Benzene-Associated Leukemia and its Risk Assessment

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Abstract: Benzene-Associated Leukemia and its Risk Assessment: Takashi KANEKO, et al. Department of Environmental Health, Medical University of Yamanashi—There is sufficient epidemiological evidence indicating that benzene is a human carcinogen. Although some reports suggest an association of benzene with multiple myeloma, malignant lymphoma and lymphatic leukemia, the closest association is found with the development of acute myeloleukemia. In contrast to epidemiological findings, benzene has induced a variety of tumors in tissues other than bone marrow in experimental animals. Attempts to induce acute myeloleukemia in rodents by benzene have not been fully successful until recently. While relatively low doses of benzene have been confirmed to induce chromosomal aberrations, sister chromatid exchanges and micronuclei in rats and mice, it has not been proven that similar changes occur in humans. For evaluation of the dose-response relation of benzene-induced leukemia, the epidemiological studies with a Pliofilm (rubber hydrochloride) cohort are considered most reliable and useful. According to recent re-examinations of the Pliofilm cohort, it is highly possible that the past epidemiological studies with this cohort underestimated the benzene exposure level, and thus overestimated the risk of benzene toward leukemia. Many extrapolation models including a linear model, a linearized multistage model, a conditioned log-logistic model, a quadratic model and a proportional hazards model have been used in the calculation of benzene risk. The risk values calculated so far with the Pliofilm cohort greatly differ according to the extrapolation models employed, and thus it cannot be determined which model is superior. While benzene exposure in the workplace is decreasing, petroleum containing a few percent of benzene has become an important source of benzene exposure for both industrial workers and the general population. Some reports suggest a positive association between gasoline exposure and the development of acute myeloleukemia.

Key words: Benzene, Gasoline, Metabolism, Species difference, Genotoxicity, Carcinogenicity, Leukemia, Risk assessment

The acute toxicity of benzene is manifested by its effect on the central nervous system (anesthetic action) and skin and mucous membrane irritations. Individuals exposed to very high concentrations of benzene (usually > 1,000 ppm) shows signs of central nervous system disorders, giddiness, euphoria, nausea and headache. If workers get used to the aromatic odor of benzene, they can work for few hours in the presence of benzene at concentrations between 500 and 1,000 ppm. In general, an 8-h exposure to around 100 ppm of benzene does not induce distinct acute toxicity.

Long-term exposure to a high concentration of benzene induces hemopoietic toxicity (myelotoxicity). Chronic benzene exposure can induce a spectrum of pathological states ranging from mild leucopenia or anemia to pancytopenia (depression of the levels of all circulating blood cell types), depending on the exposure level (concentration × duration) and individual susceptibility. Serious intoxication presents a feature of aplastic anemia, characterized by a marked decrease in hemopoietic function of the bone marrow. Clinically, anemia and bleeding tendencies (e.g., bleeding from the nose and gums, subcutaneous bleeding, and hypermenstruation) are typical symptoms.

Because benzene has been identified as a human carcinogen (leukemogen), the use of benzene in industries as a solvent has been strictly controlled and measures to decrease benzene exposure in workplaces have been introduced in most countries. Nowadays, concerns with benzene are centered on whether long-term, low-level exposure to benzene is a cause of leukemia not only in industrial workers but also in the general population. This review focuses upon the relationship between benzene and
leukemia.

Metabolism of benzene in relation to its toxicity

Benzene myelotoxicity is not induced by benzene itself; its metabolic intermediates are the cause (Fig. 1). Cytochrome P-4502E1 (CYP2E1) is a major catalyst of the oxidation of benzene in the liver (the first step of benzene metabolism) in humans, rats, mice, and in rabbits.

Benzene epoxide was once suspected as a toxic intermediate, but recent findings indicate that catechol, benzoquinone and hydroquinone may be the cause. Erexson et al. exposed human peripheral T-lymphocytes to benzene and its metabolic intermediates (phenol, catechol, 1,2,4-benzenetriol, hydroquinone, 1,4-benzoquinone and t,t-muconic acid) and observed the frequency of sister chromatid exchanges (SCE), the mitotic index and the cell cycle kinetics. The SCE frequency was increased dose-dependently by benzene, phenol, 1,2,4-benzenetriol and hydroquinone, while the mitotic index was decreased and the cell cycle kinetics was inhibited. The effects of benzene and its metabolites, when compared in terms of SCE induction, were in the order of catechol > 1,4-benzoquinone > hydroquinone > 1,2,4-benzenetriol > phenol > benzene. t,t-Muconic acid did not show any effect.

Although benzene is metabolized mainly in the liver, its main toxicity appears in the bone marrow, and thus the relation between metabolism and toxicity is complex. At present, most researchers support the hypothesis that metabolic intermediates such as phenol, catechol and hydroquinone produced in the liver are transported to the bone marrow where they are metabolically activated to ultimate toxic intermediates. The secondary activation in the bone marrow apparently plays an important role in the development of myelotoxicity including carcinogenicity, but the final steps have not yet been identified. While t,t-muconaldehyde, catechol, 1,4-benzoquinone and hydroquinone are gathering attention recently, some reports suggest the importance of hydroquinone and 1,4-benzoquinone among these.

Yager et al. examined the induction of chromosomal aberrations in human lymphocytes by metabolic intermediates of benzene (phenol, catechol, hydroquinone and 1,4-benzoquinone) in a micronucleus test. Using anti-kinetochore antibody, the cells were divided into those with the micronuclei containing whole chromosomes (kinetochore-positive) and those with the micronuclei having a part of chromosomes (kinetochore negative). All of the metabolic intermediates of benzene induced micronuclei in the lymphocytes. While the induction of micronuclei by phenol, catechol and 1,4-benzoquinone was 2- to 5-fold, that by hydroquinone was 11-fold. Since numerous micronuclei and an increased number of kinetochore-positive micronuclei were induced by low concentrations of hy-

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**Fig. 1.** Metabolic pathways of benzene. Adapted from Subrahmanyam et al.
detroquinone, Yager et al. considered that hydroquinone played a major part in the induction of chromosomal aberrations in human lymphocytes.

Glatt et al.\textsuperscript{20} reported an experimental result indicating that the mutagenicity of hydroquinone and 1,4-benzoquinone in V79 Chinese hamster cells was stronger than that of any other metabolic intermediates. Further, Post et al.\textsuperscript{21} demonstrated that phenol was oxidized by macrophage peroxidase to a metabolic intermediate which covalently bound to cellular macromolecules. They presumed this intermediate to be either hydroquinone or benzoquinone on the basis of their stronger effects on RNA synthesis.

**Interaction among benzene metabolites**

While a single metabolic intermediate of benzene (phenol, catechol, hydroquinone, 1,4-benzoquinone or \(\text{t,t-muconaldehyde}\)) induces micronuclei and SCE in human lymphocytes, myelotoxicity caused by benzene does not occur \textit{in vivo} by any of the intermediates alone. However, when phenol and hydroquinone were administered simultaneously to B6C3F\(_1\) mice, inhibition of bone marrow function occurred, which was not seen by the single administration of any other compounds\textsuperscript{22-24}. To elucidate this phenomenon, Smith et al.\textsuperscript{23} examined the mutual interaction between phenol and hydroquinone metabolism and found that phenol greatly accelerated the oxidation of hydroquinone to 1,4-benzoquinone by myeloperoxidase. To further elucidate the mutual interaction, Subrahmanyam et al.\textsuperscript{24} administered \([^{14}\text{C}]\)phenol and \([^{14}\text{C}]\)hydroquinone to mice either independently or in mixture and observed their covalent binding to the macromolecules in blood, bone marrow, liver and kidneys. The result was that phenol promoted the covalent binding of hydroquinone or its metabolites in blood and bone marrow. These authors supported the view of Smith et al., proposing that the interaction between hydroquinone and phenol on myelotoxicity was due to an acceleration of myeloperoxidase-dependent metabolism of hydroquinone to 1,4-benzoquinone by phenol.

A synergistic interaction of benzene metabolites has also been confirmed in culture cells. Robertson et al.\textsuperscript{25} examined the appearance of micronuclei in cultured human lymphocytes using phenol, catechol and hydroquinone and found that a combination of hydroquinone with catechol increased the appearance of micronuclei synergistically. This also supports the finding of Smith et al.\textsuperscript{23} that the simultaneous administration of benzene's phenolic metabolites caused myelotoxicity similar to that caused by benzene itself.

Based on the above reports, the prevailing working hypothesis is that the metabolic pathway from hydroquinone to benzoquinone with production of a semiquinone radical is associated with benzene myelotoxicity. Several intermediate metabolites of benzene seem to be involved synergistically in the myelotoxicity. The formation of oxygen radicals\textsuperscript{26} or DNA adducts\textsuperscript{27}, or the inhibition of DNA polymerase\textsuperscript{28} has also been postulated as the underlying mechanism of the hydroquinone-benzoquinone pathway-induced chromosomal aberrations.

Oliveira and Kalf\textsuperscript{29} observed the effect of hydroquinone on the differentiation of human pro-myelocytes (HL-60) into granulocytes/phagocytes. Hydroquinone specifically inhibited the differentiation of HL-60 cells to phagocytes. The authors suggested that benzene myelotoxicity could be explained in part by the inhibition of differentiation of stem cells to phagocytes by hydroquinone, because phagocytes take part in the regulation of hematopoietic function by producing interleukin (IL)-1.

Phagocytes and fibroblasts, which are interstitial cells of the bone marrow, are closely associated with the proliferation of myeloid and lymphatic cells\textsuperscript{31,30}. For example, phagocytes in the bone marrow have high peroxidase activity and metabolize hydroquinone to 1,4-benzoquinone, while the activity of DT-diaphorase, a reductase which inactivates the benzoquinone, is low\textsuperscript{31}. Twerdok et al.\textsuperscript{32} also reported that DT-diaphorase plays an important role in the defense system against the myelotoxicity of 1,4-benzoquinone. Ganousis et al.\textsuperscript{33} measured the activities of enzymes associated with the activation and inactivation of hydroquinone/benzoquinone (e.g., peroxidase, glutathione S-transferase, UDP-glucuronyl transferase and DT-diaphorase) in the bone marrow interstitial cells (phagocytes and fibroblasts) of B6C3\(_1\) mice. They found that the glutathione S-transferase and DT-diaphorase activity levels were high in fibroblasts, while the activity levels of peroxidase and UDP-glucuronyl transferase were high in phagocytes. Although the exact nature of the peroxidase involved in the hydroquinone → benzoquinone reaction was not clearly demonstrated, apparently the enzyme was not prostaglandin H synthase, as suggested earlier by Schlosser et al.\textsuperscript{34}, because the reaction progressed with peroxide but not with arachidonic acid. The report of Ganousis et al. includes the interesting result that the activation of hydroquinone to benzoquinone was greatly augmented by the simultaneous addition of catechol.

Guy et al.\textsuperscript{35} administered 1,4-benzoquinone, \(\text{t,t-muconaldehyde}\) and hydroquinone intraperitoneally
Species difference in benzene metabolism

A species difference was observed in the metabolism of benzene: mice produced active metabolic intermediates to a greater extent than did rats\(^{39, 40}\). Sabourin et al.\(^{41}\) compared benzene metabolism between F344/N rats and B6C3F\(_1\) mice repeatedly exposed to 600 ppm benzene (6 h/d, 5 d/wk, 3 wk). Benzene metabolism was evaluated by the urinary excretion of metabolic intermediates, formation of hemoglobin adducts and appearance of micronuclei in erythrocytes. No effect of cytochrome P-450 induction by repeated exposure to benzene on the metabolism was observed in any parameter. The only change induced by the repeated exposure was that gluturorurate conjugation became dominant in rats, in contrast to sulfate conjugation in mice. Comparison between the rats and mice revealed that the formation of hemoglobin adducts was similar, despite the finding that benzene metabolism in the mice was 3 times higher than that in the rats. Benzene exposure for one week significantly increased the appearance of micronucleated polychromatic erythrocytes in the mice, but not in the rats. However, the number of micronucleated erythrocytes in the mice did not increase further by additional benzene exposure.

Benzene metabolism according to exposure concentration

According to the results of recent studies, exposure concentration seems to greatly affect the metabolic pathway of benzene. The formation rate of active metabolites was higher at low than at high exposure concentrations\(^{42, 43}\).

Medinsky et al.\(^{44}\) simulated benzene metabolism in men using a physiologically-based toxicokinetic model on the basis of the data obtained from experiments with mice. In the exposure to benzene at concentrations lower than 10 ppm, the rate of metabolism was directly proportional to the concentration. Moreover, the main metabolites at such a low exposure level were hydroquinone conjugates, which are closely related to benzene toxicity. However, as the exposure level was raised, the formation of phenyl conjugates, which is a detoxification pathway, became the major metabolic route. Assuming that benzene toxicity is associated with hydroquinone formation, a linear extrapolation of the results of toxicity tests at high exposure levels would result in underestimation of the effect.

Carcinogenicity of benzene

Epidemiological studies on benzene leukemia

Four key epidemiological studies concerning benzene leukemia have been reported: 1) Aksoy’s survey of shoemakers in Turkey\(^{45, 46}\), 2) Wong’s survey of chemical factory workers\(^{47}\), 3) the survey at Dow Chemicals by Ott et al.\(^{48}\) and Bond et al.\(^{49}\), and 4) the study by Rinsky et al. in Pliofilm (rubber hydrochloride) manufacturing plants\(^{50, 51}\).

Paustenbach et al.\(^{52}\) evaluated these studies as follows: Aksoy’s study\(^{45, 46}\) reported that 51 cases...
of leukemia occurred in shoemakers exposed mainly to benzene between 1967 and 1983. The incidence (13 per 100,000) was significantly higher than that of the general population in Turkey, i.e., 2.5–3 per 100,000. However, the major drawback of this study is the lack of information related to the exposure level of benzene. In Wong’s study, the relative risk cannot be calculated because there was no death due to leukemia in the control group. In addition, the exposure group had no case of acute myeloid leukemia, which is known to be strongly associated with benzene, among the cases of death due to leukemia. While Ott et al. examined three cases of leukemia occurring among 594 workers exposed to benzene in 33 years (1940–1973), those exposed to benzene were also exposed to various other chemical compounds. In comparison to these studies, the Pliofilm cohort of Rinsky et al. is considered the best at present to assess the risk related to benzene exposure in that the exposure level was relatively well estimated and the cohort subjects were not exposed much to chemicals other than benzene. This study will be mentioned in detail later in the section of risk assessment.

Yin et al. reported an epidemiological study related to benzene leukemia carried out for the first time in China. Although the use of benzene in industries has been strictly controlled worldwide, benzene was used in various factories in China in 1970s or before. The study of Yin et al. is a retrospective cohort study conducted in 233 factories using benzene and 83 control factories in 12 cities of China. The exposure group consisted of 28,460 workers (178,556 person-years between 1972 and 1981), and the control group 28,257 workers (199,201 person-years). The exposure group had 30 cases of leukemia (25 dead and 5 alive), and the control group four cases (all dead). The majority (76.6%) of leukemia cases (myelocytic 13, monocytic 4, myelo-monocytic 2, lymphocytic 3, and erythroleukemic 1) occurring in the exposure group were of the acute type. The benzene level in the air to which the patients had been exposed was in the range between 10 and 1,000 mg/m³ (mostly between 50 and 500 mg/m³). The mortality rate from leukemia was 14/100,000 in the exposure group and 2/100,000 in the control group. The standardized mortality ratio (SMR) adjusted by the control group was 5.74 (p < 0.01 by U test).

The mortality rate from cancer as a whole in the benzene exposure group (123.2/100,000 person-years) was higher than that in the control group (54.7/100,000 person-years) and, in the male exposure group, not only the number of deaths from leukemia (SMR 5.74) but also that from lung cancer (2.31) were high. In contrast, leukemia was the only malignant tumor with a higher mortality rate in the female benzene exposure group than in that in the control group. This study supported the results of animal experiments to be described later, where benzene induced tumors in multiple organs. In this connection, among the recent epidemiological studies, there is a report suggesting that benzene is related not only to acute myeloid leukemia (specifically, nonlymphatic leukemia) but also to tumors of the lymphatic tissues including multiple myeloma, lymphoma and lymphocytic leukemia. While the studies of Yin et al. included a large number of workers exposed to benzene, its major drawback may be the lack of accurate information on the level of benzene exposure.

**Benzene carcinogenicity in animal experiments**

While the benzene-induced malignant tumor usually observed in humans is acute myeloid leukemia (AML), benzene has induced a variety of tumors in experimental animals. Based on the carcinogenicity study in progress in the Bologna Cancer Institute since 1976, Maltoni et al. reported that benzene was a multipotential carcinogen, showing tumorigenicity in various tissues including Zymbal gland, oral cavity, liver and mammary gland in rats. According to the reports of Huff et al., benzene induced tumors in various tissues including Zymbal gland (both sexes), oral cavity (both sexes) and skin (male only) in F344/N rats, and Zymbal gland (both sexes), lung (both sexes), Haderian gland (male), perpetual gland (male), mammary gland (female) and ovari (female) in B6C3F1 mice, supporting the above study by Maltoni et al.

These results demonstrate that although the relation between benzene and tumors in tissues other than hematopoietic tissues is unclear in humans, the majority of tumors caused by benzene in experimental animals are tumors other than leukemia, suggesting that the target tissues of benzene differ between humans and rodents. Except for two studies by Goldstein et al. and Cronkite et al., all studies attempting to induce AML in rodents (rats and mice) by benzene have failed.

Snyder et al. of the New York University Institute of Environmental Medicine were the first to report a case of leukemia induced by benzene in animal experiments. They exposed C57Bl/6J mice to 300 ppm benzene for 6 h/d, 5 d/wk for life and detected thymic lymphomas in 8 of 40 animals.

Goldstein et al. reported four cases of bone marrow tumors in rodents probably induced by benzene. They observed the development of one case of myelogenous leukemia, one case of acute
myeloblastic leukemia and one case of granulocytic bone marrow hyperplasia out of 40 CD-1 mice exposed to 300 ppm benzene for 6 h/d, 5 d/wk for life. They also reported one case of myeloid leukemia out of 40 Sprague-Dawley rats exposed to 100 ppm benzene under a similar experimental schedule. The authors suggested that, although the incidence was not significantly different from that in the control animals, the myeloid lesions were caused by benzene because there had been no report of spontaneous myeloid leukemia occurrence in these two species before. The report of Goldstein et al. is considered the first report suggesting the association of benzene exposure with the development of myeloid leukemia in rodents.

Cronkite et al.61) exposed female C57Bl/6 mice to 300 ppm benzene for 6 h/d, 5 d/wk for 16 weeks and continued observation throughout life thereafter. While only one of the 88 control group animals died in the control group, 10 of the 90 animals in the exposure group died, by 64 weeks after the start of the study. Thymic lymphoma was detected in six of the 10 dead mice in the exposure group and unspecified lymphoma in the other two. Another animal in the moribund state was sacrificed and confirmed not to have leukemia. The cause of death of the remaining animal that died could not be specified because a part of its body was cannibalized and tissue liquefaction had already started. The cause of death of the control animal was confirmed to be neither lymphoma nor leukemia. These results indicated that benzene could induce lymphoma in C57Bl/6 mice. This study differed from others in that the animals were exposed to benzene for only 16 weeks and the rest of life was left in the non-exposed state. However, it should be emphasized that the C57Bl/6 mice used is liable to develop leukemia by nature. In the experiment by Snyder et al.62) mentioned above, leukemia developed in C57Bl/6 mice but did not occur in AKR/J mice.

In a later study, Cronkite et al.61) exposed male CBA/Ca mice to 10, 25, 100, 300, 400 or 3,000 ppm benzene for 6 h/d, 5 d/wk for up to 16 weeks, and observed the myelotoxicity. Two-week exposure to 10 ppm benzene caused no hematological effect, but two-week exposure to 25 ppm benzene significantly decreased the number of lymphocytes in the peripheral blood. Exposure to 100, 300 or 400 ppm benzene induced a dose-dependent decrease in the number of lymphocytes, myelocytes and spleen colony-forming units (CFU-S) in the bone marrow. A marked decrease in lymphocytes and a decrease in CFU-S in the bone marrow occurred by exposure to 300 ppm benzene for two, four, eight and 16 weeks. The damage in the bone marrow caused by exposure to 3,000 ppm benzene for 8 days was less than that caused by exposure to 300 ppm for 80 days. When CBA/Ca mice were exposed to 300 ppm benzene for 6 h/d, 5 d/wk for 16 weeks, myelogenous tumor (leukemia) developed in male mice. However, Farris et al.64), in a study based on the experimental procedure employed by Cronkite et al., did not observe the development of myeloid leukemia due to benzene in CBA/Ca mice.

Farris et al.64) conducted a carcinogenicity test of benzene using CBA/Ca mice to examine benzene-induced myelogenous (granulocytic) leukemia in this strain of mouse. An exposure group consisting of 150 CBA/Ca mice was exposed to 300 ppm of benzene for 6 h/d, 5 d/wk for 16 weeks, and the same number of mice (control group) inhaled air containing no benzene for the same period of time. After 16-week exposure, the animals were kept under ordinary conditions, and dead or moribund mice were autopsied to confirm the cause of death. Observation was continued until the survival rate of the exposure group had declined to 20% (22 months after the start of exposure). At the end of the observation period, blood and bone marrow of the 24 surviving mice of the exposure group and those of 24 randomly chosen control group mice were histologically examined. At the time when the survival rate in the exposure group was 20%, 60% of the control group animals were alive (p < 0.01). The major cause of death in the exposure group during the observation period was malignant lymphoma accompanying metastasis. Throughout the observation period, malignant lymphoma occurred in 14 out of 118 animals (12%) in the exposure group and in two of 119 (2%) in the control group (p < 0.002). Preputic cell carcinoma developed in 71 mice (60%) in the exposure group while it did not occur in the control group. The incidence of lung adenoma was also significantly higher in the exposure group (36%) than in the control group (14%). The incidence of Zymbal gland carcinoma (11% vs. 1%) and that of squamous cell carcinoma in the anterior stomach (7% vs. 0%) were also higher in the exposure group, but the difference was not significantly different. No significant difference was observed in the incidence of Haderian gland adenoma (6% vs. 5%).

A striking result of this carcinogenicity study is that there was no occurrence of granulocytic leukemia in CBA/Ca mice, even though the investigators conducted the study with the same protocol as that of Cronkite et al.61), who reported the leukemia caused by benzene. In the study of Farris et al.64),
bacteria, drosophila and mammal cells were
While most mutagenicity tests of benzene using
become purulent ulcers.
preputic adenoma and Zymbal gland adenoma to
active change produced by the vulnerability of both
hyperplasia was caused not by benzene, but by a re-
control group). Farris et al. suggested that this
hyperplasia of myelogranulocytes was seen at a high
frequency in the exposed group (36% vs. 8% in the
control group). Farris et al. suggested that this
hyperplasia was caused not by benzene, but by a re-
active change produced by the vulnerability of both
preputic adenoma and Zymbal gland adenoma to

Genotoxicity (Mutagenicity)

While most mutagenicity tests of benzene using
bacteria, drosophila and mammal cells were negative,69–71, exposure to benzene induces
chromosomal aberrations in the bone marrow cells
or peripheral lymphocytes of animals and humans.
In particular, benzene is known to increase the inci-
dence of SCE and micronuclei. Experimental results
have indicated that benzene induces the
chromosomal changes at relatively low doses.
Styles and Richardson68 exposed 6–8 week-old
Wistar rats to 1, 10, 100 or 1,000 ppm of benzene
for six hours. The frequency of chromosomal aber-
rations increased significantly in rats exposed to 100
and 1,000 ppm benzene. Although no significant
increase was observed in rats exposed to 1 or 10
ppm benzene, the incidence rate of chromosomal aberrations increased with the level of benzene ex-
posure; 0.25% at 0 ppm, 0.41% at 1 ppm, 0.91% at
10 ppm, 1.23% at 100 ppm and 4.49% at 1,000
ppm, a finding which indicated a dose-effect rela-
tion between the exposure dose and the inci-
dence of chromosomal aberrations (excluding gaps).

While this study has found that a single exposure
to benzene resulted in chromosomal damage in rats,
apparently contradictory experimental results have
been documented in literature69: the same exposure
centration of benzene induced a higher inci-
dence of chromosomal aberrations in a group ex-
posed for two hours than in a group exposed for six
hours, and the aberrations occurring from a single
exposure did not appear after repeated exposure.
Thus, the chromosomal aberrations observed after a
single exposure of animals to benzene needs further
careful evaluation.

By exposing CD-1 mice to benzene (0, 40, 100 or
1,000 ppb) for 22 h/d, 7 d/wk for 6 weeks, Ward
et al.66 observed the variation in hypoxanthine-
guanine-phosphoribosyl transferase (hpdr) in
the splenic lymphocytes. Variant cells were distin-
guished by observing 3H-thymidine uptake in the
presence of 6-thioguanine by autoradiography. The
frequency of variant cells (Vf) in female mice was
7.2 × 10−6 at 0 ppb, 29.2 × 10−6 at 40 ppb, 62.5 ×
10−6 at 100 ppb and 25.0 × 10−6 at 1,000 ppb. The
Vf was higher in female mice than in males, but the
tendency was similar in both sexes. Although it is
not clear why frequency of hpdr variation increased
at 100 ppb and decreased at 1,000 ppb, a similar
tendency was also noted in chromosomal aberrations
in splenic lymphocytes in the same animals. It
is interesting that the mutagenicity test was positive
at a concentration as low as 100 ppb, but it is nec-
essary to take into account the fact that the animals
in this study were exposed to benzene for 22 h a
day, with only a two-hour break from exposure per
day.

Benzene metabolites such as hydroquinone,
catechol and 1,2,4-benzenetriol form DNA adducts
in HL-60 cells, mouse bone marrow macrophages,
and human bone marrow cells71–73. In contrast to
these in vitro findings, the evidence for the forma-
tion of DNA adducts in animals treated with ben-
zone has been contradictory. 32P-postlabeling
studies have demonstrated DNA adduct formation
in the liver of New Zealand rabbits74 and in the
bone marrow of B6C3F1 mice75 treated with ben-
zene; however, Reddy et al.76,78 were unable to
detect the formation of DNA adducts in various
tissues of either Sprague-Dawley rats or B6C3F1
mice administered benzene or its hydroxylated met-
obolites.

Lee et al.79 identified p-benzoquinone (BQ) and
1,2,4-benzenetriol (BT) as toxic metabolites of ben-
zene on the basis of their inhibitory effect on DNA
synthesis. Later, Lee and Garner80 investigated the
capability of benzene and the two metabolites to
induce DNA strand breaks was in either the in vivo
or the in vitro system by comparing the DNA elu-
tion rate on a fine membrane filter at alkaline pH.
In the in vitro system where bone marrow cells were
reacted with test chemicals for 60 min, both BQ
and BT induced a dose-related increase in alkali-
labile DNA single-strand breaks (SSBs) of bone
marrow cells. However, when glutathione (350 μg/
ml) was added to the same reaction system, the
DNA damaging effect of BQ (24 μM) and BT (24
μM) was blocked by 100 and 53%, respectively.
Catalase (130 units/ml) completely blocked the
DNA damaging effect of BT, while no protection
was afforded with BQ. Consistent with these obser-
vations, no induction of alkali-labile DNA SSBs was
observed in the in vivo system by an anesthetic dose
of benzene (1,760 mg/kg, ip or po) at 1-, 24-, and
36-h postadministration in both male and female
ICR mice. From these results Lee and Garner con-
cluded that benzene exposure would not induce
direct DNA strand breaks in vivo under realistic
work-related or accidental exposure conditions and
also that caution should be exercised in the inter-
pretation of in vitro data for whole-body toxicity
Many surveys have been conducted to clarify the effect of benzene exposure on chromosomes in peripheral lymphocytes in humans. As mentioned above, although chromosomal aberrations have been confirmed to be induced by relatively low doses of benzene in animal experiments, similar changes have not been proven to occur in humans.

Sarto et al. examined the frequency of SCE in the peripheral lymphocytes of 22 workers in the chemical industry manufacturing benzene from coal tar. These workers were exposed to 0.2–12.4 ppm benzene for 11.4 ± 7.0 years. Workers in neighboring factories where no benzene was used served as the control group. The control subjects were matched the exposure group subjects by sex, age, smoking habit and residential area. The exposure group was divided into a high-exposure group and a low-exposure group according to the benzene concentration in the expired air and urinary excretion of phenol, a metabolite of benzene. The results demonstrated that the SCE frequency, which had no significant difference between the exposure and control groups, was even lower in the high-exposure group than in the low-exposure group.

Clare et al. also examined chromosomal aberrations and SCE in workers exposed to relatively high concentrations of benzene 3 months earlier and reported that chromosomal aberrations due to benzene exposure could not be detected. In their report, nearly 1,200 gallons of benzene was accidentally leaked during a ship loading, and 10 workers were exposed to benzene. The urinary excretion of phenol in the workers after the accident was quite high. Clare et al. collected venous blood samples from these 10 workers (exposure group) and 11 workers who were engaged in similar jobs but were not exposed to benzene (control group), and examined the chromosomal aberrations in lymphocytes. Lymphocytes were cultured to promote division, and 200 cells in the middle stage of mitosis during a 49-h culture were examined for evaluation of the chromosomal damage, while 30 cells in the second stage of mitosis during a 72-h culture were examined for detection of SCE. The number of cells showing chromosomal aberrations was larger in the control group than in the exposure group, and this tendency was particularly prominent for chromosomes with chromatid gaps. However, all the values were within normal range. A few of the exposed workers showed a slightly higher incidence of SCE, leading to a positive correlation between urinary phenol excretion at the time of exposure and SCE frequency ($r=0.55$, $0.05 < p < 0.10$), but no evidence indicating that the single exposure caused continuous chromosomal damage was obtained. This indicates that either chromosomal damage was not induced by benzene exposure at such a level (the mean urinary phenol concentration at a short time after exposure was 127.3 mg/l) or that the damage which might have been present at the time of exposure disappeared over the period of 3 months. Thus, chromosomal damage by a single exposure to benzene has not been confirmed in humans, in contrast to the results of animal experiments.

Major et al. compared the incidence of chromosomal aberrations (CA), the frequency of SCE and the proliferation rate index (PRI) of the peripheral lymphocytes between workers engaged in the distillation of benzene at petroleum refineries exposed to low doses of benzene (mean concentration, 7.2 mg/m$^3$ or 2.3 ppm) and control workers. While exposure to this low dose of benzene raised the incidence of CA and SCE, it did not affect the PRI. Smoking habit was stratified according to self-statement and serum thiocyanate concentration. No influence of smoking on CA, SCE or PRI was shown. Smoking is known to cause chromosomal changes (SCE in particular), but the changes were not demonstrated in this study.

According to Santos-Mello and Cavalcante, the incidence of chromosomal aberrations in peripheral lymphocytes was significantly high among the gasoline stand refueling attendants in Rio de Janeiro and Sao Paulo, Brazil, compared to that in the control group. Although this report did not provide the benzene content in the gasoline, the subjects were considered to have inhaled quite a large amount of gasoline vapor, because only a few gasoline stands were equipped with an automatic refueling system in those cities at the time of the survey and the refueling workers were said to judge the filling of a gas tank by sound, by touching their ears to the car’s gas tank opening.
Chronic benzene toxicity in relation to carcinogenicity

Whether chronic benzene intoxication is involved in the development of leukemia is of great concern in benzene toxicology. The epidemiological study\(^5\),\(^5\)\(^4\), which Yin et al. carried out in China, indicated a relation between these two pathological states. According to these authors, out of 25 cases of death due to leukemia in the benzene exposure group, 7 had chronic benzene intoxication before developing leukemia. The mortality rate from leukemia in patients with chronic intoxication was 700/100,000 person-years, a 50-times higher rate than that in the whole exposure group (14/100,000).

Based on this observation, Yin et al. suggested that prevention of chronic benzene intoxication would be effective for prevention of benzene leukemia. However, it is uncertain whether or not chronic benzene intoxication (that is, exposure to high concentrations of benzene) is a prerequisite for the development of leukemia.

Ruiz et al.\(^1\) reported an interesting finding in relation to hypoplasia of bone marrow due to chronic exposure to benzene. Workers at a steelworks in Sao Paulo were exposed to high concentrations (8–2,000 ppm) of benzene for a long period because of a leak from the gas pipe of a smelting furnace. The jobs of 152 workers were changed due to abnormalities observed in hematological examination (mostly neutropenia). Bone marrow samples were collected from these workers and examined histologically. This examination was conducted for the workers whose peripheral neutrophil counts were less than 2,000/mm\(^3\), with the erythrocyte sedimentation rate and the reticulocyte count within the normal ranges. Their feces were tested for intestinal parasites three times, and only those whose fecal tests were negative were included in the study.

Sixty percent of the subjects complained of general malaise including asthenia, myalgia, drowsiness and vertigo. Fifty-four percent of the subjects had white blood cell counts less than 4,000/mm\(^3\) in the peripheral blood, but anemia (hemoglobin content < 13 g/100 ml) was detected only in 9%, and only one case showed thrombocytopenia (< 150,000/mm\(^3\)). However, eosinophilia (> 500/mm\(^3\)) was observed in 27% of the subjects. A typical change in bone marrow histology was hypoplasia of hemopoietic tissue; this change was observed in 82% of the subjects. Although most of the hypoplasia cases (87%) were due to a decrease in the precursor cells of granulocytes, eosinophilia in the bone marrow was observed in 71% of the cases. Ruiz et al. stated that eosinophilia was one of the characteristic symptoms of chronic benzene intoxication, since the subjects presented no allergic symptoms and were negative in parasite tests. It is not clear at present whether the changes seen in the peripheral blood and the bone marrow of the subjects with chronic benzene intoxication were caused by benzene alone or by a combination of benzene with other factors.

Biological monitoring of benzene exposure

In the likely event of lowering the occupational exposure limit of benzene from 10 ppm to 1 ppm or lower, the measurement of urinary phenol content will be useless for monitoring benzene exposure. In recent years, attempts have been made to use urinary concentrations of benzene metabolites other than phenol, \(t, t\)-muconic acid (MA) or S-phenylmercapturic acid (S-PMA), as markers of benzene exposure.

Inoue et al.\(^8\) were the first to use the urinary MA concentration for the biomonitoring of occupational exposure to benzene. Ducos et al.\(^8\) reported a method for detecting MA in urine as low as 0.05–0.1 mg/l by high pressure liquid chromatography (HPLC) using a simple clean-up procedure. According Ducos et al., MA rarely exceeds 0.5 mg/l in the urine of non-exposed persons, and benzene exposure of a few ppm can be distinguished by its measurement. An ordinary intake of sorbic acid, a food additive which is a precursor of MA, will affect the biomonitoring only insignificantly.

Bechtold et al.\(^9\) proposed a method for measuring urinary MA by gas chromatography-mass spectrometry (GC-MS) after trimethylsilyl esterification. This method is claimed to be sensitive and applicable to benzene exposure at the 1 ppm level.

Stommel et al.\(^9\) exposed rats to benzene at levels of 1 to 500 ppm and assayed the urinary concentration of S-PMA using amino acid analysis. A significant correlation existed between S-PMA and benzene exposure level. In order to confirm the possibility of monitoring benzene exposure by measuring urinary S-PMA, they determined the S-PMA concentrations in the urine of workers occupationally exposed to benzene. In workers who were exposed to benzene at the maximum level of 0.15 ppm for 8 h, the concentration of 12.0 ± 16.0 \(\mu\)g/g creatinine at the end of work. In workers exposed to 1.13 ppm benzene for 12 h, the pre-exposure concentration of 25.1 ± 25.1 increased to 70.9 ± 109.2 \(\mu\)g/g creatinine. No significant change was observed in urinary phenol at these low levels of exposure. Urinary S-PMA may be useful as a marker for benzene exposure at
levels below 1 ppm, but its complicated measurement method remains a problem.

Bechtold et al. measured a hemoglobin adduct with benzene oxide, S-phenylcysteine (SPC), by GC-MS, and examined its usefulness as a marker of benzene exposure. SPC was detected dose-dependently in rats and mice exposed to benzene. However, SPC was not detected in workers exposed to 28 ppm benzene for 8 h/d, 5 d/wk. Later, Bechtold et al. reported a method for measuring SPC as an albumin adduct by modifying the purification method of blood albumin. The SPC in albumin was detected in workers exposed to benzene. Albumin SPC levels in 7 out of 9 subjects who were not occupationally exposed to benzene were below the detection limit (0.1 pmol SPC/mg albumin), suggesting that albumin SPC may be a promising biomarker for benzene exposure at low levels.

Bechtold and Henderson compared the usefulness as a biomarkers of benzene exposure between urinary MA and albumin SPC. MA concentrations in the urine of workers exposed to 4.4 ppm benzene for 8 h (n = 8) and that of control subjects (n = 5) were 6.2 ± 3.1 and 0.27 ± 0.21 μg/g creatinine (p < 0.005), respectively, and the minimum value in the exposed group exceeded the maximum value in the control group. A regression line between albumin SPC and benzene exposure levels (0-23 ppm) had a slope of 0.044 ± 0.008 (pmol/mg albumin vs. ppm) with an intercept of 0.135 ± 0.095 pmol/mg albumin. From these results, Bechtold and Henderson concluded that urinary MA may be one of the most useful biomarkers for occupational exposure to benzene. MA can probably distinguish the exposure at 1 ppm. However, since benzene exposure in the general environment requires a biomarker for a long-standing exposure, albumin SPC (half life, 20 days) may be a promising biomarker for the general population's benzene exposure.

Risk assessment for occupational exposure to benzene

The U.S. NIOSH (United States' National Institute for Occupational Safety and Health) recommended in 1976 that benzene should be controlled as a human carcinogen and the U.S. OSHA (Occupational Safety and Health Administration) proposed in 1978 that the permissible exposure limit (PEL) for benzene should be lowered from 10 ppm to 1 ppm. This proposal was rejected by the U.S. Supreme Court in 1980 after objections from industries. However, Rinsky et al. and the IARC (International Agency for Research on Cancer) published reports stating that exposure to benzene at 10 ppm could well result in excess death from leukemia, and the U.S. OSHA proposed again in 1985 to reduce the benzene PEL to 1 ppm. The risk assessment by Crump and Allen was a direct motive for lowering the PEL by the U.S. OSHA.

Benzene was the first substance for which the U. S. EPA (Environmental Protection Agency) conducted a risk assessment on the basis of epidemiological data. The risk assessment used the data from Pliofilm and Dow Chemical cohorts, estimating benzene exposure in terms of cumulative exposure (products of concentration and time) and using a linear nonthreshold model. According to the assessment, excess death from leukemia at 45 ppm-year (1 ppm, 8 h/d, 5 d/wk for 45 years) was 14.9/1,000 from the Pliofilm and 46.4/1,000 from the Dow Chemical data. White et al. also assessed the risk from the data of Pliofilm and Dow Chemical using a one-hit model. They assumed that the exposure level was equal to its threshold limit value (TLV) at that time. In their results, excess death from leukemia at 30 ppm-year (1 ppm, 8 h/d, 5 d/wk for 30 years) was 3-11/1,000 (Pliofilm) and 3-10/1,000 (Dow Chemical).

The ACGIH (American Conference of Governmental Industrial Hygienists) changed its carcinogenicity classification of benzene from A2 (suspected human carcinogen) to A1 (confirmed human carcinogen) in May of 1990 and proposed to reduce its TLV for benzene from 10 ppm (32 mg/m³) to 0.1 ppm (0.3 mg/m³). This proposal was based on the risk assessment of Rinsky et al. for benzene-induced leukemia. Although this proposal was to be formal after one-year discussion, an ACGIH booklet of 1994-1995 TLVs noted that the TLV of benzene was changed to 0.3 ppm (0.96 mg/m³), putting off a final decision even further.

The study of Rinsky et al. which provided the basis for the ACGIH's TLV revision in 1991 was a survey in which the relation between benzene and leukemia in the Pliofilm cohort was reevaluated. The cumulative benzene exposure level was estimated individually from the record of past environmental measurement and the individual working record. The SMR of leukemia in the whole cohort was 3.37 (95% confidence interval, 1.54-6.41), and that of multiple myeloma was 4.09 (1.10-10.47). When the SMR was calculated from the cumulative exposure dose (ppm-year), it was 1.09 for 40 ppm-year or lower, 3.22 for 40-100 ppm-year, 11.86 for 200-399 ppm-year and 66.37 for 400 ppm-year and higher. A logistic regression analysis of the dose-response relation revealed that the lowering of the benzene exposure level would exponentially reduce the risk of death from leukemia.
According to the study of Rinsky et al., the risk (odds ratio) of dying from leukemia by 40-year benzene exposure was 154.5 (95% confidence interval, 3.1–7785) at 10 ppm, 1.7 (1.1–2.5) at 1 ppm and almost equal to the background risk (1.05; 1.01–1.09) at 0.1 ppm. Thus, this report indicated a risk of death from leukemia due to occupational exposure to benzene at 1 ppm.

This benzene-leukemia risk assessment by Rinsky et al.\textsuperscript{51} eliminated the ambiguity in the dose-response relation in the past Pliofilm studies. In spite of the criticism that Rinsky et al. overestimated the risk of benzene-induced leukemia, the report was epoch-making in that it suggested a possibility of leukemia development by exposure to 1 ppm benzene. The results reported by Rinsky et al. have been reassessed by Lamm et al.\textsuperscript{100}, Byrd and Barfield\textsuperscript{101}, Yardley-Jones et al.\textsuperscript{102} and Paustenbach et al.\textsuperscript{103}.

Yardley-Jones et al.\textsuperscript{102} stated as follows in regard to the risk assessment of Rinsky et al.\textsuperscript{51}: “Examination of the data of Rinsky et al. which are based on nine leukemia related deaths, shows that three cases had cumulative exposure between 470–640 ppm-years, two cases had cumulative exposure between 250 and 260 ppm-years, and the remaining four cases had cumulative exposures of 99, 50, 10 and 0.1 ppm-years, that is, effectively three different data points. Rinsky et al. stated that the shape of the best fit model was linear. If the observed and predicted probabilities of leukemia are examined, however, the data are seen to be polarized into three areas, with the response at lower levels being relatively flat. Thus, in the risk assessment of Rinsky et al., it is the three highest exposure cases that drive the model. When using a conditional logistic regression to predict the probability of leukemia at a given level of exposure, with the three cases included, the model was significant ($\chi^2=13.3, p<0.01$); after removal of the three cases (and their control), however, the model was not significant ($\chi^2=1.4, p=0.23$). Furthermore, the estimates of exposure as highlighted in the study were considered to be an underestimate, and underestimation of exposure would increase the predicted risk at any given exposure. A further confusing factor is that multiple myeloma was the cause of death in four members of the Rinsky cohort of benzene workers, three of the four were among the group with the lowest cumulative exposure to benzene (< 40 ppm-years), and all four required an exceptionally long latency period (> 20 years).\textsuperscript{56} These two factors indicate a possibility that low cumulative exposure to benzene may result in well differentiated malignancy such as multiple myeloma, whereas higher exposures lead to leukemia.\textsuperscript{56} This observation confuses even further the risk assessment procedure for occupational and environmental exposure to benzene, for if true it might justify the recent ACGIH proposal for a decrease in the TLV for benzene to 0.1 ppm.”

Paustenbach et al.\textsuperscript{103}, after reexamining the past records and interviewing workers, published their opinion that Rinsky et al.\textsuperscript{51} underestimated the level of exposure to benzene in the 1940s and in the early 1950s. According to Paustenbach et al., Rinsky et al. estimated the benzene exposure level as considerably lower (by 25–50%) than the actual level, because they did not take into account 1) short-term high-level exposures, 2) skin exposure, 3) the fact that more than 70% of the workers were working for longer than 40 h per week in the 1940s, and 4) the inefficiency of the respiratory masks used. If these suggestions are justified, the risk assessment by Rinsky et al. will lose its usefulness and the proposal of ACGIH for lowering the TLV of benzene to 0.1 or 0.3 ppm will need to be reevaluated.

The estimation of benzene exposure in the Pliofilm cohort can be summarized as follows. Rinsky et al.\textsuperscript{51} assumed that workers of the same job category were exposed to the same concentration of benzene unless there was a special reason. In other words, they calculated the risk of benzene-induced leukemia on the assumption that benzene exposure in 1945 was at the same level as that in 1966. In contrast, Crump and Allen\textsuperscript{96} estimated the cumulative exposure and the peak exposure concentration for each worker. Assuming that the ratio of measured concentration to the TLV in a period during which the data were available was applicable to other periods, they estimated the exposure level in a period without data on the basis of the TLV during the ascertainable period. Thus, their estimated value at the early stage (TLV at that time = 100 ppm) was higher than that estimated by Rinsky et al.\textsuperscript{51}. Meanwhile, Paustenbach et al.\textsuperscript{103} estimated exposure levels considering that unusually long working hours were practiced in the 1940s, the exposure during this period might be underestimated due to the lack of sufficient measurement apparatus, and that considerable amounts of benzene must have been absorbed through the skin under the working conditions at that time. In comparison to the estimates of Paustenbach et al., it may be concluded that those of Rinsky et al. underestimated the benzene exposure in almost all workers, and those of Crump and Allen overestimated the exposure in some job categories and underestimated in others.
Brett et al.\textsuperscript{104} recalculated the benzene risk by combining the Pliofilm data of Rinsky et al.\textsuperscript{51} with the exposure estimates of Crump and Allen\textsuperscript{96} and reported that the risk value of Rinsky et al. overestimated benzene carcinogenicity by 3 to 24 times (Table 1). According to this calculation, the excess death rate from leukemia would be 7.9/1,000 and 0.5/1,000 at cumulative benzene exposures of 450 and 45 ppm-years, respectively. Since Brett et al.\textsuperscript{104} obtained the results in Table 1 by applying a logistic regression analysis to the data of Rinsky et al. and Crump and Allen, the difference in risk assessment between Rinsky et al. and Crump and Allen is derived from the difference in exposure estimates between the two research groups.

Another factor greatly affecting the risk assessment of benzene-induced leukemia in the Pliofilm workers is the choice of a model used for extrapolation of the dose-response relationship. The U.S. EPA and OSHA adopted a linear model in the early 1980s. Crump and Allen\textsuperscript{96} and the U.S. EPA\textsuperscript{105} adopted a linearized multistage model. Rinsky et al.\textsuperscript{51} used a conditioned log-logistic model, and Paxton et al.\textsuperscript{106, 107} used a proportional hazards model to calculate the risk. It cannot be decided which one is superior to others.

Comparing the risk assessments by Crump and Allen\textsuperscript{96}, Rinsky et al.\textsuperscript{51} and Brett et al.\textsuperscript{104} which were all based on Pliofilm data, Crump\textsuperscript{108} stated that a more accurate risk assessment would be available by introducing the exposure estimation method of Paustenbach et al.\textsuperscript{103} Crump applied the method to the Pliofilm cohort, extending the follow-up period to 1987. The cohort consisted of 1,717 white men who had been engaged in manufacturing Pliofilm (rubber hydrochloride) for at least one day. Benzene exposure levels were expressed as both cumulative exposure for individuals and exposure period-weighted cumulative exposure. This weight was taken into account on the assumption that once leukemia develops due to DNA injury caused by benzene, benzene exposure thereafter is irrelevant to the course of leukemia. The risk of benzene-induced leukemia was calculated for occupational benzene exposure at 1 ppm for 8 h/d for 45 years for men between the ages of 20 and 65. It was assumed that the Pliofilm workers had inhaled 10 m\textsuperscript{3} air for 8 h/d, 250 d/yr. The target diseases were tumors in hemopoietic and lymphatic tissues (202-209 of ICD 9), all leukemia, or AMML (acute myelocytic leukemia and acute monocytic leukemia which corresponds to acute non-lymphatic leukemia). In the Pliofilm cohort, 21 workers died of hemopoietic and lymphatic tumors. Among those, 8 cases were definitely AMML. While a dose-response relationship was observed in all disease groups, the relation was most prominent in AMML. The relationship disappeared when AMML was excluded from either hemopoietic and lymphatic tumors or all leukemia. Thus, it can be said that only AMML was relevant to benzene exposure. Table 2 shows the risk levels of occupational exposure to benzene at 1 ppm calculated from various exposure estimates with different models. According to the results summarized in Table 2, excess death from leukemia by 45 ppm-year benzene exposure is $1.8-3.6 \times 10^{-5}$ with a nonlinear model, while it is $1.5-3.8 \times 10^{-5}$ with a linear

<table>
<thead>
<tr>
<th>Exposure assumptions</th>
<th>Control sets</th>
<th>Additional lifetime leukemia deaths per 1,000 workers due to benzene exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45 ppm-years</td>
</tr>
<tr>
<td>Rinsky et al.\textsuperscript{51}</td>
<td>1</td>
<td>5.1 (0.8-311.7)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.4 (1.2-14.7)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.2 (1.0-8.7)</td>
</tr>
<tr>
<td>Crump and Allen\textsuperscript{96} I\textsuperscript{b}</td>
<td>1</td>
<td>0.5 (0.1-1.0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.7 (0.1-1.3)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5 (0.1-1.0)</td>
</tr>
<tr>
<td>Crump and Allen\textsuperscript{96} II\textsuperscript{c}</td>
<td>1</td>
<td>1.3 (0.3-2.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.6 (0.3-3.1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.2 (0.3-2.3)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Control sets: 1, Rinsky et al.'s controls matched for date of birth and date entering Pliofilm work; 2, Brett et al.'s controls matched for date of birth and date entering Pliofilm work; 3, Brett et al. controls matched for date of birth, date entering Pliofilm work, and plant. \textsuperscript{b}Exposure assumptions originally derived by Crump and Allen. \textsuperscript{c}Crump and Allen's alternate exposure estimates in which a ceiling of 131 ppm for each job category was enforced. Numbers in parentheses are 95% confidence intervals.
Table 2. Expected number of additional deaths per 1,000 persons from occupational exposure to 1 ppm benzene from age 20 through age 65\textsuperscript{108}

<table>
<thead>
<tr>
<th></th>
<th>Linear model</th>
<th>AUC dependent</th>
<th>Intensity-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMML</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weighted exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.5</td>
<td>0.17</td>
<td>0.018</td>
</tr>
<tr>
<td>Multiplicative\textsuperscript{a}</td>
<td>1.6</td>
<td>0.75</td>
<td>0.020</td>
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<tr>
<td>Cumulative exposure (lag = 5 yr)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.8</td>
<td>0.34</td>
<td>0.021</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>2.6</td>
<td>1.0</td>
<td>0.030</td>
</tr>
<tr>
<td>All leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weighted exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.9</td>
<td>0.72</td>
<td>0.023</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>1.9</td>
<td>0.56</td>
<td>0.022</td>
</tr>
<tr>
<td>Cumulative exposure (lag = 5 yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.8</td>
<td>0.35</td>
<td>0.022</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>3.8</td>
<td>2.9</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Crump and Allen's exposure matrix\textsuperscript{109}

<table>
<thead>
<tr>
<th></th>
<th>Linear model</th>
<th>AUC dependent</th>
<th>Intensity-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMML</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weighted exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>2.2</td>
<td>0.99</td>
<td>2.0</td>
</tr>
<tr>
<td>Multiplicative\textsuperscript{a}</td>
<td>3.2</td>
<td>1.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Cumulative exposure (lag = 5 yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>2.6</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>4.3</td>
<td>3.9</td>
<td>4.3</td>
</tr>
<tr>
<td>All leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weighted exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>2.4</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>3.0</td>
<td>2.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Cumulative exposure (lag = 5 yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>2.6</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>5.1</td>
<td>5.0</td>
<td>5.1</td>
</tr>
</tbody>
</table>

AMML = acute myelocytic or acute monocytic leukemia. \textsuperscript{a}Methods that provided the best fits to the Pi- ofilm cohort data.

Table 3. Additional leukemia deaths\textsuperscript{a} estimated by means of the proportional hazards model with various exposure assessments\textsuperscript{b,103}

<table>
<thead>
<tr>
<th>Exposure estimates</th>
<th>Cumulative occupational benzene exposure (ppm-years)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Rinsky et al\textsuperscript{51}</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(0.032-0.21)</td>
</tr>
<tr>
<td>Crump and Allen\textsuperscript{109}</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>(0-0.051)</td>
</tr>
<tr>
<td>Paustenbach et al\textsuperscript{103}</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>(0.016-0.080)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Estimated mean number of leukemia deaths over the lifetime of 1,000 exposed individuals in excess of an assumed background rate of 7.07 (95% confidence interval). \textsuperscript{b}All estimates based upon the updated information on all 15 leukemia cases and the 650 controls. \textsuperscript{c}Stated occupational exposure assumed to result from exposure to a concentration of 0.1, 1, 2.5, or 10 ppm for 8 h/d, 5 d/wk, 50 wk/yr.
Paxton et al.\textsuperscript{106, 107} calculated the risk of benzene-induced leukemia applying a proportional hazards model to a group consisting of 15 cases of death from leukemia among the Pliofilm workers and to a group of 650 control subjects matched to the case group by factory, sex, age and starting year of work (Table 3). For the exposure levels, the values estimated by Rinsky et al.\textsuperscript{51}, Crump and Allen\textsuperscript{96}, and Paustenbach et al.\textsuperscript{103} were employed. The risk value of Paxton et al. thus calculated was $1.3 \times 10^{-3}$ for 45 ppm-year benzene exposure, almost a quarter that of Rinsky et al.\textsuperscript{51} ($5.1 \times 10^{-3}$). Furthermore, when the exposure estimates proposed by Crump and Allen and Paustenbach et al. were used, the excess death at 45 ppm-year were $0.26 \times 10^{-3}$ and $0.49 \times 10^{-3}$, respectively, which were each less than 1/10 of the risk by Rinsky et al. Paxton et al. evaluated the exposure estimate of Crump and Allen and that of Paustenbach et al. highly, and emphasized the advantage of risk estimation with a proportional hazards model. According to Paxton et al., 45 ppm-year occupational exposure to benzene causes 0.3–0.5 excess death from leukemia per 1,000 exposed individuals, with a 95% confidence interval of 0–8.

The exposure estimation of Paustenbach et al.\textsuperscript{103} was based on more detailed observation than that of Rinsky et al.\textsuperscript{51} or Crump and Allen\textsuperscript{96}, and is considered at present to have estimated the benzene exposure in the Pliofilm cohort most adequately. However, there seem to be some uncertain points in the exposure estimation of Paustenbach et al., particularly concerning the exposure in the very early stage. According to Paxton et al.\textsuperscript{106}, the exposure level estimated by Paustenbach et al. at this stage was twice as high as that by Crump and Allen and almost 4 times that of Rinsky et al. According to Crump\textsuperscript{108}, the dose-response relationship was nonlinear when the exposure estimates of Paustenbach et al. were employed. Whether this relation is linear or nonlinear is an important issue, since an 80- to 90-fold difference was present between the risk values calculated with a linear and a nonlinear model, even when the same exposure estimates were used\textsuperscript{108}. There are many difficulties in determining precisely from the data available at present to what degree the dose-response relationship between benzene and leukemia is apart from nonlinearity. A further follow-up study of the Pliofilm cohort is needed to clarify the relationship.

**Risk assessment for benzene exposure in general population**

The U.S. EPA\textsuperscript{109} reported a value of $2.6 \times 10^{-2}$ as the unit risk of benzene exposure (1 ppm, 24 h/d for 70 years) by applying the linearized multistage model of Crump and Allen\textsuperscript{96} to the data of Pliofilm\textsuperscript{50} and Dow Chemicals\textsuperscript{44}. From this risk assessment, the benzene concentration corresponding to $10^{-6}$ excess death from leukemia was about 40 ppt.

The WHO (World Health Organization) Regional Office for Europe\textsuperscript{109} calculated the unit risk (1 $\mu g/m^3$) using an average relative risk model with the Pliofilm data reported by Rinsky et al.\textsuperscript{50}, Infante\textsuperscript{103}, Infante and White\textsuperscript{111}, and White et al.\textsuperscript{94}. Based on the assumption that the SMR of leukemia in the cohort was 5.60, the background death rate from leukemia was 0.007 for life, the benzene exposure level in the cohort was 30–300 mg/m$^3$, and the period of exposure was 8.5 years, the calculation resulted in a unit risk of $4 \times 10^{-6}$. The WHO Office also calculated the unit risk as $3.8 \times 10^{-6}$ with the same model based on the reports of Ott et al.\textsuperscript{48} and Bond et al.\textsuperscript{49} concerning the Dow Chemical cohort by assuming that the SMR of myeloleukemia was 4.40, the background death rate from the leukemia 0.003 for life, the benzene exposure level in the cohort was 100 mg/m$^3$, and the exposure period was 8.5 years. On the basis of these values, the WHO Office proposed a value of $4 \times 10^{-6}$ as the unit risk for leukemia of benzene in the environmental atmosphere.

The risk assessments of the EPA and WHO may be altered in the future, because the Pliofilm cohort has since been modified by Rinsky et al.\textsuperscript{51}, Paustenbach et al.\textsuperscript{103}, Crump\textsuperscript{108}, Paxton et al.\textsuperscript{106, 107}, and Thorslund et al.\textsuperscript{112}.

Thorslund et al.\textsuperscript{112} recommended a quadratic model for the risk assessment of benzene-induced leukemia because the dose-response relationship between benzene and myeloleukemia in the Pliofilm cohort fitted better to a nonlinear model than to a linear model. With this model, they estimated the unit risk (1 ppm) of benzene exposure (24 h/d for 70 years) to be $1.43 \times 10^{-4}$. By using a linear-quadratic model (combination of a quadratic model for high-concentration exposure with a linear model for low-concentration exposure), they also reported $0.007 \times 10^{-3}$ as the unit risk. Thorslund et al. gave two reasons for using a quadratic model: 1) The Pliofilm data fit well to a quadratic equation, and 2) the mechanism of benzene carcinogenicity is explained better by a two-hit than a one-hit model (two molecules of benzene or its metabolites bind to DNA, inducing leukemia).
Automobile gasoline as a source of benzene exposure

While benzene exposure in the workplace is decreasing at present, petroleum containing a few percent of benzene has become an important source of benzene exposure for both industrial workers and the general population. It is worthwhile to discuss automobile gasoline in relation to benzene exposure and its association with benzene-induced leukemia.

Tironi and Hodgkins\textsuperscript{113} measured benzene concentration at a gasoline refueling stand during the winter (February) and summer (August and September). Benzene content (volume %) in the gasoline was 2.64 in winter and 2.66% in summer. The atmospheric concentration of gasoline in winter was in the range between 0.79 and 734 ppm, and that of benzene measured simultaneously was between 0.01 and 2.88 ppm; the values in summer were 1.08–266 ppm and 0.1–2.30 ppm, respectively. The relation between benzene (Y) and gasoline concentrations (X) was \( Y = 0.0041X + 0.027 \) in winter and \( Y = 0.00905X - 0.0116 \) in summer. The authors stated that the slower slope during winter was due to the addition of hydrocarbons with low boiling points such as butane and pentane as well as to the low atmospheric temperature. The present TLV of gasoline recommended by the ACGIH is 300 ppm. By applying the above equations to the air at a refueling stand containing 300 ppm gasoline, the benzene concentration is 1.3 ppm in winter and 2.7 ppm in summer. If the TLV of benzene proposed by ACGIH (0.1 or 0.3 ppm) is realized, the permissible level of gasoline will also have to be lowered.

Foo\textsuperscript{114} reported that the automobile gasoline sold in Singapore contained 1.8–3.7% benzene, and that benzene exposure derived from gasoline in terms of an 8-h TWA (time-weighted average) concentration amounted to 0.028–0.71 ppm among refueling workers, 0.014–1.7 ppm among automobile repairmen and 0.08–2.37 ppm among drivers of gasoline tank trucks.

The ACGIH's TLV for benzene has a skin notation, in consideration of the potential contribution of skin absorption to the overall exposure. According to Blank and McAuliffe\textsuperscript{115}, 1.5 \( \mu \)l/h of benzene was absorbed through the skin of an adult working in an environment containing 10 ppm benzene. They also reported that 7.0 \( \mu \)l/h of benzene was absorbed by contact of 100 cm\(^2\) of skin with petroleum gasoline containing 5% benzene.

In the U.S.A., the modified Clean Air Act was enacted in 1990 to control the release of benzene by technology-based standards and to limit the benzene content in petroleum gasoline to below 1% (v/v) by 1995. Further, the California Air Resources Board has proposed to limit benzene content in gasoline to below 0.8% by 1996\textsuperscript{116}.

Gasoline and leukemia

At a meeting on the biological effects of petroleum held in 1991, three papers were presented concerning leukemia among workers engaged in petroleum transport. They suggested excess death due to leukemia, particularly acute myeloid leukemia (AML), among workers engaged in this work. In relation to AML, the SMR given by Rushton\textsuperscript{117} was 1.21 (25 cases), that for gasoline tank truck drivers by Schnatter et al.\textsuperscript{118} 3.35 (5 cases), and that for workers engaged in land transport by Wong et al.\textsuperscript{119} 1.50 (13 cases), although none of these results showed a statistically significant increase. Whether petroleum exposure is associated with leukemia may depend on the benzene content of the petroleum, but none of the above reports discussed the death from leukemia in relation to benzene content.

Jakobsson et al.\textsuperscript{120} examined the incidence of acute myeloid leukemia (AML) with respect to occupation using the Swedish Cancer Environment Registry. Subjects of this survey were 2,308,444 male and female employees aged between 20 and 64 years on the basis of the national census conducted in 1970. Their occupations were classified into 295 categories according to the Nordic Occupational Classification. Examination of the cancer registration in Sweden in 1971–1984 revealed 1,275 cases of AML. The subjects were stratified by sex, age (5-year interval), county (n = 24) and degree of urbanization (n = 2), and the occupational career was expressed in person-years. In the occupations in which the observed incidence of AML exceeded the expected value, individual information relating to the job category and the job history was obtained by sending a questionnaire to the worker or the surviving family or by interviewing them on telephone. The only occupation in which the observed mortality rate from AML exceeded the expected rate was male petroleum station attendants. There were 9,000 workers classified in this job category, and 10 cases of death from AML were observed, with an SMR of 3.6 (95% confidence interval, 1.7–6.6). The cause of death as AML was confirmed by histopathological examination of bone marrow biopsy specimens. Among those 10 patients with AML, 6 were petrol station attendants at the time of the 1970 Swedish census. One of the remaining patients was also working at a petrol stand around 1970, and another had been a petrol stand worker and later moved to a rubber industry (liable to ben-
zene exposure). The remaining two patients were handling airplane fuel (benzene content not exceeding 1%) and diesel and light oils (benzene content not exceeding 0.05%), respectively. The period of time that passed between the time of the first occupational handling of petroleum and the time of AML diagnosis was 9 to 36 years (median 16 years), a period which is similar to that of the 3.5 to 37 years reported by Rinsky et al.\textsuperscript{31}.

No components other than benzene are conceivable factors which can explain the excess death from AML among petrol stand attendants. One factor in this issue is the benzene content in gasoline. While gasoline sold in the U.S.A. contains 2% or less benzene, that sold in Europe contains 4-5% benzene\textsuperscript{29}.

Jakobsson et al.\textsuperscript{120} stated as follows in relation to gasoline-associated leukemia. Gasoline sold in Sweden contains as much as 5% benzene and 700 mg of benzene would volatilize in the air during refueling 30 liters of gasoline containing 5% benzene. Benzene content in the exhaust gas during idling of a car engine is high; the emission of benzene amounts to 10 mg/min. Since self-service refueling pumps came into general use during the 1970s in Sweden, the exposure of petrol stand attendants to benzene has been lowered compared to that in 1970. In addition, around 1970, many petrol stand attendants and car repairmen had a bad habit of washing oil dirt with gasoline, and this senseless practice (skin absorption as well as inhalation of benzene) might be associated with the development of AML.

A significant correlation between petroleum exposure and AML was also observed in another case-control study in Sweden\textsuperscript{21}). Excess death due to leukemia including AML was also observed among automobile repairmen in the U.S.A.\textsuperscript{122}). Although a survey conducted in Denmark did not reveal excess death from leukemia (but did from lung cancer) among petrol stand attendants\textsuperscript{123}), case reports of leukemia (particularly AML) developed in workers exposed to petroleum\textsuperscript{124,125} suggest an association between benzene in petroleum and leukemia.

Wolff\textsuperscript{126} examined the relationship between the incidence of leukemia and lymphoma (1984–1988) and the rate of automobile possession in 1981 in 22 counties in the United Kingdom. The result revealed a significant correlation (Spearman's rank correlation coefficient, 0.68) between the incidence of non-Hodgkin's lymphoma (follicular lymphoma) and the rate of automobile possession/1,000 population. Moreover, when the rate of automobile possession was expressed per family, significant correlations were shown with follicular lymphoma (0.66), acute lymphoblastic leukemia (0.44), chronic lymphocytic leukemia (0.41), total lymphatic tissue proliferation diseases (0.40) and acute myeloid leukemia (0.38). The petroleum engine is an important source of nonoccupational benzene exposure, but a hasty conclusion should not be made from this study since numerous compounds other than benzene are contained in car exhaust gas. However, this study is interesting in that it infers a correlation between the use of automobiles and the incidence of leukemia.

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