the potentially harmful health effects of benzene over the past 50 years has dramatically reduced the magnitude of worker exposures here in the United States. The lack of reports finding increased prevalence of disease in workers exposed after 1970 indicates that few workers in Western society are currently at increased risk. In developing nations, where occupational and environmental controls are often less restrictive, cumulative exposures may still be leading to measurable benzene-related health risks (Ruchirawat, 2010; Bao et al., 2009).

Our knowledge regarding the impact of benzene on human health continues to evolve. Hopefully, as we better understand at the molecular level how benzene and its metabolites may relate to the process of carcinogenesis, we will be in a better position to evaluate the risks associated with low-level, chronic exposure to doses of benzene (less than 40 ppm-years) with respect to the development of hematopoietic and other disease processes.

Declaration of interest

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opinions and perspectives are those of the authors, some of whom have served, and may continue to serve on expert scientific
panels that evaluate the potential health hazards posed by benzene, and may act as expert witnesses in litigation or while addressing governmental bodies. The authors wish to extend their deep thanks to Carrie Kahn for her keen technical assistance during the preparation of this manuscript.

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