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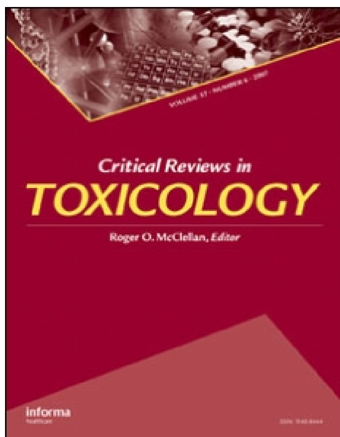

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The Nature of Chromosomal Aberrations Detected in Humans Exposed to Benzene

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The Nature of Chromosomal Aberrations Detected in Humans Exposed to Benzene

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KEY WORDS: benzene, chromosomal aberrations, cytogenetics, FISH, human, leukemia.

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ABBREVIATIONS: AML: acute myeloid leukemia; **ALL**: acute lymphocytic leukemia; **CA**: chromosomal aberrations; **CML**: chronic myeloid leukemia; C_s: stable chromosomal aberrations; C_u: unstable chromosomal aberrations; **FISH**: fluorescence *in situ* hybridization; **MDS**: myelodysplastic syndromes; **MN**: micronuclei; **NCA**: numerical chromosomal aberrations; **NCI**: National Cancer Institute; **Ph**: Philadelphia chromosome; **PHA**: phytohemagglutinin A; **ppm**: parts per million; **PWM**: pokeweed mitogen; **SCA**: structural chromosomal aberrations; **SCEs**: sister chromatid exchanges; **+C**: trisomy C; and **–C**: monosomy C.

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GLOSSARY

Chromosomal Aberrations: All types of changes in chromosome structure and chromosome number;

Chromosome-type Aberrations: Structural chromosome changes in which both chromatids of the chromosome are involved, including isochromatid gaps, breaks, fragments, minutes, and dicentrics;

Chromatid-type Aberrations: Chromosome structural changes in which only a single chromatid of the chromosome is involved, including chromatid gaps, breaks, and exchanges;

Aneuploidy: Numerical chromosome changes, including the loss and/or gain of individual chromosomes in mitosis or meiosis leading to the formation of nuclei with hypoploid and/or hyperploid chromosome numbers, and the loss or gain of whole chromosome sets;

Polyploidy: The gain of one or more complete chromosome sets in diploid somatic cells, including triploidy, tetraploidy and pentaploidy etc.

ABSTRACT: Benzene is an established cause of human leukemia that is thought to act by producing chromosomal aberrations and altered in cell differentiation. In several recent studies increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially hematological malignancies. Thus, chromosomal aberrations may be a predictor of future leukemia risk. Previous studies exploring whether benzene exposure induces chromosomal aberrations have yielded mostly positive results. However, it remains unclear whether the chromosomal aberrations induced by benzene occur in a distinct pattern. Here, we thoroughly review the major chromosome studies published to date in benzene-exposed workers, benzene-poisoned and preleukemia patients, and leukemia cases associated with benzene expose. Although three cytogenetic markers (chromosomal aberrations, sister chromatid exchanges, and micronuclei) are commonly examined, our primary focus is on studies of chromosomal aberrations, because only this marker has so far been correlated with increased cancer risk. This review surveys the published literature, analyzes the study results, and discusses the characteristics of effects reported. In most studies of currently exposed workers, increases in chromosomal aberrations were observed. However, due to the relatively small number of affected individuals and variability in the reported aberrations, firm conclusions cannot be made about the involvement of specific chromosomes or chromosome regions. Further, in leukemia cases associated with benzene exposure, there is no evidence of a unique pattern of benzene-induced chromosomal aberrations in humans. Leukemia cases associated with benzene exposure are, however, more likely to contain clonal chromosome aberrations then those arising *de novo* in the general population.

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I. HISTORICAL BACKGROUND

Benzene, a volatile, colorless, highly flammable liquid, was first discovered in 1825 by Michael Faraday, who isolated it from a liquid condensed from compressed oil gas (Gist and Burg, 1997). Forty years later, after struggling to determine its structural formula, Friedrich Kekulé had a dream one night in which the benzene molecule appeared as a snake biting its tail while in whirling motion; he said,

> One of the snakes had seized hold of its own tail, and the form whirled mockingly before my eyes . . . Let us learn to dream . . . and then perhaps we shall learn the truth.
> (Morrison and Boyd, 1966).

From that vision his concept of the benzene ring was born. The molecular structure of benzene consists of six carbon atoms with three unsaturated bonds arranged in a radially symmetric ring. Many industrial applications have been found for benzene, and since its discovery it has been widely used as an industrial solvent. Today, 98% of the benzene produced is derived from the petrochemical and petroleum refining industries (Gist and Burg, 1997). As a result, human exposure to benzene takes place in factories, refineries, and other industrial settings. Although only a relatively small number of individuals are occupationally exposed to benzene, the general population is exposed to benzene contained in gasoline, automobile exhaust, and diesel fuel (Knott, 1994; Muzyka et al., 1998). Furthermore, benzene is present in cigarette smoke, and smoking is the main source of benzene exposure for many people (Gist and Burg, 1997).

In 1897, Santesson first described nine cases of chronic benzene hematotoxicity (Santesson, 1897); that same year, Le Noire and Claude also reported a case of a blood disorder (purpura) consistent with benzene poisoning (Le Noire and Claude, 1897). The hematotoxic effects of benzene were further documented about 20 years later in studies by Selling (Selling, 1916) and Weiskotten (Weiskotten et al., 1920; Weiskotten et al., 1916). The first case of benzene-associated leukemia was described by Delore and Borgomano in 1928 (Delore and Borgomano, 1928). Since then, many leukemia cases associated with benzene exposure have been reported (Aksoy et al., 1972; Cronkite, 1987; Goldstein, 1977; Vigliani and Saita, 1964), and by 1961 benzene had been identified as one of two industrial leukemogens, the other being ionizing radiation (Cronkite, 1961). Chromosome changes in leukemia patients were not detected until after the karyotype of normal somatic cells had been established in 1956 (Ford and Hamerton, 1956; Tjio and Levan, 1956). Shortly thereafter, the first evidence emerged of the presence of nonrandom chromosomal aberrations (CA) in leukemia cases, such as the Philadelphia (Ph) chromosome (Nowell and Hungerford, 1960), which was further characterized by Rowley as t(9;22) (Rowley, 1973). Thus, by the early 1960s chromosome studies could be conducted for benzene-associated leukemia and preleukemia cases. Pollini and Colombi described increased rates of aneuploid cells, some with structural aberrations, in bone marrow and peripheral blood lymphocytes from patients with severe benzene-associated hematological disorders, including leukopenia or pancytopenia, aplastic anemia, and "blood dysplasia", which was most likely a myelodysplastic syndrome, MDS (Pollini et al., 1969; Pollini and Colombi, 1964a; Pollini and Colombi, 1964b; Pollini et al., 1964c).

Since the foregoing studies, chromosome damage caused by benzene exposure has been independently reviewed by Forni and Aksoy (Aksoy, 1988; Forni, 1979). Several factors indicate the importance of a current review of benzene-induced CA in humans. First, a considerable amount of research has been conducted since the previous reviews by Forni and Aksoy. Second, the development of new molecular cytogenetic techniques such as fluorescence *in situ* hybridization (FISH), which allows the detection of specific CA and the evaluation of more cells than conventional techniques, has facilitated investigations in this area. Third, the frequently repeated but questionably supported conclusions about the involvement of specific chromosomes in benzene-associated leukemias warrant analysis with consideration of the actual published data. Finally, several recent European and Chinese studies have reported a correlation between classic CA and increased cancer risk (Bonassi et al., 1995; Hagmar et al., 1998; Hagmar et al., 1994; Liou et al., 1999).

II. CHROMOSOMAL ABERRATIONS AS A BIOMARKER OF CANCER RISK

Various biomarkers of leukemia risk using benzene as a model leukemogen have been described previously (Smith and Zhang, 1998). These biomarkers can be classified into three categories: biomarkers of exposure, susceptibility, and early effect. Chromosomal aberrations appear to be a particularly promising early-effect biomarker of chemical exposure. Early cytogenetic studies indicated that humans exposed to carcinogenic agents exhibited elevated frequencies of CA in their peripheral blood lymphocytes (Schinzel and Schmid, 1976), which was reviewed by Sorsa (Sorsa et al., 1992). Confirming and extending these earlier theories, Hagmar and colleagues in 1994 reported that increased levels of CA in human lymphocytes were associated with future cancer risk (Hagmar et al., 1994). A similar association was also found between frequency of chromosome changes in lymphocytes (non-target tissues) and risk of cancer, particularly lymphocytic and hematopoietic tissue malignancies (Bonassi et al., 1995). In a recent update to these studies, these earlier results were confirmed by both groups (Hagmar et al., 1998). Additional studies from Taiwan and the Czech Republic have further strengthened the association between increased levels of CA in human lymphocytes and future cancer incidence and mortality (Liou et al., 1999; Smerhovsky et al., 2001). Hence, CA appear to be a useful predictor of future cancer risk, at least at the group level. Further studies are needed to evaluate their usefulness at the individual level. It is also important to note that the increased cancer risk is not associated with a specific exposure, with the exception of radon in one recent study (Smerhovsky et al., 2001). However, because CA are detected mostly in peripheral blood, a tissue affected by leukemia, one would expect a particularly close association between frequency of CA and leukemia risk. By monitoring the level of chromosome damage in a population, especially workers exposed to genotoxic chemicals, it is possible that we may effectively identify individuals or groups with elevated risk of leukemia so that appropriate measures may be taken to reduce their exposure.

In addition to CA, other cytogenetic endpoints, primarily sister chromatid exchanges (SCEs) and micronuclei (MN) have been used to assess human exposure to genotoxic agents. However, SCEs do not appear to have a significant association with cancer risk (Hagmar et al., 1994; Liou et al., 1999; Sorsa et al., 1990; Sorsa et al., 1992). Likewise, the link between MN and cancer risk is indeterminate, perhaps due to insufficient published data (Hagmar et al., 1994; Sorsa et al., 1992). Therefore, CA appear to be the most valid cytogenetic endpoint now available for predicting future leukemia risk.

Many studies have identified particular types of CA in cases of leukemia associated with benzene exposure (Aksoy, 1988; Forni, 1979). However, whether these chromosome changes were caused directly by benzene exposure or by genetic changes that took place as a consequence of the development of leukemia has been an unsettled question. In an attempt to answer that question, many researchers have examined chromosome damage in patients with benzene hematotoxicity before the onset or in the absence of leukemia. As a result of these studies, certain chromosome changes have been detected in preleukemia cases and benzene-poisoned patients. Other studies have found increased levels of CA in benzene-exposed but otherwise healthy workers. In the current review, we first describe the studies that have detected chromosome changes in leukemia cases associated with benzene exposure and compare their findings to those in leukemia cases arising *de novo* in the general population. Next, we trace the studies measuring chromosome damage in preleukemia and benzene-poisoned patients. Finally, we survey CA studies in benzene-exposed workers without evident disease.

III. CHROMOSOMAL ABERRATIONS REPORTED IN BENZENE-ASSOCIATED LEUKEMIA CASES

In order to determine the types of chromosome damage that characterize leukemias associated with benzene exposure, we have summarized 23 cases from 19 cytogenetic reports in Table 1. While additional studies of benzene-associated leukemia exist in the litera-

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TABLE 1
Chromosomal Aberrations Detected in Leukemia Cases Ascribed to Benzene Exposure by the Authors

Reference	Age / Gender	Leukemia classification	Occupation	Benzene Exposure (yr)	Cell # tested	Specimen	Numerical CA	Structural CA
Di Guglielmo, 1958	M	AML-M6	Ink press	4	N/A	PB	Polyploidy	
Forni, 1967	38/F	AML-M1/2	Electric cable finisher	22	150*	PB	Trisomy	t(C;D;G); b, f, d
					39	PB/BM	+C, G, D	
					32	PB	+C (+9)	
Rozman, 1968	58/M	AML-M6	Varnisher	30	N/A	BM	Polyploidy	
Forni, 1969	37/F	AML-M6	Case mounting	7	11	PB	–E; –A, B, C, D; Poly-	mar 1, mar 2
					12	BM	–E; Polyploidy	
Hartwich, 1969	53/M	AML-M1/2	Oil refinery	4	100	PB		g, b, f, m
Forni, 1970**	55/M	AML-M6	Shoe repairer	7	22	PB	Hypo-, –C, -16	C_s, mar 1, mar 2
Sellyei, 1971	35/M	AML-M2/4	Car repair	1.5	27	BM	+C, D, E; –G, A	f, d, r
					5	PB	+D	
Robustelli, 1972	61/M	AML-M6	Shoemaker	6	N/A	PB	Hypo- / hyper-, +/–E	
Liaudet, 1973	35/M	CML	Petro-chemist	17	N/A	N/A		possible t(9;22)
Erdogan, 1973	42/M	AML-M4/5	Glue user	10	N/A	PB/BM	Polyploidy (4%)	b
Aksoy, 1974	24/M	ALL	Shoemaker	6	100	N/A		g, b, d
Aksoy, 1974	43/M	AML-M1/2	Shoemaker	6	100	PB/BM	+C (13%)	g, b, d
Erdogan, 1975	48	AML	N/A	3	N/A	N/A	–C (40%)	d
Erdogan, 1975	23	AML-M6	N/A	11	N/A	N/A		g, d, b
Erdogan, 1975	36	AML-M6	N/A	15	N/A	N/A		g
Van den Berghe, 1979	25/M	AML-M4	Steel draftsman	4	20	PB		t(9;10)(p24?;p12) &
					15	BM		t(4;15)(p13;q14)
Solé, 1990	55/F	Early-B ALL	Furniture	35	7	PB		t(4;11)(q21;q23)
Lumley, 1990	58/M	AML-M2	Truck driver	~30	N/A	N/A	–17, 18, 19, 20	5q-,t(6q;7p), 8p- etc.
Tasaka, 1992	64/M	AML-M4	Painter	40	N/A	BM		t(3;21)(q26;q22)
Mahendra, 1996	42/M	AML	Printer	20	20	BM		t(9;13)(q34;q12)
Colovic, 1996	30/M	AML-M2	Driver / lacquerer	10	N/A	PB	normal karyotype	
Colovic, 1996	37/M	T-ALL	Driver / lacquerer	10	N/A	PB	normal karyotype	
Siena, 1999	49/M	a-CML***	Petro-worker	27	18/49	PB/BM		t(5;10)(q23;q22)

Abbreviations: +: trisomy, –: monosomy, Poly-: polyploidy, Hypo-: hypodiploidy, Hyper-: hyperdiploidy, g: gaps, b: breaks, f: fragments, m: minutes, d: dicentric, r: rings, mar: marker, PB: peripheral blood, BM: bone marrow, C_u: unstable CA (e.g. acentric fragments, ring and dicentric chromosomes), C_s: stable CA (e.g. deletions and translocations), N/A: information not available. *: Cells sampled during preleukemic phase. **: Unpublished data (used by permission) from personal communication with author. ***: Atypical CML.

ture, we generally report only those that included chromosome analysis. The list in Table 1 is extensive, but not exhaustive. It is made up largely of cases of shoe workers in Italy and Turkey. The relative risks in these studies exceeded 10, so we can be confident with a >90% certainty that they were caused by benzene. Most of the reported benzene-induced leukemia cases (18 out of 23) were classified as acute myeloid leukemia (AML).* All cases selected for study involved occupational exposure to benzene or benzene-containing solvents, and many involved long-term exposures (up to 40 years). Most early studies were performed by traditional cytogenetic techniques, which could not identify individual chromosomes. Later studies employing banding technology, on the other hand, were able to detect specific chromosome changes. Cytogenetic results were obtained from both bone marrow and peripheral blood lymphocytes.

Despite technological limitations, one thing becomes immediately apparent: Almost all of the cases (21/23) have some type of clonal chromosome aberrations and only 2 cases have a normal karyotype. Further, in the AML cases specifically only 1 out of 18 cases has a normal karyotype. This contrasts sharply with *de novo* AML in the general population. At the 4th International Workshop on Chromosomes in Leukemia held in Chicago in 1982, only 54% of *de novo* AMLs (n = 660) had chromosomal abnormalities as detected by banding analysis of their bone marrow (Sandberg, 1990). In contrast, all (100%) of the AML cases diagnosed prior to 1982 and associated with benzene in Table 1 had clonal chromosome abnormalities. This suggests that benzene-associated leukemias are more likely to harbor chromosomal abnormalities than *de novo* leukemias arising in the general population. The question then becomes whether a specific type of abnormality is associated with benzene exposure. A description of the types of chromosome aberrations observed in leukemia cases associated with benzene exposure follows.

A. Gain of Chromosomes

The gain of one C-group chromosome (chromosomes 6 to 12, X)**, trisomy C (+C), was the most frequent aberration detected in the cases associated with benzene exposure in Table 1. This may reflect a selective effect of benzene on C-group chromosomes, or may be due to the fact that the C-chromosomes are the largest group of chromosomes. Forni and Moreo performed a total of 8 cytogenetic analyses over a year at different stages in the development of AML in a 38-year-old female patient (Forni and Moreo, 1967). Trisomy was detectable at the first analysis, before the onset of leukemia (3 out of 150 cells sampled). At about the time the patient developed leukemia, a majority of the cells sampled had +C; several months later, near-tetraploid cells appeared, along with +C, in some cases identifiable as +9. The authors suggested that these results support the hypothesis that the development of a clone with +C, in particular +9, is involved in leukemogenesis (Forni and Moreo, 1967).

Trisomy C was also detected in several other cases (Aksoy et al., 1974; Sellyei and Kelemen, 1971; Vigliani and Forni, 1969). Aksoy reported the cases of two shoemakers, related as uncle and nephew, with the same duration of exposure to benzene. The uncle developed AML with detectable +C (13% of cells); the nephew developed acute lymphocytic leukemia (ALL), but no aneuploidy was detected in his blood samples (Aksoy et al., 1974). Another pair of leukemia cases, one with AML and one with ALL, was found among two brothers, both of whom drove buses and worked with lacquers. However, normal karyotypes were found in samples from each patient (Colovic et al., 1996). The gain of chromosomes in groups other than C has been reported (Erdogan and Aksoy, 1973; Forni and Moreo, 1967; Robustelli della Cuna et al., 1972; Sellyei and Kelemen, 1971). Hyperdiploidy (mostly trisomy) of D-group chromosomes was found in 100% of cells examined in one case of AML associated with benzene exposure (Sellyei and Kelemen, 1971). Trisomy E was the most frequent form of hyperdiploidy observed in an AML case that developed from hypoplastic

* Most early studies did not specify the AML subtype; however, where the case reports contained sufficient clinical information, the AML has been classified by likely subtype according to the French-American-British (FAB) system.

** For ease of reference, all human chromosome pairs are grouped according to their conventional classification, as follows: Group A: chromosomes 1, 2, 3; Group B: 4, 5; Group C: 6, 7, 8, 9, 10, 11, 12, X; Group D: 13, 14, 15; Group E: 16, 17, 18; Group F: 19, 20; Group G: 21, 22, Y (Barch, 1991).

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anemia associated with benzene exposure (Robustelli della Cuna et al., 1972).

B. Loss of Chromosomes

The loss of chromosomes, mostly in the form of monosomy, has been primarily observed in groups with smaller chromosomes, such as E and F. Loss of E-group chromosomes, especially 17 and 18, occurred more frequently than that of others (Forni and Moreo, 1969; Lumley et al., 1990; Robustelli della Cuna et al., 1972). In a case studied by Forni and Moreo, a 37-year-old woman who worked in a factory manufacturing beauty cases developed AML-M6. Loss of E-group chromosomes, particularly 17 and 18, took place in all leukemic cells examined in peripheral blood (Forni and Moreo, 1969). Loss of both copies of chromosome 17 was observed in this subject (Forni and Moreo, 1969) and in another case of AML which evolved from hypoplastic anemia (Robustelli della Cuna et al., 1972). In an additional unpublished case report, the loss of chromosome 16 was also observed (personal communication with Dr. Alessandra Forni). Loss of one copy of chromosomes 17, 18, 19, and 20 was reported in a 58-year-old male with AML-M2 who had regularly washed his hands with gasoline at work (Lumley et al., 1990). Monosomy C (-C) was found in the bone marrow and blood of several patients with AML associated with benzene exposure (Erdogan and Aksoy, 1975; Forni and Moreo, 1969; Robustelli della Cuna et al., 1972). Finally, the loss of G-group chromosomes was found in the majority of cells in one case of AML associated with benzene exposure (Sellyei and Kelemen, 1971).

C. Polyploidy

Polyploidy was observed in three early cases of erythroleukemia associated with benzene exposure, currently classified as AML-M6 under the French-American-British (FAB) system (Di Guglielmo and Iannaccone, 1958; Forni and Moreo, 1969; Rozman et al., 1968). In the first case, polyploid cells with multiple nuclei and atypical karyokineses were observed in a patient who was occupationally exposed to benzene vapors for 4 years and had suffered a previous episode of acute benzene poisoning. In addition, there was an inhibition of mitosis at the earliest stage of metaphase of the erythroblasts (Di Guglielmo and Iannaccone, 1958). In another case, a 58-year-old varnisher previously exposed to benzene for 30 years acquired benzene poisoning and later developed acute erythroleukemia. Giant polyploid forms, diploerythroblasts, atypical mitoses, and cytoplasmatic vacuoles were observed in samples of his bone marrow (Rozman et al., 1968). In both studies, no further changes were reported, although the analysis performed was not extensive. Near-tetraploidy, in addition to aneuploidy, was detected in the 37-year-old AML-M6 patient who manufactured beauty cases (Forni and Moreo, 1969). Polyploidy was found in another case of AML, albeit not AML-M6 (Erdogan and Aksoy, 1973). In four other cases of AML-M6, one of which was personally communicated to us by Forni, no polyploidy was detected (Erdogan and Aksoy, 1975; Robustelli della Cuna et al., 1972). Thus, polyploidy was found in three out of seven patients with AML-M6 and in a case of AML-M4/M5 associated with benzene exposure. This is much higher than would generally be expected in these subtypes of AML arising in the general population.

D. Structural Chromosome Changes

Early cytogenetic analyses were generally unable to locate CA on specific chromosomes and could only determine the type of induced structural chromosome damage at metaphase: chromosome-type or chromatid-type. Chromosome-type aberrations (the damage is mostly on both chromatids at the same locus) included isochromatid gaps and breaks, fragments, minutes, and dicentrics, while chromatid-type aberrations (usually only a single chromatid is involved) included breaks, gaps and exchanges (Hartwich et al., 1969; Rieger et al., 1991).

In the earliest reported cases of leukemia associated with benzene exposure, more cytogenetic changes were detected as chromosomal in type than chromatid in type (Aksoy et al., 1974; Erdogan and Aksoy, 1973; Erdogan and Aksoy,

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1975; Forni and Moreo, 1967; Hartwich et al., 1969; Sellyei and Kelemen, 1971). Forni and Moreo reported that 13 out of 150 cells examined during the preleukemic phase in one case had chromosome-type aberrations, while only 4 cells had chromatid-type changes (Forni and Moreo, 1967). Another study observed 14 out of 100 cells analyzed with chromosome-type aberrations, and 9 with chromatid type (Hartwich et al., 1969). In the cases of the uncle and nephew reported by Aksoy, the same types of structural changes (gaps, breaks, and dicentric) were detected in each patient, even though they had different classes of leukemia (Aksoy et al., 1974). In the above reports, the majority of aberrations detected were isochromatid gaps and breaks (Aksoy et al., 1974; Erdogan and Aksoy, 1973; Erdogan and Aksoy, 1975; Forni and Moreo, 1967; Hartwich et al., 1969).

E. Specific Translocations and Deletions

None of the studies of structural changes discussed thus far identified the chromosomes affected or the specific chromosomal location of those changes, although one early study using prebanding techniques detected translocations involving three chromosomes from the C, D, and G groups in cultured lymphocytes in the preleukemic phase (Forni and Moreo, 1967). Another relatively early report suggested the possible existence of the Ph chromosome in the case of a 35-year-old petroleum chemist with chronic myeloid leukemia (CML); however, this case report was not a cytogenetic study (Liaudet and Combaz, 1973). Banding technology, developed in 1970, has allowed the detection of specific structural changes on specific chromosomes (Caspersson et al., 1970). For example, a translocation involving the *MLL* gene, t(4;11)(q21;q23), was detected in 100% of lymphocytes examined from a benzene-exposed patient who developed early-B ALL. The patient, a furniture worker, had previously suffered an episode of benzene poisoning that lasted 3 months (Solé et al., 1990). A different translocation, t(3;21)(q26;q22), was detected in the bone marrow of a 64-year-old painter who was diagnosed with refractory anemia with excess blasts after a history of exposure to organic solvents, including benzene (Tasaka et al., 1992). The same translocation was again detected when the patient was diagnosed with AML-M4 about 16 months later (Tasaka et al., 1992). Similarly, t(9;13)(q34;q12) was detected in most bone marrow samples from a 42-year-old painter who developed pancytopenia as a result of occupational exposure to benzene and other organic solvents for 20 years (Mahendra et al., 1996). The translocation was still present in all bone marrow cells examined, but not in cultured peripheral blood lymphocytes after he developed AML (Mahendra et al., 1996). In another patient who developed preleukemic symptoms after having used petrol to clean metal sheets for about 4 years, translocation t(9;10)(p24?;p12), in addition to a familial t(3;16)(q11;p11), was found in 100% of blood and bone marrow cells tested (Van den Berghe et al., 1979). At the time of his diagnosis of AML-M4 about 7 months later, these translocations were still present, and t(4;15)(p13;q14) was also detected in all cells examined (Van den Berghe et al., 1979). Another study identified a translocation involving the long arm of chromosome 6 and the short arm of chromosome 7 (Lumley et al., 1990). Specific deletions of the long arm of chromosome 5 and the short-arm of chromosome 8 were also reported (Lumley et al., 1990). In a recent case report, t(5;10) was detected in a petroleum worker who developed atypical CML after exposure to benzene for 27 years (Siena et al., 1999).

F. Chromosome Changes in Leukemia Patients with Likely Prior Benzene Exposure

In contrast to the case reports described above, several large-scale leukemia studies have been conducted that surveyed their subjects for information on prior chemical exposures (Cuneo et al., 1992; Golomb et al., 1982; Groupe, 1984; Iurlo et al., 1989; Li et al., 1989; Mitelman et al., 1978; Mitelman et al., 1981; Richardson et al., 1992). These studies compared chromosome changes in leukemia patients with various occupational chemical exposures and in those without exposure. From these studies, we have consolidated karyotypes from patients with likely prior expo-

TABLE 2
Abnormal Karyotypes of Selected Leukemia Patients with Likely Prior Exposure to Benzene[a]

Reference	Age/ sex	Leukemia	Probable exposure	Karotype
Mitelman, 1981	44/F	AML	Printing	45,XX,–7 / 46,XX
	38/M	AML	Printing	46-50,XY,+Y,+del(7)(p11),–8,+9,del(12)(p11),+17,21q+,+22 / 46,XY
	82/M	AML	Printing	45,X,–Y,t(8;21)(q21;q22)
	74/F	AML	Paints	48,XX,+3,+8 / 46,XX
	42/M	AML-M4	Glues	45-46,XY,–7,+17 / 46,XY
	53/M	AML	Glues	46,XY,del(7)(q31),del(11)(q14),del(12)(p12)
	65/M	AML-M3	Rubber	45-51,XY,t(3;12)(p14;q24),t(13;18)(q11;p11),+1,+6,+16,+19,+21, +22
	57/M	AML	Exhaust	44,XY,–5,t(5;12)(q12;q24),iso(18q),–19
	57/M	AML-M6	Exhaust	45-49,XY,–4,–7,–8,–10,+14,+16,–21,–22,+1-2mar
Golumb, 1982	21/M	AML-M4	Paints	46,XY,t(3;5)(q25;q31 or 32)
	45/M	AML-M2	Exhaust	45,XY–7 / 46,XY
	28/M	AML-M2	Exhaust	45,X,–Y,t(8;21)(q22;q22) / 46,XY
	49/M	AML-M1	Exhaust	44,XY,del(5)(q?),–7,–12,–13,–17,8q+,17p–,+mar(B?), +dic.t(12;17),(p?;p?)
	43/M	AML-M4	Exhaust	42,XY,–7,i(8q),–16,–17,–18,tan(21;21)(q22;q11),+mar,+1-2 ace / 43,XY,–7,i(8q),+i(8q),–16,–17,–18,tan(21;21),+mar,+1-2 ace
	81/M	AML-M4	Exhaust	45,XY,–7/46,XY
	38/M	AML-M3	Exhaust	46,XY,t(15;17)(q24–5;q21)/46,XY,del(7)(q22),del(9)(q22),t(15;17)
	64/F	AML-M2	Leather industry	43,XX,–3,–4,del(5)(q15?),–7,17p+,–19,+mar/46,XX
Groupe Français, 1984	37/M	AML-M1	Benzene	46,XY,–?8,–21,+mar1,+mar2 / 45,XY,–?8,–17,–21,+mar1,+mar2
	40/M	AML-M2	Benzene	44,XY,–5,–8,–17,6q-,7q-,10p+,+r
	42/M	MDS	Solvents	43,XY,–17,–18,–18,t(1;5),5q-,t(7;18),r(11),t(21;21)
Iurlo, 1989	18/M	AML-M2	Benzene	46,XY,del(3)(q14q22) (40%) / 46,XY
	78/M	MDS	Leather industry	46,X,-Y,-7,+8,-13,del(5)(q14q34),del(6)(p22),inv(12)(p12q23), del(17)(p11),+2mar (50%) / 46,XY
Li, 1989	37/M	AML-M2	Petroleum	45,X,–Y,t(8;21)(q22;q22)
	36/M	AML-M2	Petroleum	45,X,–Y,t(8;21)(q22;q22)
	19/M	AML-M5	Petroleum	46,XY,del(11)(q23q25)
	22/M	AML-M3	Benzene	46,XY,17q-
Cuneo, 1992	70	AML-M3	Solvents	47,XY,ins(14;2)(q23,q13q37),t(5;?)(q31;?),t(11;12)(p14;q12),+21
	63	AML-M5	Solvents	47,XY,+10
	33	AML-M6	Solvents	49,XY,+11,t(13;?)(p11;?),+M1,+M2 / 50,XY,+11,t(13;?)(p11;?), +M1,+M2,+M3
	55	AML-M2	Solvents	44,XX,del(5)(q13q33),del(16)(q22),t(17;?)(p12;?),–18, t(20;?)(p11;?),–21/45,XX,del(5)(q13q33),t(17;?)(p12;?), t(20;?)(p11;?),–18,–21,+M
	43	AML-M2	Solvents	46,XY,t(6;9)(p23;q34)
	40	AML-M6	Solvents	45,XY,t(13;?)(q14;?),del(20)(q11q13),–8
	74	AML-M5	Solvents	47,XY,+21
	26	AML-M2	Solvents	47,XX,t(8;21)(q22;q22),del(15)(q11q15),+20
	71	AML-M1	Solvents	45,XX,–21
Richardson, 1992[b]	N/A	CML	Benzene	t(9;22)(q34;q11)
	N/A	CML	Benzene	t(9;22)(q34;q11)
	N/A	AML / CML[c]	Benzene	t(9;22)(q34;q11)
	N/A	ALL	Benzene	t(9;22)(q34;q11)
	N/A	AML-M3	Benzene	t(15;17)(q22;q11)
	N/A	N/A	Benzene	Aneuploidy (41-47 chromosomes)[d]

[a] Only patients (n = 41) with abnormal karyotypes presented.
[b] Unpublished cytogenetic data, used by permision, from personal communication with author.
[c] Case involved a primary acute myelogenous leukemia, then, after remission, a chronic myeloid leukemia.
[d] No banding analysis performed.

sure to benzene according to their occupational histories (Table 2). While these studies also included patients with normal karyotypes, we only selected those patients with abnormal karyotypes for presentation in Table 2. The results from these cases are generally consistent with those from the leukemia case reports described above (see Table 1). Because these studies date from the early 1980s, they were able to detect specific chromosome changes through advances in banding technology. For instance, t(8;21) was commonly found in AML-M2 cases and t(9;22) was found in CML. Aneuploidy of C-group chromosomes, such as the loss of chromosome 7 and the gain of chromosomes 8 and 9, was relatively frequent among these cases. In addition to studies listed in Table 2, a recent study has shown that exposure to organic solvents was associated with an increased risk of AML and especially high risk (OR 11, 95% CI 2.7–42) was found for AML with trisomy

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8 as the sole aberration (Albin et al., 2000). Numerical and structural changes of chromosome 3, such as monosomy 3, trisomy 3 and del(3)(p13p21) etc. in AML patients have also been reported recently to be associated with occupational exposure to organic solvents and/or petroleum products (Lindquist et al., 2000). Loss of E-group chromosomes, including chromosomes 17 and 18, was detected in several studies (Golomb et al., 1982; Groupe, 1984). More detailed analysis of the abnormal karyotypes shown in Table 2 will be presented in the Discussion section.

In summary, aneuploidy (hypodiploidy and/or hyperdiploidy) and polyploidy have been detected in leukemia cases associated with benzene exposure. Trisomy of C-group chromosomes was especially common, and the loss of chromosomes 17 and 18 was found in three independent studies. Most structural changes detected were gaps and breaks, with the majority being chromosome type rather than chromatid type. While specific structural changes can now be detected with banding technology, no particular translocation has been observed in more than one case (see Table 1). Results from patients whose leukemia probably resulted from benzene exposure appeared largely consistent with those from the benzene-associated leukemia case reports. However, among cases with possible prior exposure to benzene, several subjects displayed the same translocation. Nevertheless, there is insufficient data to conclude that benzene induces specific structural changes in patients with benzene-associated leukemia.

IV. CHROMOSOMAL ABERRATIONS DETECTED IN PRELEUKEMIA AND BENZENE-POISONED PATIENTS

Short of inducing leukemia, benzene exposure also causes a variety of preleukemic hematological disorders, including aplastic anemia, blood dyscrasia, hemopathy, and myelofibrosis. The criteria for the diagnosis of benzene poisoning,* another potential result of chronic exposure to benzene, varies in practice. For instance, Chinese clinicians diagnose benzene poisoning when a patient's white blood cells count falls below 4000/mm^3 (Ding et al., 1983). Under the "Rule of Four" in European clinical practice, however, benzene poisoning is indicated by the presence of any of the following conditions: white blood cells below 4000/mm^3, red blood cells below 4,000,000/mm^3, or neutrophils below 40% (Saita, 1973). Because in some cases it is difficult to distinguish benzene poisoning from other benzene-related hematological disorders, all of these conditions have been consolidated in Table 3. We have located 18 studies of chromosome changes in cases of benzene poisoning, both current and past, and preleukemia (Table 3). Early studies were mostly performed by the Italian scientists Pollini and Forni working independently. Table 3 reports the duration of exposure to benzene, although this information was not available in some studies. Most exposures in the assembled cases were occupationally related and are more likely than not associated with benzene exposure.

A. Numerical Chromosome Changes

From 40 to 70% of the dividing cells analyzed in Pollini's studies were reported as abnormal. In one study, the percentage of abnormal karyotypes fell from 70% to 40% 5 years after hemopathic diagnosis (Pollini et al., 1969). Aneuploidy, both the loss and gain of chromosomes, was found in most abnormal cells examined (Table 3). Generally, hypodiploidy was much more frequent than hyperdiploidy (Ding et al., 1983; Haberlandt and Mente, 1971; Liniecki et al., 1971; Maugeri et al., 1965; Pollini et al., 1976; Pollini and Colombi, 1964a; Pollini and Colombi, 1964b). This finding, however, may partially result from the overreporting of hypodiploidy that commonly occurs during the preparation of metaphase spreads in the classic cytogenetic assay. A 19.4% incidence of aneuploid cells was detected in patients with previous benzene hemopathy, compared with 9.9% in controls (Forni, 1966). Hyperdiploidy was a particularly frequent form of aneuploidy in the bone marrow of some patients (Forni, 1966). Two trisomic clones (+19 and +21) were detected in two cases of hemopathy (Pollini et al., 1969).

Loss and/or gain also occurred in E-group chromosomes (Ding et al., 1983; Pollini et al.,

* For a recent report of a fatal case of benzene poisoning caused by acute benzene exposure, see Barbera et al. (Barbera, et al., 1998).

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TABLE 3
Chromosomal Aberrations Detected in Preleukemic and Benzene-Poisoned Patients

Reference	n	Sex	Diagnosis	Benzene exposure (yr)	Specimen	Cell #	Abnormal	Numerical CA	Structural CA
Pollini, 1964 a	5	N/A	Poisoning	N/A	BM	342	70%	Hypo- (63%), hyper- (4%); poly-	f
Pollini, 1964 b	6	N/A	Aplastic anemia	> 5	PB	514	70%	Hypo- (66%), hyper- (3%), +C, E, F; +4, 21; -X, 1, 3, 7, 15, 17, 20; --5, 15, 22; poly-	
Pollini, 1964 c	4	N/A	Blood dyscrasia	N/A	PB/BM	N/A	70%	Aneuploidy, -C, D, E, G; --5, 18, 20, 22; +15	
Magueri, 1965	132	N/A	Hemopathy	N/A	PB/BM	N/A	N/A	Hypo-, hyper-, poly-, -3, 5, 21, 22	f
Rozman, 1965	20	5 F	Aplastic anemia	1.5 - 17	PB/BM	N/A	N/A	Polyploidy	
Forni, 1966	1	M	Poisoning	Acute	PB	100	6%		C_u
	3	1 F	Hemopathy	N/A	PB/BM	271	7%	+C, E, F, G	C_u, C_s
	12	7 F	Past hemopathy	N/A	PB	784	6%	Aneuploidy (Hyper-)	C_u, C_s
Pollini, 1969	5	F	Hemopathy	N/A	PB	349	70%	Aneuploidy, +19, +21	C_u, C_s
			5 yr later	N/A	PB		40%	+19, 21; -A to C	C_u, C_s
Forni, 1971 a	25*	12 F	Past hemopathy	N/A	PB	2380	~19%	Aneuploidy, poly-	C_u, C_s
	7	3 F	Poisoning	N/A	PB/BM			Aneuploidy	C_u, C_s
Haberlandt, 1971	12	N/A	AA, panmyelopathy	N/A	PB	40 - 50	~22%	Hypo- (20%), hyper- (2%)	g, b (A to E)
Liniecki, 1971	12	11 F	Past poisoning	2 - 5	PB	1911	13%	Hypo- (12%), hyper- (0.6%)	f, d
Hartwich, 1972	1	N/A	Panmyelopathy	7	PB	125	14.4%	Polyploidy	g, b
Erdogan, 1973	10	1 F	Pancytopenia	3 - 10	PB/BM	N/A	≤ 16%	Aneuploidy, poly-, +/-C, +9	C_u, b, f
Erdogan, 1975	4	N/A	Preleukemia	4 - 10	PB/BM	N/A	22%	Polyploidy, +C	g, f, d, b, t(9;22)
Pollini, 1976	4	F	Hemopathy	N/A	PB	349	38%	Hypo- (33%), hyper- (5%)	
Van den Berghe, 1979	1	M	Aplastic anemia	N/A	PB/BM	19	95%	-7	
Ding, 1983	21	13 F	Poisoning	1 - 28	PB	1032	4%	Hypo- (11%), hyper- (1.4%) -C, E, G; +C, E; poly-	g, b, etc.
Antonucci, 1989	1	M	Myelofibrosis	7	BM	16	N/A	++8	del(12)(p11-12)
Li, 1990	35	23 F	Poisoning	1.7 - 18	PB	350	N/A	+6, +X, -X	2q-, 6q-, 12q-; t(3;3)
				1.7 - 18	BM	99	N/A	Aneuploidy	2q-, 6q-

Abbreviations: -: monosomy, --: loss of both copies, +: trisomy, ++: tetrasomy, q-: q-arm deletion, g: gaps, b: breaks, f: fragments, C_u: unstable CA (e.g. acentric fragments, ring chromosomes, and dicentric chromosomes), C_s: stable CA (e.g. deletions and translocations, etc.), PB: peripheral blood, BM: bone marrow, Hypo-: hypodiploidy, Hyper-: hyperdiploidy, Poly-: polyploidy, AA: aplastic anemia, N/A: information not available. *: Most of these past hemopathy cases were first reported in Forni, 1966.

1964c). Aneuploidy of chromosomes 10, 14, and 22 was observed in the bone marrow cells of patients in a more recent study (Li and Ding, 1990). Polyploidy has also been found, but in a limited number of cells (Ding et al., 1983; Erdogan and Aksoy, 1973; Erdogan and Aksoy, 1975; Forni et al., 1971a; Hartwich et al., 1969; Maugeri et al., 1965; Pollini and Colombi, 1964a; Pollini and Colombi, 1964b; Rozman et al., 1965).

B. Aneuploidy of C-Group Chromosomes

The loss and/or gain of C-group chromosomes has been observed in several studies (Antonucci et al., 1989; Ding et al., 1983; Erdogan and Aksoy, 1973; Erdogan and Aksoy, 1975; Forni, 1966; Li and Ding, 1990; Pollini and Colombi, 1964b; Pollini et al., 1964c; Van den Berghe et al., 1979). Monosomy 7 was detected in 95% of peripheral blood and bone marrow cells sampled from a patient with benzene-induced aplastic anemia; however, 2 months later, the clonal anomaly had entirely disappeared (Van den Berghe et al., 1979). Trisomy C appeared in three of ten pancytopenia patients and in one case was identified as +9 (Erdogan and Aksoy, 1973). In benzene-poisoned patients, monosomy and trisomy were both more commonly detected in the C-group than in other chromosomes (Ding et al., 1983), but this may simply be a reflection of the fact that the C-group is the largest chromosome group, containing 7 of the 24 chromosomes. Monosomy, trisomy, and hyperdiploidy X were found in poisoned patients in another study (Li and Ding, 1990). In addition, trisomy 6 was found in one poisoning case (Li and Ding, 1990). The gain of two copies of chromosome 8 (tetrasomy 8) was detected in a 55-year-old male who acquired benzene-induced aplastic anemia 20 years before he developed myelofibrosis (Antonucci et al., 1989).

C. Structural Chromosome Changes

As in leukemia cases associated with benzene exposure, gaps, breaks, and fragments have been detected by classic nonbanding cytogenetic analyses in cases of hematological disorders associated with benzene exposure (Ding et al., 1983; Erdogan and Aksoy, 1973; Erdogan and Aksoy, 1975; Haberlandt and Mente, 1971; Hartwich and Schwanitz, 1972; Liniecki et al., 1971; Maugeri et al., 1965; Pollini and Colombi, 1964a). Most gaps and breaks seemed to occur in the chromosomes of groups A through E (Haberlandt and Mente, 1971). Nine out of 10 cases of pancytopenia in one study had structural CA (Erdogan and Aksoy, 1973), which have been categorized into stable CA (C_s, including deletions and translocations) and unstable CA (C_u, including acentric fragments, ring chromosomes, and dicentric chromosomes) (Dabney, 1981; Forni, 1978). Stable CA can be transmitted to further generations of cells. High rates of both stable and unstable CA have been found in benzene-poisoned and preleukemia patients (Forni, 1966; Forni et al., 1971a; Pollini et al., 1969). However, in one earlier benzene poisoning case and several pancytopenia cases, a high number of C_u, but no C_s, were detected (Erdogan and Aksoy, 1973; Forni, 1966).

The Ph chromosome was observed in a case of preleukemia (leukopenia) resulting from 4 years of chronic exposure to benzene without indications of leukemia. Interestingly, after 4 years without exposure, the aberration disappeared (Erdogan and Aksoy, 1975). Chromosome deletions have been found in various studies. Deletion of the short-arm of chromosome 12 was found in one study (Antonucci et al., 1989), and deletion of its long-arm was found in another (Li and Ding, 1990). In the latter report, the long-arm deletions of chromosomes 2 and 6 were also detected (Li and Ding, 1990). Gaps and deletions of the long-arm of chromosome 6 were more commonly detected than those of other chromosomes in poisoning cases (Li and Ding, 1990). Translocation t(3;3) was observed in one poisoning case (Li and Ding, 1990).

D. A Unique Follow-Up Cytogenetic Study

In an earlier report, CA were analyzed in 32 case subjects with past benzene hemopathy or benzene poisoning and in matched controls (Forni et al., 1971a). Both numerical and structural CA were detected in this study (Table 3). To investigate

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whether increased CA in lymphocytes were predictive of future cancer risk and whether CA persist over time in these subjects, the same author conducted a follow-up study of these same subjects (one was lost to study) about 25 years later (Forni, 1996). Forni subclassified the subjects according to vital status and regrouped the original cytogenetic data. Chromosome-type aberrations were found in 2.3% of cells among the 20 subjects with past benzene hemopathy or benzene poisoning still living. Among the 11 subjects who had died prior to the follow-up study, 3.9% of cells had chromosome-type aberrations (4.4% among 5 subjects with neoplastic diseases, including one with AML-M6, and 3.5% among the remainder). Living and deceased controls both had the same level (1%) of chromosome-type aberrations. These results suggest that increased CA may indicate higher cancer risk, although the author emphasizes that this may be at a group rather than individual level (Forni, 1984). At the time of the follow-up study, CA rates were remeasured in the lymphocytes of four subjects with past hemopathy, now recovered, and seven controls. Hyperdiploidy, which was absent in controls, was detected at similar rates in case subjects in the initial study and in the follow-up (Forni, 1996). Chromosome-type aberrations persisted at similar levels in the later study (Forni, 1996). Prior to this follow-up study, however, another report found rates of aneuploid cells, mainly hypodiploid, returning to normal levels in four subjects who had been studied during benzene hemopathy 12 years earlier (Pollini and Biscaldi, 1977).

E. Summary of Chromosomal Changes in Preleukemia and Hematotoxicity Associated with Benzene Exposure

Aneuploidy, including hypodiploidy and hyperdiploidy, has been observed in the peripheral blood lymphocytes of patients with preleukemia and hematotoxcity associated with benzene exposure. Trisomy and monosomy of C- and E-group chromosomes were particularly common. Abnormal cells were reported in as few as 4% of samples in one analysis and as many as 95% in another. Nonspecific structural changes, including unstable and stable CA, have been detected, and two studies have found specific deletions, including those on chromosomes 2, 6, and 12. The unique follow-up study by Forni suggests that increased chromosome-type aberrations may be a good indicator of future cancer risk and that elevated levels persist for years after recovery from benzene hematotoxicity.

V. CYTOGENETIC CHANGES DETECTED IN BENZENE-EXPOSED (NON-DISEASED) INDIVIDUALS

Since the 1960s, approximately 50 cytogenetic studies in benzene-exposed individuals have been conducted. Most studies have shown a positive association between benzene exposure and increased CA (Table 4). However, eight of those studies reported negative results (Table 5). Other cytogenetic markers, SCEs and MN, were applied to measure chromosome changes resulting from benzene exposure (Table 6). In addition, six studies examined cytogenetic aberrations in individuals exposed to fuel, organic solvents, or other sources suspected to contain benzene. These studies are presented separately (Table 7). All cytogenetic studies but one compared results from unexposed controls, on the one hand, and currently or previously exposed but otherwise healthy individuals on the other; the single exception analyzed exposed workers without reference to controls (Girard et al., 1970). Three other studies that included controls did not provide their number or other data (Fredga et al., 1979; Hartwich and Schwanitz, 1972; Lalchev et al., 1997). A wide range of benzene exposure, from less than 1 to more than 500 ppm (parts per million), was reported in the CA studies, while in some cases the levels of exposure were unknown. Duration of exposure also varied widely, from a few months up to 50 years, and was unknown in a few cases. Virtually all studies examined peripheral blood lymphocytes, because these specimens are easily obtained; only a few studies sampled other types of cells.

A. Studies Finding Increased Chromosomal Aberrations

Over the last 3 decades, more than two dozens studies have found an increase in chromo-

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TABLE 4
Summary of Studies Showing Positive Results of Benzene Exposure on CA

Reference	Subject #		Benzene exposure		Cell # per subject	Endpoints		
	Exposed	Controls	Level, ppm (Mean)	Duration, yr (Mean)		NCA	SCA	
							C_u	C_s
Tough, 1965	20	43[a]	N/A	1 - 20	100		↑	↑ (NS)
Forni, 1966	3	15	N/A	N/A	100		↑	↑
Tough, 1970	20	5	25 - 150	1 - 17	100		↑	x
	12	6	25 - 150	4 - 23			x	x
	20	5	12	2 - 26			x	x
Girard, 1970	50	0	14	N/A		Poly-	b	
Forni, 1971 b	10	34	125 - 532[b]	1 - 22	100	↑ (Hyper-, poly-)[c]	↑	↑
Hartwich, 1972	9	N/A	< 25 MAC[h]	1 - 7	100	↑ (Poly-)	↑ (g, b)	
Khan, 1973	7	14	N/A	11 - 20	50		↑↑	
	8			2 - 5			↑	
Funes-Cravioto, 1977	15[d]	42	N/A	N/A	100[e]		↑ (b)	
Fredga, 1979	65	N/A	0.02 - 10	0.1 - 43	100	↑ (Aneu-, poly-)	↑ (g, b)	
Picciano, 1979	52	44	< 10 (2.1)	0.1 - 26 (4.72)	200		↑ (b, mar)	
Ding, 1983	6	20	N/A	1 - 28 (6)	50	↑ (Hypo-)		
Sasiadek, 1984	30	16	≤ 31	11 - 23	30		↑ (b)	
Sarto, 1984	22	22	0.2 - 12.4	3 - 35	80 -100		↑ (b, f)	
Damrau, 1986	16	26	15.6 - 23.4	(18)	N/A		↑	
	44		1.6 - 3.1	(14)			↑ (NS)	
Werner, 1986	26	21	N/A	1 - 30	~ 100	↑ (Aneu-)	↑ (g, b)	
Jablonická, 1987	66	20	0.5 - 12	Long-term	100		↑ (b) (NS)	
Gundy, 1989	10	211	2.2 - 10.8 (8.2)[f]	1 - 27 (11.4)	100		↑	

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Sasiadek, 1989	33	15	< 31	3 - 23	100		↑ (g, b) b: 2, 4, 9 g: 1 & 2
Sasiadek, 1990	16	10	< 31	15 - 30	100		↑ (g, b) (2, 4)
Yardley-Jones, 1990	48	29	1 - 10[g]	> 5	< 100	x	↑ (g, b)
Sasiadek, 1992	56	20	< 10[h]	10 - 20	70 - 100		↑ (g,b) (2,4,7)
Major, 1994	42	42	0.3 - 15.3 (2.2)	≥ 2	100	x	↑ (b)
Tompa, 1994	49	213[i]	1 - 21 [1990] 0.5 - 8.5 [1991]	0 - 2 2 - 10	100 100	↑	↑
Tunca, 1994/1996	58	20	N/A	5 - 50 (23)	20 - 30	↑ (NS)	↑ (g, b)
Gao, 1994	12 7	10 7	4 - 60 9 - 49	2 - 21 N/A	100		↑ ↑
Karacic, 1995	38 45	35	2 - 15[f] 2 - 13[f]	2 - 31 (13.4) 1 - 33 (17.7)	200		↑ (d) ↑ (d)
Bogadi-Sare, 1997	49	27	≤ 15[j]	1 - 33 (17)	200		↑ (d)

↑ = increase; ↑↑ = large increase; x = no association; NS = not significant; Poly- = polyploidy; Aneu- = aneuploidy; Hyper- = hyperdiploidy; Hypo- = hypodiploidy; g = gaps; b = breaks; d = dicentric; f = fragments; mar = markers; N/A = information not available.

[a] Two groups of controls: general controls (n=38) and industrial controls (n=5). [b] Exposed to benzene, then toluene. [c] Statistical analysis, reperformed by Fisher's exact test, showed significance. [d] Group B (exposed primarily to benzene). [e] Only cells with 46 chromosomes examined. [f] Also exposed to toluene. [g] With peak exposure from 10 - 100 ppm for brief periods. [h] Not measured, but lower than maximum allowable concentration (MAC). [i] Two groups of controls: general controls (n=91) and industrial controls (n=122). [j] Concurrently exposed to ≤ 50 ppm toluene.

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TABLE 5
Summary of Studies Not Showing Positive Results of Benzene Exposure on CA

Reference	Subject #		Benzene Exposure		Cell # per subject	Endpoints	
	Exposed	Controls	Level, ppm (Mean)	Duration, yr (Mean)		NCA	SCA
Watanabe, 1980	9 7	7	Trace - 9 3 - 50[a]	1 - 20 2 - 12	30 - 50 30 - 50	x x / ↓ slight	x x / ↓ slight
Clare, 1984	10	11[b]	Acute	Accidental	200	x (poly-)	↓ slight
de Jong, 1988	32	108	0.03 - 0.8 (0.1)	1 - 13 (7)	90 - 100		x
Yardley-Jones, 1990	48	29	1 - 10[c]	> 5	≤ 100	x (hyper-)	↑ (g, b)
Nise, 1991	21	21	0.2 - 5[d]	0.5 - 37	100		x
Major, 1994	42	42	0.3 - 15.3	≥ 2	100	x	↑ (b)
Carere, 1995	23	24	0.03 - 4.1	3 - 42	200		x
Carere, 1998a	12	15	(1.7)	(8.8)	N/A		x / ↓ slight

↑ = increase; x = no association; ↓ = decrease; poly- = polyploidy; hyper- = hyperdiploidy; g = gaps; b = breaks; N/A = information not available.

[a] With peak exposure up to 177 ppm.
[b] Although the study analyzes results from 11 "acting controls" from a comparable shift, it is possible that these workers might also have been chronically exposed to benzene during cleanup.
[c] Occasionally exposed to peak levels of 10 - 100 ppm for brief periods.
[d] Workers were exposed to benzene and toluene. Benzene level is calculated as 0.5% of average toluene exposure level (150 mg/m^3), although some older workers may have been exposed to levels up to 5 ppm based on higher levels of benzene in toluene during the 1950s.

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TABLE 6
Summary of Studies of Effect of Benzene Exposure on SCEs and MN

Reference	Subject #		Benzene exposure		Cell # per subject	Endpoints	
	Exposed	Controls	Level, ppm (Mean)	Duration, yr (Mean)		SCEs	MN
Watanabe, 1980	9	7	Trace - 9	1 - 20	30 - 50	↓ slight	
	7		3 - 50[a]	2 - 12	30 - 50	↓	
Clare, 1984	10	11	Acute	Accidental	30	↑ slight	
Sarto, 1984	22	22	0.2 - 12.4	3 - 35	15	x	
Yardley-Jones, 1988	28	23	1 - 10[b]	> 5	25	x	
Gundy, 1989	10	211	2.2 - 10.8 (8.2)[c]	1 - 27 (11.4)	50	↑	
Seiji, 1990	36	11	50	(5.5)	25	x	
Nise, 1991	21	21	0.2 - 5[d]	0.5 - 37	4000		x (PHA-)
					4000		↑ (PWM-)
Hogstedt, 1991	15	15	0.3[e]	N/A	1000		x (PHA-)
							↑ (PWM-)
Popp, 1992	20	20	< 5	≥ 8 (18)[f]	25	↑	
Major, 1994	42	42	1 - 6	≥ 2	50	↑	
Tompa, 1994	10	91 / 122[g]	1 - 21	0 - 2	50	↑	
	22			2 - 10		↑↑	
	17			> 10		↑	
Gao, 1994	12	10	4 - 60	2 - 21	1000		↑
	7	7	9 - 49	N/A			↑
Xu, 1995	59	40	5 - 68	N/A	1000		↑
Carere, 1995	23	24	0.03 - 4.1	3 - 42	75	x	
					2000		x (PHA-)
					1000		x (PWM-)
Karacic, 1995	38	35	2 - 15	2 - 31	200	↑	
	45		2 - 13	1 - 33		x	
Liu, 1996	87	30	0.8 - 133[h]	N/A	2000		↑
Zhang, 1996a	89	150	0.02 - 9.14	7	1000		↑
Surralés, 1997	38	13	1	N/A	2000		x
	18	15	1	N/A	1000[i]		x

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TABLE 6 (continued)

Bogadi-Sare, 1997	49	27	< 15[j]	1 - 33	200	x	
Xu, 1998	23	22	0.7	N/A	N/A	x	
Carere, 1998a	12	15	(1.7)	(8.8)	25	x	
					2000		↓ (PHA-)
					1000		↓ (PWM-)
	12	8	(1.3)	(6.6)	25	x	
					2000		↓ (PHA-)
					1000		↓ (PWM-)
Holland, 1999	43	44	1.6 - 328.5	0.7 - 16 (6.3)	1000		x

↑ = increase; ↑↑ = large increase; x = no association; ↓ = decrease; PHA- = phytohemagglutinin; PWM- = pokeweed mitogen; N/A = information not available.

[a] With peak exposure up to 177 ppm. [b] Occasionally exposed to peak levels of 10 - 100 ppm for brief periods. [c] Workers exposed to benzene and toluene. [d] Workers exposed to benzene and toluene. Benzene level is calculated as 0.5% of average toluene exposure level (150 mg/m^3), although some older workers may have been exposed to levels up to 5 ppm based on higher levels of benzene in toluene during the 1950s. [e] Occasionally exposed to peak levels up to 6 ppm. [f] One subject had only 3 years of exposure. [g] Two separate groups of controls: general controls (n=91) and industrial controls (n=122). [h] Exposed to benzene, toluene, and xylene. [i] Buccal cells. [j] Concurrently exposed to ≤ 50 ppm toluene.

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TABLE 7
Representative Studies of Workers Exposed to Petroleum Fuels or Organic Solvents

Reference	Subject #		Chemical exposure		Cell # per subject	Endpoints			
	Exposed	Controls	Type or source	Duration, yr (Mean)		NCA	SCA	SCEs	MN
Santos-Mello, 1992	49	24	Fuel vapors, auto emissions	0.5 – 36	150		↑ (d)		
Betancourt, 1995	4	6	Gasoline fumes	Acute	50		↑ (b)		
Silva, 1996	25	20	Car paints	1 – 39	200	↑	↑ (d)		
Lalchev, 1997	13	N/A	Organic solvents (higher)	N/A	100		↑		
					30			↑	
					1000				↑
	26	N/A	Organic solvents (lower)	N/A	100		↑		
					30			x	
Bukvic, 1998	22	19	Fuel vapors, auto emissions	1 – 42	≥ 25			x	
					1000*				↑
Carere, 1998b	12	12	Fuel vapors, auto emissions	(24.1)	2000				x

↑ = increase; x = no association; d = dicentric; b = breaks; N/A = information not available. *: MN assay performed among 21 exposed subjects.

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some changes in benzene-exposed populations (Table 4). For the most part, these studies analyzed different groups of exposed workers, separated by exposure level, duration, occupation, and/or time of sampling (Damrau et al., 1986; Fredga et al., 1979; Gao et al., 1994; Karacic et al., 1995; Khan and Khan, 1973; Tompa et al., 1994; Tough et al., 1970; Werner, 1986). For the sake of convenience we have generally consolidated these different exposed groups in Table 4, particularly where similar results were reported among groups (Fredga et al., 1979; Tompa et al., 1994; Werner, 1986). In some studies, control subjects were classified into general and "industrial" controls (Tompa et al., 1994; Tough and Court Brown, 1965). Around 30 to 200 metaphase cells were analyzed in the assembled cases. In this review, we recorded numerical and structural aberrations separately in the tables in order to evaluate patterns of chromosome changes.

1. Structural Chromosomal Aberrations

Most of the studies employing traditional cytogenetic techniques observed structural CA. These structural changes were detected as C_u and C_s, both of which were increased in benzene-exposed workers (Forni, 1966; Forni, 1971b; Tough and Court Brown, 1965), although in one study C_s were not increased significantly (Tough and Court Brown, 1965). Five years later another study by the same authors found increased C_u, but not C_s, in one group of exposed subjects (Tough et al., 1970). Increased structural changes in general have been detected in benzene-exposed workers (Damrau et al., 1986; Gao et al., 1994; Gundy, 1989; Khan and Khan, 1973; Tompa et al., 1994), with much larger increases reported among a group of subjects with longer exposure periods (Khan and Khan, 1973). Gaps and breaks were the types of structural CA most frequently increased in exposed workers (Fredga et al., 1979; Funes-Cravioto et al., 1977; Hartwich and Schwanitz, 1972; Jablonická et al., 1987; Major et al., 1994; Picciano, 1979; Sarto et al., 1984; Sasiadek, 1984; Sasiadek, 1992; Sasiadek and Jagielski, 1990; Sasiadek et al., 1989; Tunca and Egeli, 1994; Tunca and Egeli, 1996; Werner, 1986). In Sasiadek's series of studies from 1989 to 1992, higher than expected levels of breakage were located on chromosomes 2, 4, 7, and 9 (Sasiadek, 1992; Sasiadek and Jagielski, 1990; Sasiadek et al., 1989). Increased rates of dicentric chromosomes were found in two recent reports (Bogadi-Sare et al., 1997; Karacic et al., 1995). Finally, another study reported increased marker chromosomes in exposed workers (Picciano, 1979).

2. Numerical Chromosomal Aberrations

Numerical CA encompasses aneuploidy, including hyperdiploidy and hypodiploidy, and polyploidy (Table 4). Perhaps due to the limitations in classical cytogenetic studies and analyses, relatively few studies (fewer than half) reported increases in numerical CA. Both aneuploidy and polyploidy have been detected in benzene-exposed workers (Ding et al., 1983; Forni, 1971b; Fredga et al., 1979; Girard et al., 1970; Hartwich and Schwanitz, 1972; Werner, 1986). Recent studies have found possible increases in numerical chromosome changes in exposed workers, but according to statistical analyses performed by the reporting authors, these increases were not significant (Tunca and Egeli, 1994; Tunca and Egeli, 1996). An earlier study reported increases in hyperdiploidy and polyploidy, and although the statistical analyses applied by the authors showed no significance (Forni, 1971b), we found these changes to be significant when we reanalyzed the results using the Fisher's exact test. The increase detected in hyperdiploidy was greater than that in polyploidy. Hypodiploidy has also been increased in exposed workers (Ding et al., 1983). While most studies have shown that benzene increases CA in exposed subjects, several studies, reported below, have yielded only a weak association between CA and benzene exposure.

B. Studies Not Finding Increased Chromosomal Aberrations

Several studies reported no increase in CA resulting from benzene exposure (Table 5). Numerical CA, including aneuploidy and polyploidy, were not increased in benzene-exposed subjects in four studies (Clare et al., 1984; Major et al.,

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1994; Watanabe et al., 1980; Yardley-Jones et al., 1990). Among these studies, at least two showed an increase in structural CA (mostly gaps and breaks), as described in the previous section (Major et al., 1994; Yardley-Jones et al., 1990). No significant increase in structural changes was found in five reports in which workers had been exposed to trace and lower levels of benzene (Carere et al., 1995; Carere et al., 1998a; de Jong et al., 1988; Nise et al., 1991; Watanabe et al., 1980). These negative results may simply be due to a lack statistical power. Further, the increase in overall SCA in one study was of borderline statistical significance, and a significant trend was observed when subjects were divided into controls and lower and higher exposure groups (Carere et al., 1995). Another study found slightly decreased SCA in workers acutely exposed following a benzene spill (Clare et al., 1984). For reasons that are unclear, both types of CA were slightly decreased in workers exposed to high levels of benzene (up to 177 ppm) (Watanabe et al., 1980).

While the reasons for the negative results found in these studies are unclear, the following factors may have affected the results: (1) a small number of study subjects and examination of relatively few cells (Watanabe et al., 1980); (2) acute benzene exposure of short duration (Clare et al., 1984); (3) very low levels of benzene exposure (de Jong et al., 1988), or low levels of exposure combined with concurrent exposure to toluene (Nise et al., 1991) or other chemicals (Carere et al., 1995); or (4) ethnic differences between control and exposed subjects (Carere et al., 1998a). Such factors may have reduced the statistical power of these studies. Despite these occasional negative studies, the overall weight of the evidence strongly indicates that benzene induces CA in the peripheral blood lymphocytes of exposed workers.

C. Studies Examining Sister Chromatid Exchanges and Micronuclei

Besides CA, other cytogenetic markers such as SCEs and MN have also been used to measure chromosome changes. Although not the primary focus of this review, 14 studies analyzing SCEs and ten measuring MN are included to provide a point of comparison for CA studies of benzene-exposed individuals (Table 6). We find that results from studies examining SCEs and MN are much more variable than those from studies analyzing CA.

1. Sister Chromatid Exchanges (SCEs)

Studies measuring SCEs are relatively recent, dating back only to 1980 (Table 6). While previously SCEs were measured in smaller numbers of cells (about 15 to 30) per subject, they have more recently been measured in as many as 200. Whether SCEs are increased in benzene-exposed workers is unsettled. Early studies conducted during the 1980s showed mostly negative results (Sarto et al., 1984; Seiji et al., 1990; Yardley-Jones et al., 1988). In one report, a slight decrease in SCEs was detected (Watanabe et al., 1980). However, slight increases in SCEs were found in another case involving an accidental benzene spill that resulted in acute exposure (Clare et al., 1984). Several more recent studies have detected increased SCEs following exposure to benzene (Gundy, 1989; Major et al., 1994; Popp et al., 1992; Tompa et al., 1994). One study analyzed cells only at the first division in two groups of exposed workers with similar levels and duration of exposure. Increased SCEs were found in the first group, but not the second (Karacic et al., 1995). Four recent reports found no increase in SCEs in benzene-exposed workers compared with controls (Bogadi-Sare et al., 1997; Carere et al., 1995; Carere et al., 1998a; Xu et al., 1998). Therefore, based on the conflicting results from these studies, the effect of benzene exposure on SCE induction remains unclear. This point is well illustrated by Karacic's study, which found different results for two groups of workers with similar parameters of exposure.

2. Micronuclei (MN)

The MN assay has been applied in studies of benzene exposure only during the last decade (Table 6) (Carere et al., 1995; Carere et al., 1998a; Gao et al., 1994; Högstedt et al., 1991; Holland et

al., 1999; Liu et al., 1996; Nise et al., 1991; Surrallés et al., 1997; Xu et al., 1995; Zhang, 1996a). More than half of these studies found no association between increased MN frequency and exposure to benzene (Carere et al., 1995; Carere et al., 1998a; Högstedt et al., 1991; Holland et al., 1999; Nise et al., 1991; Surrallés et al., 1997), but almost all of these negative studies involved relatively low levels of exposure (< 5 ppm) (Carere et al., 1995; Carere et al., 1998a; Högstedt et al., 1991; Nise et al., 1991; Surrallés et al., 1997). On the other hand, increased MN frequencies in pokeweed mitogen (PWM) stimulated lymphocytes of exposed workers were detected in two studies (Högstedt et al., 1991; Nise et al., 1991). In addition, four other reports by Chinese investigators have reported increased MN in benzene-exposed workers (Gao et al., 1994; Liu et al., 1996; Xu et al., 1995; Zhang, 1996a); in one study this increase was dose-dependent (Liu et al., 1996). Thus, whether increased frequencies of MN are associated with benzene exposure remains controversial.

D. Representative Studies in Workers Exposed to Petroleum Fuels or Organic Solvents

Several studies examined cytogenetic endpoints in subjects exposed to petroleum fuels, auto exhaust, or other organic solvents (Table 7). While benzene is likely to have been a component in these exposures, the benzene level was not ascertainable or available in these cases. Furthermore, individuals in these studies were subjected to a combination of chemical exposures. For these reasons, these studies are presented together in Table 7 (Betancourt et al., 1995; Carere et al., 1998b; Lalchev et al., 1997; Santos-Mello and Cavalcante, 1992; Silva and Santos-Mello, 1996; Bukvic et al. 1998). Increased numerical CA were found in subjects working with auto paints, which used to contain benzene and other organic solvents (Silva and Santos-Mello, 1996). Structural CA, mostly in the form of dicentrics and breaks, were increased in exposed subjects in a majority of studies (Betancourt et al., 1995; Lalchev et al., 1997; Santos-Mello and Cavalcante, 1992; Silva and Santos-Mello, 1996). SCEs were increased in the higher but not the lower exposure group in one preliminary study of workers exposed to organic solvents (Lalchev et al., 1997). While MN were increased in the higher exposure group of subjects in this same study (Lalchev et al., 1997), there was no change in MN frequency in another study that examined MN among gas station attendants using a FISH probe (Carere et al., 1998b). Finally, a recent study among 22 gas station attendants found a significant increase in MN, but not in SCEs, comparing with control subjects (Bukvic et al., 1998).

In summary, benzene exposure has been strongly associated with increased CA in the lymphocytes of nondiseased individuals. Most structural CA detected were gaps and breaks. Elevated breakage reported in one series of studies was located on chromosomes 2, 4, 7, and 9. While numerical CA have been increased in exposed workers, the association between benzene exposure and NCA seems relatively weak. Several studies did not find increased CA in benzene-exposed workers, but these studies may lack statistical power for various reasons. In contrast, increases in SCEs and MN as a result of benzene exposure have not been established. Similar results for all three cytogenetic markers have been detected in workers exposed to petroleum fuels or organic solvents.

VI. APPLICATION OF FISH IN BENZENE-EXPOSED WORKERS

The results reported above were mostly obtained with classic cytogenetic techniques. However, this procedure has several drawbacks for the detection of CA. For example, cells must be cultured so that metaphase spreads may be obtained, only a limited number (25 to 100) of cells can typically be examined, and recognition of specific chromosomes is problematic. These problems can now be overcome by fluorescence *in situ* hybridization (FISH), which can be used to measure aneuploidy of specific chromosomes in large numbers of cells, perhaps 1000 or more (Eastmond and Pinkel, 1990; Gray and Pinkel, 1992). A significant advantage of FISH is that it can be used to analyze both metaphase spreads and interphase cells, unlike classic techniques,

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which can only examine dividing cells. FISH is now a widely used tool in the analysis of chromosome changes in human cancers, including leukemias, and in prenatal diagnostics (Cohen et al., 1993; Gray and Pinkel, 1992). It has been used extensively to analyze chromosome damage induced by exposure to ionizing radiation (Gray et al., 1994; Tucker and Senft, 1994) and has also been gradually applied to populations exposed to chemicals and various carcinogens (Dulout et al., 1996; Rupa et al., 1995; Zhang et al., 1996b). FISH-based analysis of interphase cells can be used to detect numerical chromosome changes or specific rearrangements. Recently, several studies have applied interphase FISH to analyze aneuploidy of specific chromosomes in the lymphocytes of benzene-exposed workers.

A. Aneuploidy Detected by Interphase Cytogenetics

The first report of benzene-induced numerical changes detected by interphase cytogenetics emerged in 1996 (Zhang et al., 1996b). Along with colleagues Drs. Nathaniel Rothman and Richard Hayes at the National Cancer Institute (NCI), Drs. Li and Yin at the Chinese Academy of Preventive Medicine in Beijing, and others at the Shanghai Anti-Epidemic Center as well as other institutions in the United States, we applied FISH techniques in this collaborative study of 43 Chinese workers highly exposed to benzene (median exposure level = 31 ppm, 8 h TWA) and 44 matched controls. Hyperdiploidy of chromosome 9 was detected at significantly increased levels in the lymphocytes of highly exposed benzene workers (> 31 ppm) but not in the lower-exposed group (Zhang et al., 1996b). The level of hyperdiploidy in exposed workers correlated with their urinary phenol level, a measure of internal benzene dose. A significant correlation was also found between hyperdiploidy of chromosome 9 and decreased absolute lymphocyte count, an indicator of benzene hematotoxicity, in the benzene-exposed group (Zhang et al., 1996b). Since the first study, hyperdiploidy of chromosomes 7 and 8 has also been analyzed in the same benzene-exposed population (Zhang et al., 1999). Hyperdiploidy frequencies for chromosomes 7, 8, and 9 are illustrated together in Figure 1. Hyperdiploidy was significantly increased in all three chromosomes in highly exposed workers (> 31 ppm benzene) (Figure 1). In the lower exposure group (≤ 31 ppm), only chromosome 7 showed a significant increase in hyperdiploidy. A significant trend was detected in hyperdiploidy of chromosomes 7 and 8.

Compared with the benzene levels in the above studies, most workers, particularly in developed countries, are presently exposed to lower concentrations of benzene; for instance, the permissible exposure level for benzene in the workplace in the United States was 1 ppm (OSHA, 1987) and the American Conference of Governmental Industrial Hygienists (ACGIH) has recently lowered its recommended threshold limit value (TLV) for occupational exposure to 0.5 ppm (ACGIH, 1999). As a result, recent scientific attention has begun to focus on the health effects of benzene at or near these lower levels. Interphase FISH has been employed to measure hyperdiploidy of chromosomes 7, 11, 18, and X in the lymphocytes of workers exposed to 0.1 ppm benzene (Carere et al., 1998b). No increased levels of hyperdiploidy were detected in any chromosome; in fact, slightly lower levels in comparison with controls were observed. Similarly, no increase in hyperdiploidy of chromosome 9 was detected in the buccal cells of workers exposed to 1 ppm benzene (Surrallés et al., 1997). Hyperdiploidy and breakage of chromosomes 1 and 9 were examined in different cell populations of workers exposed to about 1 to 2 ppm benzene in a recent study (Carere et al., 1998a; Marcon et al., 1999). No significant increase in either hyperdiploidy or breakage in chromosome 1 was detected in lymphocytes or granulocytes of exposed workers, both obtained from blood smear slides (Table 8). An increase of borderline significance was detected in heterochromatin breakage, but not hyperdiploidy, of chromosomes 1 and 9 in cultured lymphocytes.

Thus, recent studies by interphase FISH have not detected significant increases in hyperdiploidy resulting from exposure to very low levels of benzene. Potential explanations for this result may include the following possibilities: that a relatively small number of study subjects produces insufficient statistical power; that benzene in fact does not induce hyperdiploidy at lower exposure levels; or that interphase FISH is not a

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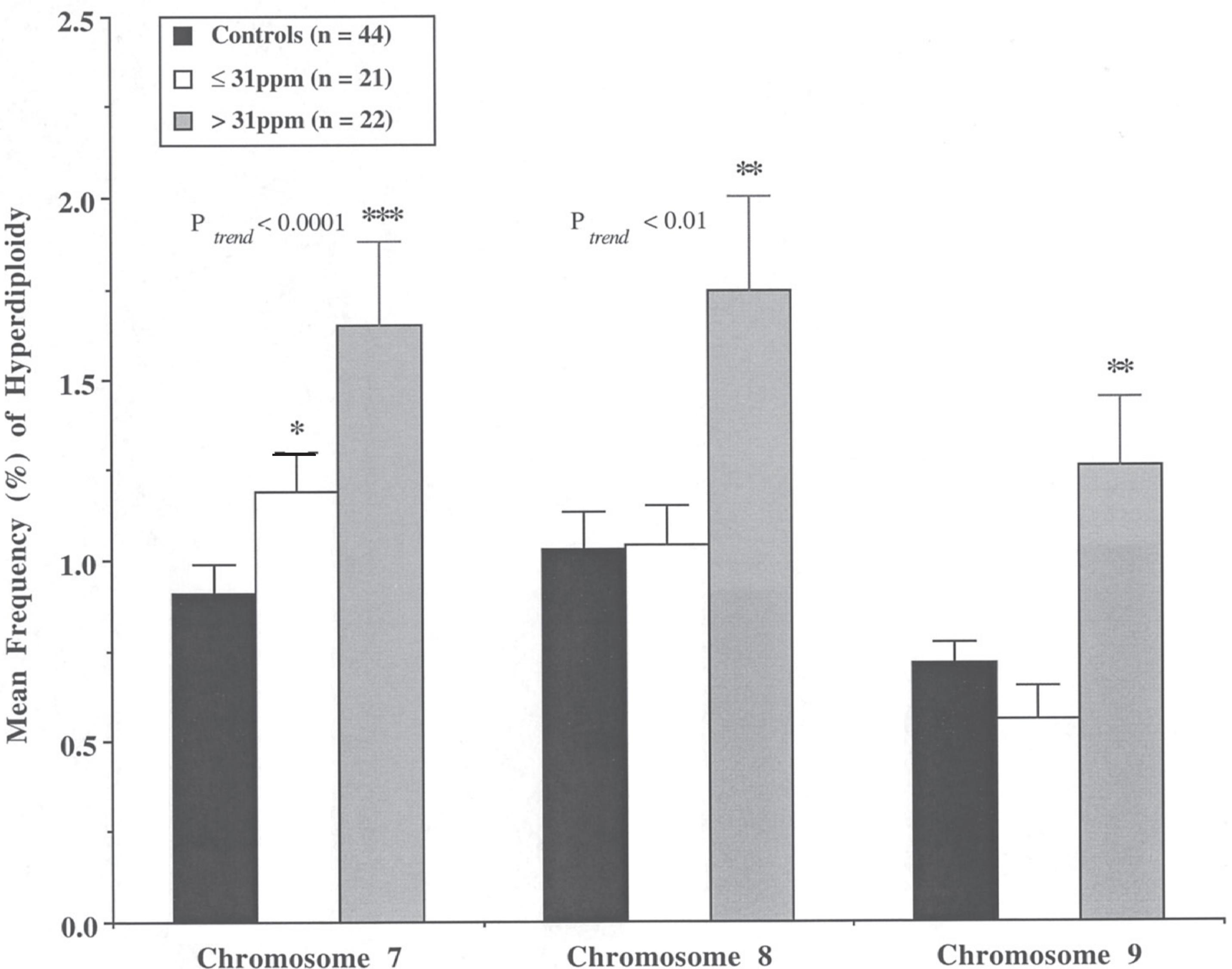

FIGURE 1. Hyperdiploidy of chromosomes 7, 8, and 9 in workers exposed to benzene and controls. Data presented are mean hyperdiploid frequency (%). Error bar represents S.E. Black bar represents control, white bar represents ≤ 31 ppm group, and gray bar represents > 31 ppm group. Test for trend was performed by linear regression on square-root transformed data. Hypodiploidy values in controls were compared to values in lower- and higher-exposed workers by analysis of variance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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TABLE 8
Breakage and Hyperdiploidy of Chromosomes 1 and 9 in Granulocytes and Lymphocytes of Workers Exposed to Low Benzene Levels[a]

Cell type	Controls (n = 8)		Exposed (n = 12)	
	Chromosome 1	Chromosome 9	Chromosome 1	Chromosome 9
Granulocytes in smear				
Breakage	0.83[b]		1.22	
Hyperdiploidy	0.13		0.33	
Lymphocytes in smear				
Breakage	0.60		0.81	
Hyperdiploidy	0.06		0.12	
Lymphocytes in culture (48 h)				
Breakage	0.30	0.59	0.65[c†]	0.95[c†]
Hyperdiploidy	0.01	0.00	0.06	0.05
Total aberrant	0.31	0.59	0.71[c*]	0.99[c*]

[a] Workers exposed to mean level of ~1-2 ppm benzene (Carere, 1998a; Marcon et al., 1999).
[b] Frequency per 100 cells.
[c] Statistical significance of the difference exposed vs. controls, estimated by one-way ANOVA on transformed outcome variables; † $P \sim 0.05$; * $P < 0.05$.

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sensitive assay for the detection of hyperdiploidy, particularly at lower doses. Analysis and comparison of results obtained by metaphase FISH is likely to be helpful in the evaluation of these possibilities.

Interphase FISH offers a number of advantages over classic cytogenetics (Eastmond et al., 1995). First, interphase FISH allows analysis of nondividing cells. Second, a much larger number of cells, as many as 1000 or more, may be analyzed. Third, the detection of aneuploidy is facilitated by simply counting the number of labeled regions representing a particular chromosome of interest within the isolated interphase nucleus. However, interphase FISH cannot readily detect the loss of chromosomes due to the problem of probe overlap artifact (Eastmond and Pinkel, 1990). In addition, interphase FISH may underestimate gain of chromosomes (Zhang et al., 1999). By contrast, metaphase FISH, because it analyzes dividing cells, does not suffer from the overlap problem and therefore can readily detect aneuploidy, the loss and gain of chromosomes. Furthermore, metaphase FISH can easily detect various types of structural rearrangements in addition to aneuploidy. In order to compare the sensitivity of both assays in detecting the same types of chromosome changes, several studies have applied both interphase and metaphase FISH in the same subjects.

B. Comparison of Data Obtained by Interphase and Metaphase FISH

While hyperdiploidy of selected chromosomes has been detected by interphase cytogenetics, as described above, aneuploidy of chromosomes 1, 7, and 8 has also been examined by metaphase FISH in the same highly exposed Chinese workers. Table 9 directly compares interphase and metaphase data for both monosomy and trisomy of chromosomes 1, 7, and 8. For chromosome 1 analysis, interphase FISH was applied in the Eastmond laboratory at the University of California, Riverside (Eastmond et al., 2001), while metaphase FISH was applied in the Smith laboratory at the University of California, Berkeley (Zhang et al., 1998a). No increase in monosomy 1 was detected in interphase cells or metaphase spreads in exposed workers (Table 9). At higher levels of exposure (> 31 ppm), a significant increase in trisomy 1 was detected by metaphase FISH but not interphase FISH, whereas in the lower exposed group (≤ 31 ppm) no increase in trisomy was detected by either method (Table 9). While no increase in monosomy 7 or 8 was observed by interphase FISH, increases in both were detected by metaphase FISH, but once again only in highly exposed workers. Both methods detected increases in trisomy 7 and 8, with more significant increases detected by metaphase FISH (Zhang et al., 1999).

Levels of monosomy 7 and 8 detected by interphase FISH were strikingly higher than those obtained by metaphase FISH (Table 9). Probe overlap artifact as well as less efficient hybridizations may explain the high levels of monosomy 7 and 8 detected in interphase cells. On the other hand, interphase and metaphase FISH data for monosomy 1, although obtained in different labs, were in good agreement. However, the performance of interphase and metaphase FISH in human biomonitoring studies has not been standardized with different probes, hybridization procedures, and scoring criteria being used by different research groups. These sources undoubtedly contribute to the variation in results reported by the various laboratories (Eastmond et al., 1995). Until more thoroughly standardized and validated, FISH results, particularly interphase FISH results, should be considered as approximations of the actual levels of aneuploidy and aberrations within cells.

One recent study applied both interphase and metaphase FISH to examine cells of workers exposed to very low levels (0.1 ppm) of benzene (Table 10). No increase in hyperdiploidy of chromosomes 7, 11, 18, or X was detected either in interphase or metaphase cells (Carere et al., 1998b). Levels of polyploidy in exposed subjects were similar to those in controls when detected by either method (Carere et al., 1998b). However, it is difficult to draw conclusions about the relative sensitivities of interphase and metaphase FISH from this report because of the very small number of study subjects (six) and the fact that the benzene exposure was relatively low (0.1 ppm).

Thus, while preliminary research suggests that metaphase FISH is more sensitive and effective

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TABLE 9
Aneusomy 1, 7, and 8 in Interphase and Metaphase Cells of Workers Exposed to High Levels of Benzene

Benzene Exposure	Monosomy 1	Monosomy 7	Monosomy 8	Trisomy 1	Trisomy 7	Trisomy 8
Control (n = 44)						
Interphase	4.27±0.13[a]	15.95±1.07	14.97±1.16	0.18±0.02	0.76±0.07	0.86±0.09
Metaphase	3.66±0.30	2.72±0.19	5.76±0.48	0.27±0.04	0.87±0.09	0.80±0.09
≤ 31 ppm (n = 21)						
Interphase	4.54±0.20	15.58±1.29	13.59±1.21	0.11±0.02	0.98±0.10[†b]	0.83±0.09
Metaphase	3.05±0.53	3.79±0.63	6.26±0.59	0.36±0.07	1.38±0.20*	1.08±0.13
> 31 ppm (n = 22)						
Interphase	3.94±0.24	17.22±1.51	15.58±1.23	0.21±0.03	1.29±0.16**[b]	1.36±0.17**
Metaphase	4.00±0.47	5.90±0.85****	8.85±1.03***	0.68±0.12***	1.92±0.34****	1.86±0.22****

[a] Data presented as mean frequency (%) ± S.E. (Zhang, 1998a; Zhang, 1999; and Eastmond 1999).
[b] Statistical significance of the difference between controls and lower exposed workers, and between controls and higher exposed workers, estimated by analysis of variance on transformed outcome variable; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P \leq 0.0001$; † $P = 0.05 - 0.10$.

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TABLE 10
Hyperdiploidy of Chromosomes 7, 11, 18, and X in Interphase and Metaphase Cells of Workers Exposed to Very Low Levels of Benzene and Matched Controls[a]

Subjects (n)	Chromosome 7	Chromosome 11	Chromosome 18	Chromosome X	Polyploidy
Controls (6)					
Interphase	0.07 ± 0.03[b,c]	0.16 ± 0.05	0.43 ± 0.07	0.17 ± 0.09	0.02 ± 0.01
Metaphase	0.23 ± 0.16[d]	0.17 ± 0.08	0.03 ± 0.03	0.07 ± 0.07	0.07 ± 0.03
Exposed (6)					
Interphase	0.05 ± 0.03	0.15 ± 0.03	0.14 ± 0.05	0.09 ± 0.04	0.03 ± 0.02
Metaphase	0.03 ± 0.03	0.10 ± 0.04	0.13 ± 0.10	0.17 ± 0.06	0.07 ± 0.02

[a] Mean benzene exposure level of *0.1 ppm* (Carere, 1998b).
[b] Data presented as mean (%) ± S.D.
[c] Total of 1000 - 2000 interphase cells examined.
[d] Total of 500 metaphase spreads examined.

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than interphase FISH in the detection of both monosomy and trisomy in human lymphocytes (Zhang et al., 1999), the small number of studies and subjects examined make it premature to conclude whether interphase FISH can be effectively used to detect aneuploidy in lower-exposed workers. However, based on the data available, metaphase FISH appears more promising for the detection of aneuploidy caused by lower levels of benzene exposure. For this reason, in collaboration with NCI and Chinese investigators, we propose to use metaphase FISH in a future study of many more workers exposed to benzene in the 1 to 10 ppm range.

C. Benzene-Induced Chromosomal Aberrations Detected by Metaphase FISH

To date, we have examined 5 chromosomes by metaphase FISH in the previously discussed groups of 43 highly exposed Chinese workers and their matched controls. Numerical and structural changes in these 5 chromosomes among the study subjects are summarized in Figures 2 to 4. Frequencies of monosomy 5, 7, and 8, but not 1 or 21, increased with elevated exposure levels (Figure 2), whereas a significant trend was observed for trisomy of all five chromosomes (Figure 3). The most striking dose-dependent increases were found in monosomy 7 and trisomy 7, 8, and 21 (Figures 2 and 3).

Specific structural CA can be detected by targeting particular chromosomal regions or by painting entire chromosomes. The most common structural changes detected among chromosomes 1, 5, 7, 8, and 21 were t(8;21), t(8;?) (translocation between chromosome 8 and another unidentified chromosome), breakage of chromosome 8, and deletions of the long (q) arms of chromosomes 5 and 7 (Figure 4). A significant trend was observed for all these changes (Smith et al., 1998; Zhang et al., 1998a). The loss and long arm deletion of chromosomes 5 and 7, two of the most common cytogenetic changes in therapy- and chemical-related leukemia, were significantly increased in benzene-exposed workers over controls (Zhang et al., 1998a). Similar results for these changes on chromosomes 5 and 7 were obtained in cultured human lymphocytes treated with the benzene metabolites hydroquinone and 1,2,4-benzenetriol (Zhang et al., 1998b). In addition, the rate of t(21;?) (translocation between chromosome 21 and one or more unidentified chromosomes) was increased in highly exposed workers (Smith et al., 1998). There was a slight but not significant increase in centromere breakage of chromosome 1, with a modest trend and almost twofold increase observed (Zhang et al., 1998a). A somewhat similar result was obtained in interphase cells (Eastmond et al., 2001).

Table 11 summarizes the results from all studies, to date, of interphase and metaphase FISH applied mostly in the lymphocytes of individuals exposed to benzene in order to analyze aneuploidy and structural changes of specific chromosomes. How specific these changes really are remains to be established as not all chromosomes have been analyzed. Studies are currently underway aimed at examining all 22 autosomes in the blood cells of benzene-exposed workers and treated human cell cultures.

VII. COMPARISON OF BENZENE TO OTHER LEUKEMOGENS

In contrast to the apparent lack of a unique pattern among benzene-induced CA, there appear to be distinctive patterns of specific CA among leukemias secondary to treatment with chemotherapeutic drugs. Like benzene, several classes of leukemogens, including chemotherapeutic drugs and radiation, induce CA. In order to place the findings on benzene's effects in context with those of other leukemogens, we now briefly discuss CA in therapy-related leukemia cases and patients with previous chemical exposure.

Clonal chromosomal aberrations, which are associated with human cancer (Hagmar et al., 1998), are the hallmark of leukemia (Hagemeijer and Grosveld, 1996; Sandberg, 1990). Specific CA are found in up to 70 to 80% of patients with acute leukemias (Look, 1997). Changes in chromosomes 5, 7, 8, 11, 15, 16, 17, and 21 are most often observed in AML and MDS, with del(5q), monosomy 7, trisomy 8, t(21q22), t(11q23), t(15;17), and inv(16) (inversion of chromosome 16) being the most prevalent (Rowley, 1998). Up

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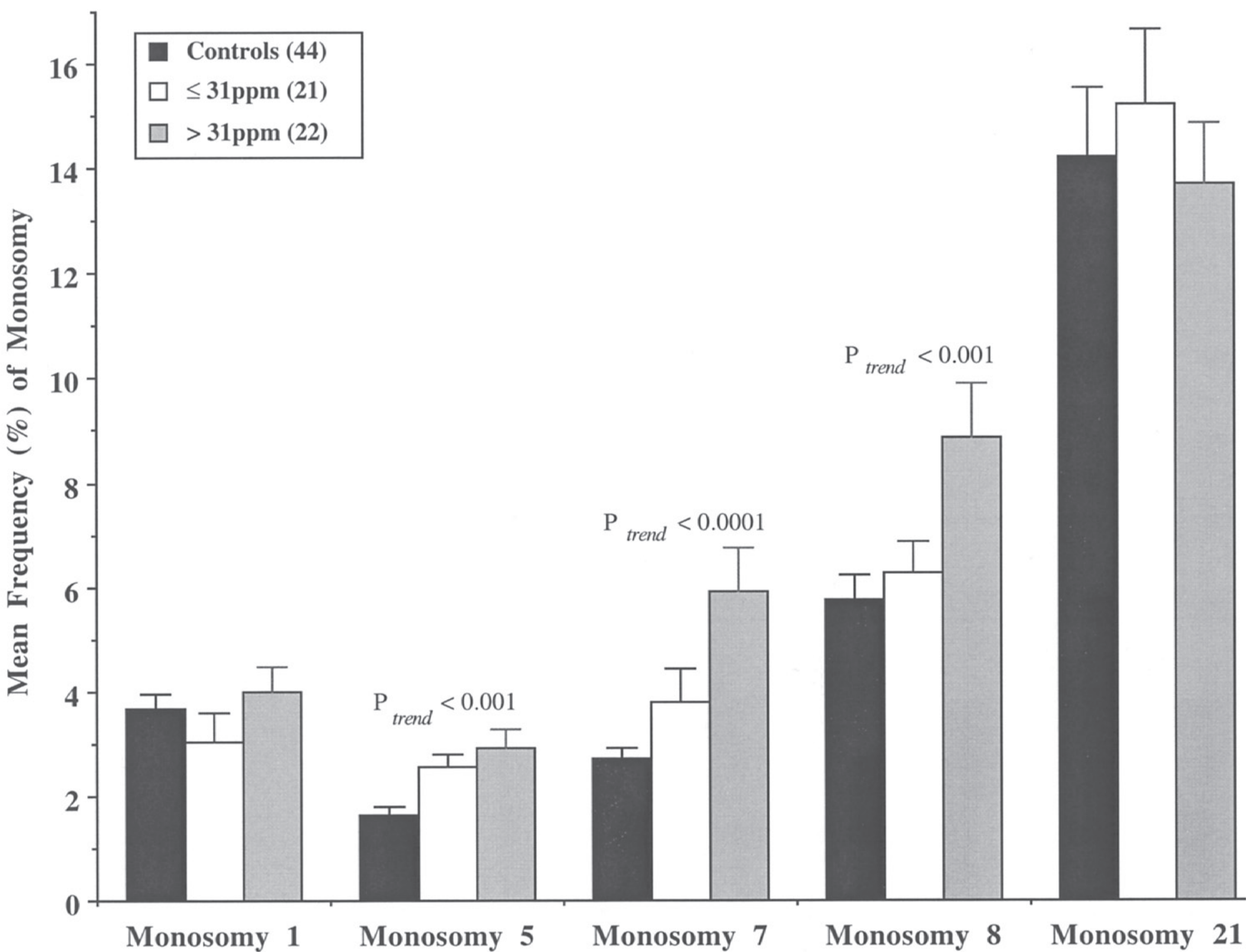

FIGURE 2. Monosomy of chromosomes 1, 5, 7, 8, and 21 in workers exposed to benzene and controls. Data presented are mean monosomy frequency (%). Error bar represents S.E. Black bar represents control, white bar represents ≤ 31 ppm group, and gray bar represents > 31 ppm group. Test for trend was performed by linear regression on square-root transformed data.

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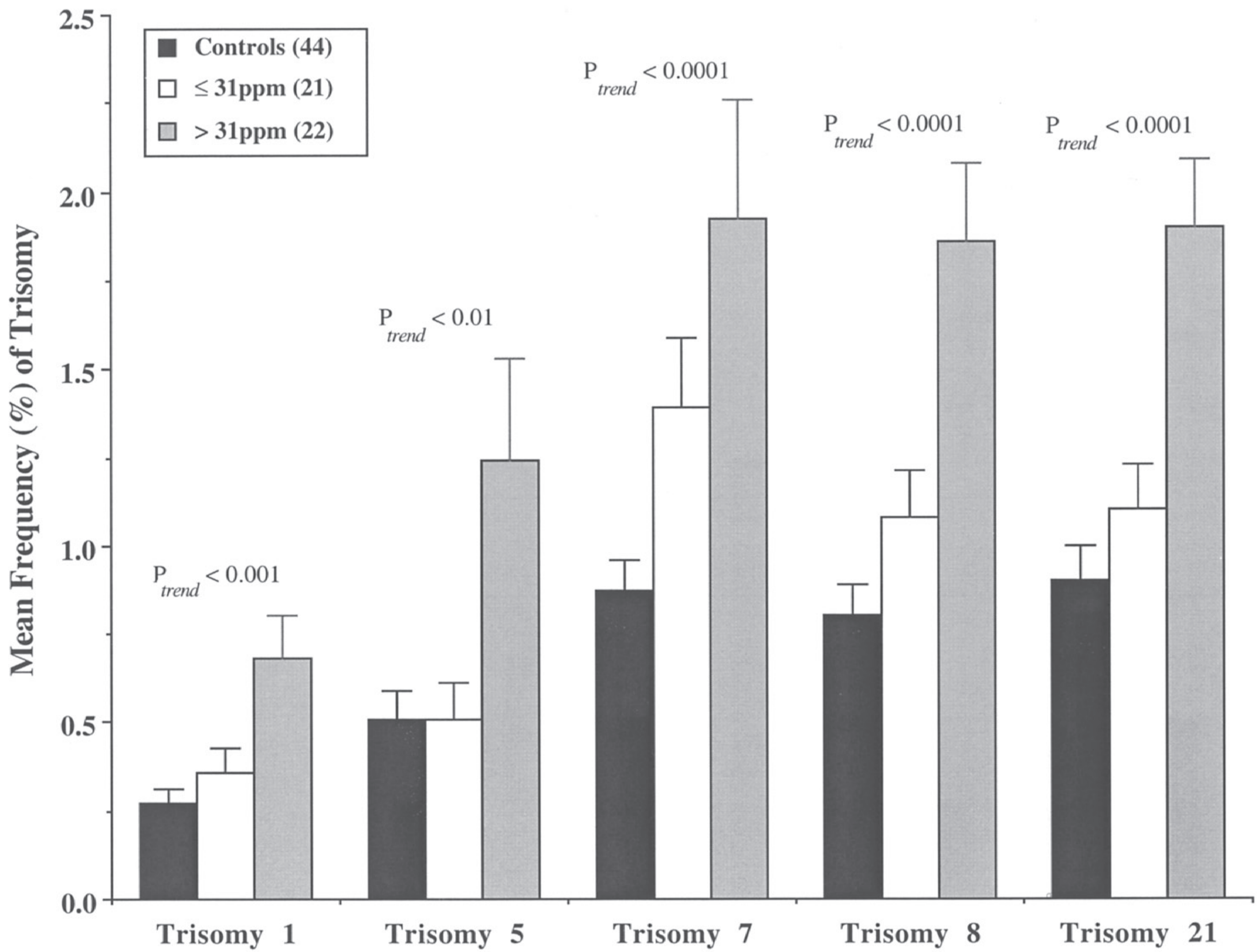

FIGURE 3. Trisomy of chromosomes 1, 5, 7, 8, and 21 in workers exposed to benzene and controls. Data presented are mean trisomy frequency (%). Error bar represents S.E. Black bar represents control, white bar represents ≤ 31 ppm group, and gray bar represents > 31 ppm group. Test for trend was performed by linear regression on square-root transformed data.

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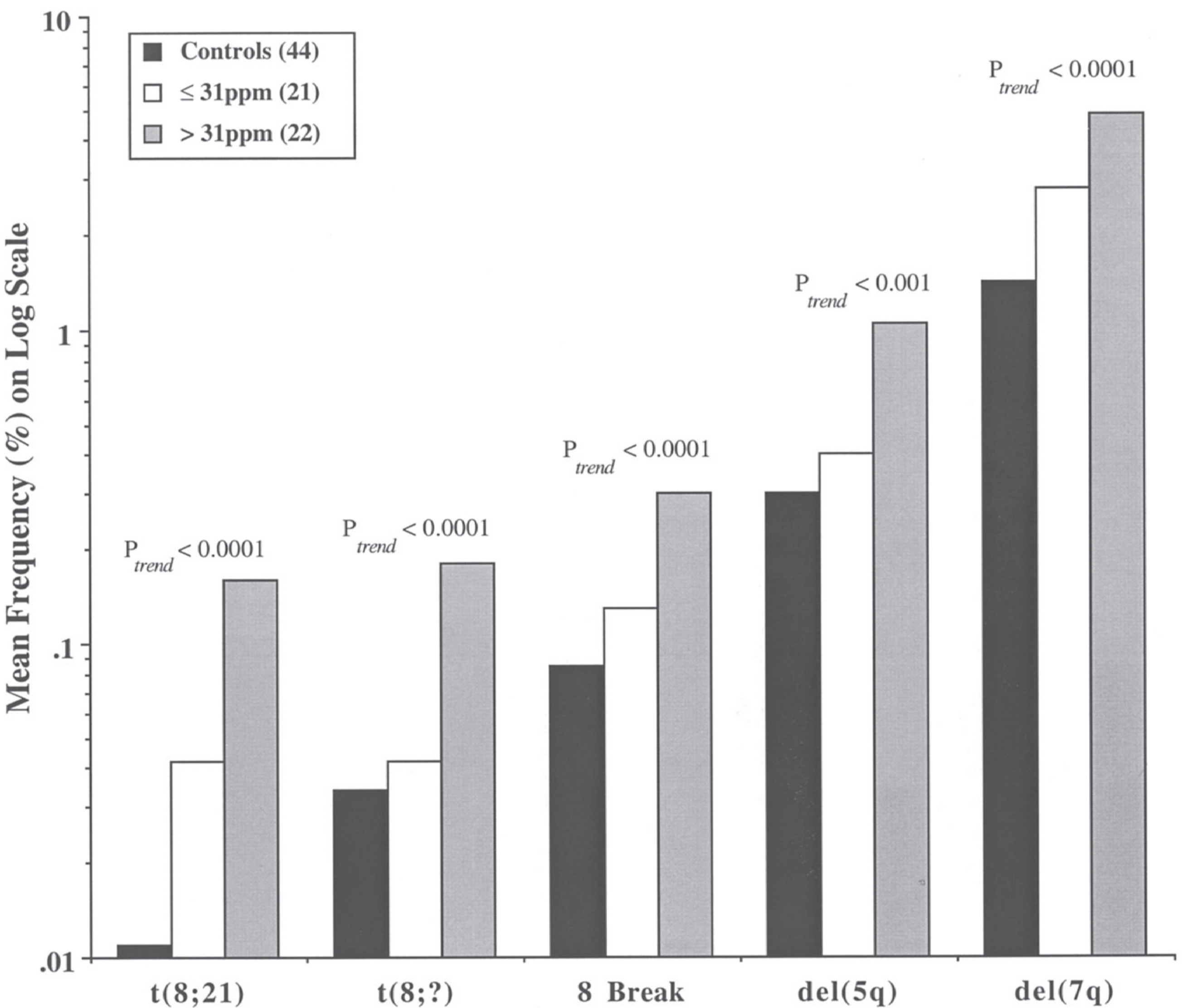

FIGURE 4. Structural changes of chromosomes 5, 7, 8, and 21 in workers exposed to benzene and controls. Data presented are mean structural change frequency (%). Error bar represents S.E. Black bar represents control, white bar represents ≤ 31 ppm group, and gray bar represents > 31 ppm group. Translocations between chromosomes 8 and 21 are represented as t(8;21); translocations between chromosome 8 and an unidentified chromosome are represented as t(8;?); breakage on chromosome 8 is represented as 8 Break; deletions of the long (q) arms of chromosomes 5 and 7 are represented as del(5q) and del(7q). Test for trend was performed by linear regression on square-root transformed data.

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TABLE 11
Benzene-Induced Chromosomal Aberrations Detected by FISH

Reference	Subject #		Benzene Exposure (Mean)		Cell type	Cell # per subject	FISH	Chromosome	Endpoints	
	Exposed	Controls	Level (ppm)	Duration (yr)					NCA	SCA
Carere, 1998b	12	12	0.1	24	Lymphocytes	1000	Inter-[a]	7, 11	x	
						2000	Inter-	18, X	x	
	6	6	0.1	24	Lymphocytes	500	Meta-[b]	7, 11	x	
							Meta-	18, X	x	
Surrales, 1997	18	13	1	N/A	Buccal cells	1000	Inter-	9	x	
Carere, 1998a	12	8	1.3	6.6	Lymphocytes	1000	Inter-	1, 9	x	↑ (NS)
					Lymphocytes[c]	300 - 500[d]	Inter-	1	x	x
					Granulocytes[c]	500	Inter-	1	x	x
Zhang, 1999	43	44	31[e]	6.3	Lymphocytes	1000	Inter-	7, 8	↑ (Hyper-)	
Zhang, 1996b	43	44	31[e]	6.3	Lymphocytes	1000	Inter-	9	↑ (Hyper-)	
Eastmond, 1999	43	44	31[e]	6.3	Lymphocytes	1000	Inter-	1	↑ (NS)	x
Zhang, 1998a	43	44	31[e]	6.3	Lymphocytes	~ 350	Meta-	1	↑ (Trisomy)	↑ (NS)
								5, 7	↑	↑
Smith, 1998	43	44	31[e]	6.3	Lymphocytes	~ 480	Meta-	8, 21	↑	↑

↑ = increase; x = no association; Hyper- = hyperdiploidy; NS = not significant; N/A = not available.
[a] Interphase FISH.
[b] Metaphase FISH.
[c] Cells examined on peripheral blood smear slides.
[d] Exposed: ~500 lymphocytes, Controls: ~300 lymphocytes.
[e] Median exposure level.

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to 15% of all AML and MDS in the U.S. are caused by cancer therapy with radiation and chemotherapeutic drugs (Pedersen-Bjergaard and Philip, 1991). These therapy-related malignancies commonly show specific cytogenetic changes that are related to the nature of the therapy given.

Alkylating agents, such as melphalan and cyclophosphamide, tend to cause leukemias and MDS with primary changes in chromosomes 5 and 7 (Pedersen-Bjergaard and Rowley, 1994), whereas topoisomerase inhibitors, such as etoposide and doxorubicin, are associated with leukemias containing translocations at 11q23, which disrupt the *MLL* gene (Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1996). Etoposide tends to cause leukemias with balanced translocations at 11q23, such as t(4;11), t(6;11), and t(11;19) (Pedersen-Bjergaard and Rowley, 1994). Topoisomerase II inhibitors like etoposide cause inhibition of the enzyme and the formation of a cleavable complex. Other topoisomerase II inhibitors, such as bimolane, inhibit the enzyme but do not form a cleavable complex. These latter inhibitors have been associated in a series of leukemias with the production of translocations at 21q22, such as t(8;21) and t(3;21) (Smith et al., 1996). Translocation (8;21) and another common cytogenetic change in AML, inv(16), are also commonly found in so-called *de novo* leukemias in which no clear etiology is known. Other clonal chromosome aberrations associated with chemotherapy include [–7,+der(7)t(1;7)(p11;p11)] and chromosome abnormalities of 3q and 12p (Rooney and Czepulkowski, 1992).

Several studies have found an association between CA and chemical exposure among leukemia patients (Crane et al., 1989; Cuneo et al., 1992; Golomb et al., 1982; Groupe, 1984), although several negative studies have been reported (Ciccone et al., 1993; Vineis et al., 1990). Early reports suggested that occupational exposure to chemicals was associated, like exposure to alkylating agents, with changes in chromosomes 5 and 7 (Golomb et al., 1982; Groupe, 1984; Mitelman et al., 1978). It subsequently became generally accepted that leukemias associated with occupational chemicals would most likely possess cytogenetic changes in chromosomes 5 and 7. However, a more recent detailed study failed to find such a firm association between cytogenetic changes in chromosomes 5 and 7 and occupational exposure to organic chemicals, although these changes may be associated with exposure to paints (Crane et al., 1996). Additional studies in leukemias associated with exposure to organic chemicals have detected many of the other major changes found in AML (e.g., t(8;21), t(11q23), and trisomy 8) in addition to changes in chromosomes 5 and 7 (Albin et al., 2000; Crane et al., 1989; Cuneo et al., 1992; Li et al., 1989). Thus, recent research does not appear to support an exclusive association between cytogenetic changes in chromosomes 5 and 7 and occupational exposure to organic chemicals.

The review of the literature on all published cases of leukemia associated with benzene exposure (Table 1) and cases with likely prior exposure to benzene (Table 2) presented here reveals no unique pattern of clonal chromosomal changes related to benzene exposure. For instance, various chromosome translocations were detected in 8 cases of leukemia associated with benzene exposure (35%), while aneuploidy mostly in C- and E-group chromosomes was found at the same rate (Table 1). This result may partly derive from limitations of outdated technologies, as many of the cases were studied before G-banding was available. However, this limitation would only affect the identification of specific chromosome changes. While no unique pattern of specific cytogenetic changes is discernible, several similarities between the types of chromosome changes detected by either the classic technique or G-banding in leukemias associated with benzene exposure are apparent. For example, the cytogenetic changes observed by G-banding (shown in Table 2) among benzene-related leukemia cases include many instances of translocations (37%), deletions (24%), and aneuploidy (49%), again mostly in the C-group chromosomes (32%) (Table 12). In addition, the loss and long-arm deletion of chromosomes 5 and 7 have been detected frequently in many leukemia patients with likely prior exposure to benzene (29%, Table 12). This is much lower than the 69% incidences of clonal chromosome 5 and 7 abnormalities reported in leukemia patients previously treated with alkylating agent-based chemotherapy (Larson et al., 1996; Pedersen-Bjergaard et al., 1995). Thus, the literature to date does not support the hypothesis that benzene-induced leukemias and preleukemic states are associated exclusively with these changes in chromosomes 5 and 7 (Tables 1 to 3, 12).

TABLE 12
Prevelance of Specific Chromosomal Aberrations in Leukemia Patients with Likely Prior Exposure to Benzene[a]

Chromosomal Aberration	Case Number	Rate (%) (n=41, abnormal only)[b]	Rate (%) (n=59, All)[c]
Translocations	**22**	**54 (22/41)**	**37 (22/59)**
t(21q22)	7	17	12
t(8;21)	5	12	8
t(9;22)	4	10	7
t(15;17)	2	5	3
t(12q)	4	10	7
t(17p)	2	5	3
Deletions	**14**	**34**	**24**
del(11q)	2	5	3
del(12p)	2	5	3
del(17p)	2	5	3
-5/5q- & -7/7q-	**17**	**41**	**29**
-5/5q-	9	22	15
-7/7q-	12	29	20
5q-/7q-	**11**	**27**	**19**
5q-/t(5q)	8	20	14
5q-	5	12	8
t(5q)	3	7	5
7q-	3	7	5
-5/-7	**11**	**27**	**19**
-5	2	5	3
-7	9	22	15
Aneuploidy	**29**	**71**	**49**
±C	19	46	32
+C	6	15	10
-C	14	34	24
±8	7 (2/5)	17	12
±21	7 (3/4)	17	12
-17/-18	6	15	10
±Y	6	15	10

[a] The most frequent abnormal karyotypes shown in Table 2 are summarized into each chromosomal aberration category.
[b] The frequency of specific chromosome changes in the total of 41 patients with abnormal karyotypes only.
[c] The frequency of specific chromosome changes in the total of 59 patients with normal and abnormal karyotypes.

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In the analysis presented in Table 12, translocations were commonly found in leukemia patients with likely prior exposure to benzene and translocations involving 21q22 were the most prevalent (12%). On the other hand, translocations at 11q23 were found in only one ALL case associated with benzene exposure but not in any AML cases (Table 1). Further, no t(11q23) were detected in leukemia patients with likely prior exposure to benzene (Table 2). This is of interest because, as mentioned above, t(11q23) is associated with leukemia caused by topoisomerase II inhibitors that form cleavable complexes, such as etoposide and adriamycin (Pui et al., 1991). Benzene metabolites are inhibitors of topoisomerase II, but do not form cleavable complexes (Chen and Eastmond, 1995; Frantz et al., 1996). In this respect they are similar to bimolane, which induces leukemias with t(21q22). This may explain why benzene also appears to induce leukemias with t(21q22) but not t(11q23). Further support for this argument comes from the fact that we have observed t(21q22) in workers exposed to benzene (Smith et al., 1998), but not t(11q23) (Zhang et al., 2000).

Therefore, benzene appears to produce a variety of chromosome changes that may lead or contribute to leukemogenesis. A possible explanation for this phenomenon may be that benzene produces genomic instability by causing recombination, double strand breaks and mitotic spindle disruption, which leads to formation of translocations, inversions, deletions, and chromosome loss and gain (Smith, 1996). As a result, one would not necessarily expect to see a specific pattern of cytogenetics associated with benzene exposure. The only means by which this question can be definitively answered is through much closer examination, using modern cytogenetic and molecular techniques, of the specific changes found in benzene-associated leukemia. A systematic effort to collect and analyze biological material from leukemias and preleukemias is necessary in order to establish their true association with benzene exposure. Although leukemias associated with benzene exposure do not appear to have a specific cytogenetic karyotype, it is clear that benzene is capable of producing both numerical and structural CA, and that leukemias associated with benzene exposure are more likely to have clonal chromosomal changes.

VIII. CONCLUSION AND FUTURE DIRECTIONS

Benzene-induced CA have been detected in many human studies. Structural chromosome changes were mostly detected as gaps and breaks. Both stable and unstable chromosome changes, particularly unstable changes, have been detected in leukemia and poisoned patients and in exposed subjects. Aneuploidy, including hyperdiploidy and hypodiploidy, has also been observed frequently. The loss and gain of chromosomes in the C-group were especially common among benzene-associated leukemia and preleukemia patients.

Results from patients whose leukemia probably resulted from prior benzene exposure appeared largely consistent with those from the benzene-associated leukemia case reports. Like aneuploidy, nonspecific structural changes are also characteristic of benzene poisoning and preleukemia cases. A recent study by Forni (Forni, 1996) among benzene hemopathy cases suggests that increased chromosome-type aberrations persist years after recovery and may be a good indicator of elevated cancer risk. The large majority of the numerous studies in exposed but otherwise healthy subjects have shown a positive association between benzene exposure and increased numerical and structural CA.

The recent development of molecular cytogenetics, namely, FISH, has facilitated analysis of specific CA among benzene-exposed populations. Increased levels of aneuploidy and structural changes associated with leukemogenesis have been detected by FISH in the majority of studies performed to date among highly exposed workers. Ongoing and future FISH studies promise a complete analysis of every chromosome. At this point, however, there does not appear to be a unique pattern of benzene-induced CA as detected by either FISH or classic cytogenetics.

In conclusion, findings in the literature to date indicate that benzene may act like both alkylating agent causing alterations in chromosomes 5 and 7, and a topoisomerase II inhibitor inducing t(21q22). To test this hypothesis, future studies especially among greater numbers of workers exposed to low levels of benzene are needed and are currently being planned in collaboration with NCI and Chinese investigators. The fact that increased CA are generally observed among benzene-exposed but otherwise healthy workers,

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preleukemia patients, and leukemia patients suggests that benzene-induced CA are a potential pathway of benzene-associated leukemogenesis.

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REFERENCES

ACGIH (1999) Guide to Occupational Exposure Values, American Conference of Governmental Industrial Hygienists, Inc. (ACGIH), Cincinnati, OH.

Aksoy, M. (1988) Benzene carcinogenicity, in M. Aksoy (Ed.), *Benzene Carcinogenicity*, CRC Press, Inc., Boca Raton, Florida, pp. 113–51.

Aksoy, M., K. Dincol, S. Erdem, and G. Dincol (1972) Acute leukemia due to chronic exposure to benzene, *Am J Med,* **52**, 160–6.

Aksoy, M., S. Erdem, G. Erdogan, and G. Dincol (1974) Acute leukaemia in two generations following chronic exposure to benzene, *Hum Hered*, **24**, 70–4.

Albin, M., J. Björk, H. Welinder, H. Tinnerberg, N. Mauritzson, B. Johansson, R. Billström, U. Strömberg, Z. Mikoczy, T. Ahlgren, P.G. Nilsson, F. Mitelman, and L. Hagmar (2000) Acute myeloid leukemia and clonal chromosome aberrations in relation to past exposure to organic solvents, *Scand. J. Work Environ. Health,* **26**, 482–91.

Antonucci, R., R. Walker, and J. Herion (1989) Myelofibrosis and aplastic anemia: first report of the two disorders occurring sequentially in the same person, *Am J Med*, **86**, 352–5.

Barbera, N., G. Bulla, and G. Romano (1998) A fatal case of benzene poisoning, *J. Forensic Sci*., **43**, 1250–1.

Barch, M.J. (1991) *The ACT Cytogenetics Laboratory Manual,* Raven Press, New York.

Betancourt, M., G. Olaiz, R. Ortiz, G. Castro, C. Gonzalez, and P. Cravioto (1995) Evaluation of chromosomal aberrations in lymphocytes of individuals accidentally exposed to gasoline fumes, *Med Sci Res*, **23**, 367–8.

Bogadi-Sare, A., V. Brumen, R. Turk, V. Karacic, and M. Zavalic (1997) Genotoxic effects in workers exposed to benzene: with special reference to exposure biomarkers and confounding factors, *Ind Health*, **35**, 367–73.

Bonassi, S., A. Abbondandolo, L. Camurri, L. Dal Pra, M. De Ferrari, F. Degrassi, A. Forni, L. Lamberti, C. Lando, P. Padovani, I. Sbrana, D. Vecchio, and R. Puntoni (1995) Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study, *Cancer Genet Cytogenet,* **79**, 133–5.

Bukvic, N., P. Bavaro, G. Elia, F. Cassano, M. Fanelli, and G. Guanti (1998) Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of gasoline station attendants, *Mutat. Res.*, **415**, 25–33.

Carere, A., A. Antoccia, D. Cimini, R. Crebelli, F. Degrassi, P. Leopardi, F. Marcon, A. Sgura, C. Tanzarella, and A. Zijno (1998b) Genetic effects of petroleum fuels. II. Analysis of chromosome loss and hyperploidy in peripheral lymphocytes of gasoline station attendants, *Environ Mol Mutagen*, **32**, 130–8.

Carere, A., A. Antoccia, R. Crebelli, F. Degrassi, M. Fiore, I. Iavarone, G. Isacchi, S. Lagorio, P. Leopardi, F. Marcon, F. Palitti, C. Tanzarella, and A. Zijno (1995) Genetic effects of petroleum fuels: cytogenetic monitoring of gasoline station attendants, *Mutat Res,* **332**, 17–26.

Carere, A., R. Crebelli, A. Zijno, P. Leopardi, F. Marcon, S. Lagorio, I. Iavarone, A. Antoccia, C. Tanzarella, D. Eastmond, R. Parks, M. Schuler, K. Peltonen, M. Sorsa, and T. Veidebaum (1998a) Cytogenetic monitoring of oil shale petrochemistry workers, in: A. Carere, Crebelli, R. (Eds.), Biomarkers for environmental and occupational exposure to aromatic mutagens and carcinogens from emissions of oil shale petrochemistry, Report of the EC PECO Program, pp. 30–42.

Caspersson, T., L. Zech, and C. Johansson (1970) Differential binding of alkylating fluorochromes in human chromosomes, *Exp Cell Res*, **60**, 315–9.

Chen, H. and D.A. Eastmond (1995) Topoisomerase inhibition by phenolic metabolites: a potential mechanism for benzene's clastogenic effects, *Carcinogenesis*, **16**, 2301–7.

Ciccone, G., D. Mirabelli, A. Levis, P. Gavarotti, C.G. Rege, L. Davico, and P. Vineis (1993) Myeloid leukemias and myelodysplastic syndromes: chemical exposure, histologic subtype and cytogenetics in a case-control study, *Cancer Genet Cytogenet*, **68**, 135–9.

Clare, M.G., A. Yardley-Jones, A.C. Maclean, and B.J. Dean (1984) Chromosome analysis from peripheral blood lymphocytes of workers after an acute exposure to benzene, *Br J Ind Med,* **41**, 249–53.

Cohen, M.M., L.S. Rosenblum-Vos, and G. Prabhakar (1993) Human cytogenetics. A current overview, *Am J Dis Child,* **147**, 1159–66.

Colovic, M., J. Gradimir, N. Suvajdzic, and N. Kraguljac (1996) Cytologically distinct acute leukemia in sibs exposed to the same leukemogen, *Haematologia*, **27**, 209–10.

Crane, M., S. Strom, S. Halabi, E. Berman, J. Fueger, M. Spitz, and M. Keating (1996) Correlation between selected environmental exposures and karyotype in acute myelocytic leukemia, *Cancer Epidemiology, Biomarkers and Prevention*, **5**, 639–44.

Crane, M.M., M.J. Keating, J.M. Trujillo, D.R. Labarthe, and R.F. Frankowski (1989) Environmental exposures in cytogenetically defined subsets of acute nonlymphocytic leukemia [published erratum appears in JAMA 1990 Feb 2;263(5):662], *JAMA*, **262**, 634–9.

Cronkite, E.P. (1961) Evidence for radiation and chemicals as leukemogenic agents, *Arch Environ Health*, **3**, 297–303.

Cronkite, E.P. (1987) Chemical leukemogenesis: benzene as a model, *Semin Hematol*, **24**, 2–11.

Cuneo, A., F. Fagioli, I. Pazzi, A. Tallarico, R. Previati, N. Piva, M.G. Carli, M. Balboni and G. Castoldi (1992) Morphologic, immunologic and cytogenetic studies in acute myeloid leukemia following occupational exposure to pesticides and organic solvents, *Leuk Res,* **16**, 789–96.

Dabney, B.J. (1981) The role of human genetic monitoring in the workplace, *J. Occupat. Med.,* **23**, 626–31.

Damrau, J., U. Renker, N. Werner, W. Wohlrab, and K.D. Wozniak (1986) (Immunologic and clastogenic changes in benzene-exposed workers), *Zeitschrift fur die Gesamte Hygiene und Ihre Grenzgebiete*, **32**, 693–5.

de Jong, G., N.J. van Sittert, and A.T. Natarajan (1988) Cytogenetic monitoring of industrial populations potentially exposed to genotoxic chemicals and of control populations, *Mutat Res*, **204**, 451–64.

Delore, P. and C. Borgomano (1928) Leucemie aigue au cours de l'intoxication benzenique: sur l'origine toxique de certaines leucemies aigues et leurs relations avec les anemies graves, *J Med Lyon*, **9**, 227–33.

Di Guglielmo, G. and A. Iannaccone (1958) Inhibition of mitosis and regressive changes of erythroblasts in acute erythropathy caused by occupational benzene poisoning, *Acta Haemat*, **19**, 144–47.

Ding, X.J., Y. Li, Y. Ding, and H.Z. Yang (1983) Chromosome changes in patients with chronic benzene poisoning, *Chin Med J (Engl)*, **96**, 681–5.

Dulout, F.N., C.A. Grillo, A.I. Seoane, C.R. Maderna, R. Nilsson, M. Vahter, F. Darroudi and A.T. Natarajan (1996) Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from northwestern Argentina exposed to arsenic in drinking water, *Mutat Res*, **370**, 151–8.

Eastmond, D.A. and D. Pinkel (1990) Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence *in situ* hydridization with chromosome-specific DNA probes, *Mutat Res,* **234**, 303–318.

Eastmond, D.A., M. Schuler, C. Frantz, H.W. Chen, R. Parks, L. Wang, and L. Hasegawa (2001) Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans, *Health Effects Institute*, Research Report 103.

Eastmond, D.A., M. Schuler, and D.S. Rupa (1995) Advantages and limitations of using fluorescence *in situ* hybridization for the detection of aneuploidy in interphase human cells, *Mutat Res*, **348**, 153–62.

Erdogan, G. and M. Aksoy (1973) Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene, *New Istanbul Contrib Clin Sci*, **10**, 230–47.

Erdogan, G. and M. Aksoy (1975) Cytogenetic studies in 20 patients with pancytopenia and leukaemia with long-term exposure to benzene, 3rd Meeting, European and African Division of the International Society of Hematology (As cited in Aksoy, M (1988). *Benzene Carcinogenicity*. CRC Press, Inc., Boca Raton, Florida), London, pp. 134.

Ford, C.E. and J.L. Hamerton (1956) The chromosomes of man, *Nature*, **178**, 1020–1023.

Forni, A. (1966) Chromosome changes due to chronic exposure to benzene, International Congress on Occupational Health, Vienna, pp. 437–439.

Forni, A. (1971b) Chromosome studies in workers exposed to benzene or toluene or both, *Arch Environ Health*, **22**, 373–8.

Forni, A. (1978) (Significance and limitations of the study of chromosome aberrations in occupational medicine), *Med Lav*, **69**, 331–40.

Forni, A. (1979) Chromosome changes and benzene exposure: a review, *Rev Environ Health*, **3**, 5–17.

Forni, A. (1984) Chromosomal aberrations in monitoring exposure to mutagens-carcinogens, in: A. Berlin, Draper, M., Hemminki, K., Vainio, H. (Eds.), *Monitoring Human Exposure to Carcinogenic and Mutagenic Agents*, International Agency for Research on Cancer, Lyon, pp. 325–337.

Forni, A. (1996) Benzene-induced chromosome aberrations: a follow-up study, *Environ Health Perspect,* 104 Suppl **6**, 1309–12.

Forni, A. and L. Moreo (1967) Cytogenetic studies in a case of benzene leukaemia, *Eur J Cancer*, **3**, 251–5.

Forni, A. and L. Moreo (1969) Chromosome studies in a case of benzene-induced erythroleukaemia, *Eur J Cancer,* **5**, 459–63.

Forni, A.M., A. Cappellini, E. Pacifico, and E.C. Vigliani (1971a) Chromosome changes and their evolution in subjects with past exposure to benzene, *Arch Environ Health*, **23**, 385–91.

Frantz, C.E., H. Chen and D.A. Eastmond (1996) Inhibition of human topoisomerase II in vitro by bioactive benzene metabolites, *Environ. Health Perspect.,* **104** Suppl 6, 1319–23.

Fredga, K., J. Reitalu and M. Berlin (1979) Chromosome studies in workers exposed to benzene, in: K. Berg (Ed.), *Genetic Damage in Man Caused by Environmental Agents,* Academic Press, New York, pp. 187–203.

Funes-Cravioto, F., C. Zapata-Gayon, B. Kolmodin-Hedman, B. Lambert, J. Lindsten, E. Norberg, M. Nordenskjold,

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R. Olin, and A. Swensson (1977) Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers, *Lancet*, **2**, 322–5.

Gao, G., H. Xu and S. Fu (1994) Cytogenetic study on peripheral blood lymphocytes of workers exposed to benzene, *China Environ Sci*, **14**, 318–20.

Girard, R., M.L. Mallein, J. Bertholon, P. Coeur, and J.C. Evreux (1970) (Study of leukocyte alkaline phosphatase and of the karyotype of workers exposed to benzene), *Arch Mal Prof*, **31**, 31–7.

Gist, G.L. and J.R. Burg (1997) Benzene — a review of the literature from a health effects perspective, *Toxicol Ind Health,* **13**, 661–714.

Goldstein, B.D. (1977) Benzene toxicity: a critical evaluation: hematotoxicity in humans, *J Toxicol Environ Health Suppl*, **2**, 69–105.

Golomb, H.M., G. Alimena, J.D. Rowley, J.W. Vardiman, J.R. Testa, and C. Sovik (1982) Correlation of occupation and karyotype in adults with acute nonlymphocytic leukemia, *Blood*, **60**, 404–11.

Gray, J.W. and D. Pinkel (1992) Molecular cytogenetics in human cancer diagnosis, *Cancer*, **69**, 1536–42.

Gray, J.W., D. Pinkel and J.M. Brown (1994) Fluorescence *in situ* hybridization in cancer and radiation biology, *Radiat Res,* **137**, 275–89.

Groupe (1984) Chromosome analysis of 63 cases of secondary nonlymphoid blood disorders: a cooperative study. Groupe Francais de Cytogénétique Hématologique, *Cancer Genetics and Cytogenetics*, **12**, 95–104.

Gundy, S. (1989) Cytogenetical studies on a large control population and on persons occupationally exposed to radiation and/or to chemicals, *Annali dell Istituto Superiore di Sanita,* **25**, 549–55.

Haberlandt, W. and B. Mente (1971) (Aberrations of chromosome number and structure in industrial workers exposed to benzene), *Zentralbl Arbeitsmed*, **21**, 338–41.

Hagemeijer, A. and G. Grosveld (1996) Molecular cytogenetics of leukemia, in: E. Henderson, T. Lister and M. Greaves (Eds.), *Leukemia*, Saunders, Philadelphia, pp. 131–144.

Hagmar, L., S. Bonassi, U. Strömberg, A. Brøgger, L.E. Knudsen, H. Norppa, and C. Reuterwall (1998) Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH), *Cancer Res,* **58**, 4117–21.

Hagmar, L., A. Brogger, I.L. Hansteen, S. Heim, B. Hogstedt, L. Knudsen, B. Lambert, K. Linnainmaa, F. Mitelman, I. Nordenson, C. Reuterwall, S. Salomaa, S. Skerfving, and M. Sorsa (1994) Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage, *Cancer Res,* **54**, 2919–22.

Hartwich, G. and G. Schwanitz (1972) (Chromosome studies after chronic benzol exposure), *Dtsch Med Wochenschr*, **97**, 45–9.

Hartwich, G., G. Schwanitz, and J. Becker (1969) Chromosome anomalies in a case of benzene leukaemia, *Ger Med Mon,* **14**, 449–50.

Högstedt, B., A. Holmén, A. Karlsson, G. Raihle, K. Nillius, and K. Vestlund (1991) Gasoline pump mechanics had increased frequencies and sizes of micronuclei in lymphocytes stimulated by pokeweed mitogen, *Mutat Res,* **263**, 51–5.

Holland, N.T., N. Rothman, G. Li, L. Zhang, R.B. Hayes, S. Yin, and M.T. Smith (1999) The micronucleus assay does not effectively detect genotoxicity of occupational exposure to benzene, *Environmen Mol Mutagen*, **33** Suppl.30, #91.

Iurlo, A., C. Mecucci, A. Van Orshoven, J.L. Michaux, M. Boogaerts, L. Noens, A. Bosly, A. Louwagie, and H. Van Den Berghe (1989) Cytogenetic and clinical investigations in 76 cases with therapy-related leukemia and myelodysplastic syndrome, *Cancer Genet Cytogenet*, **43**, 227–41.

Jablonická, A., M. Vargova, and J. Karelova (1987) Cytogenetic analysis of peripheral blood lymphocytes in workers exposed to benzene, *J Hyg Epidemiol Microbiol Immunol,* **31**, 127–32.

Karacic, V., L. Skender, B. Bosner-Cucancic, and A. Bogadi-Sare (1995) Possible genotoxicity in low level benzene exposure, *Am J Ind Med,* **27**, 379–88.

Khan, H. and M.H. Khan (1973) (Cytogenetic studies following chronic exposure to benzene), *Archiv fur Toxikologie,* **31**, 39–49.

Knott, D. (1994) U.K. unleaded gasoline draws fire in Parliament, *Oil Gas J*, **92**, 40–41.

Lalchev, S., V. Georgieva, A. Vaglenov, E. Mirkova, and M. Nosko (1997) Cytogenetic investigation of people occupationally exposed to organic solvents, *Cytogenet Cell Genet*, **77**, 71.

Larson, R.A., M.M. LeBeau, J.W. Vardiman, and J.D. Rowley (1996) Myeloid leukemia after hematotoxins, *Environmental Health Perspectives,* **104** Suppl 6, 1303–7.

Le Noire, M.M. and H. Claude (1897) Sur un case de purpura attribue a l'intoxication par le benzene, *Bull Mem Soc Med Hop Paris,* **3**, 1251.

Li, Y. and Y. Ding (1990) Chromosome changes by G-banding in patients with chronic benzene poisoning, *Chin J Ind Med*, **3**, 29–31.

Li, Y.S., Y.L. Zhao, Q.P. Jiang, and C.L. Yang (1989) Specific chromosome changes and nonoccupational exposure to potentially carcinogenic agents in acute leukemia in China, *Leuk Res,* **13**, 367–76.

Liaudet, J. and M. Combaz (1973) (Chronic myeloid leukemia in a 35 year old petroleum chemist, handling benzene since the age of 18 years), *Eur J Toxicol Hyg Environ,* **6**, 309–13.

Lindquist, R., A.M. Forsblom, A. Ost, and G. Gahrton (2000) Mutagen exposures and chromosome 3 aberrations in acute myelocytic leukemia, *Leukemia*, **14**, 112–8.

Liniecki, J., A. Bajerska and M. Glusczowa (1971) Analiza kariologiczna limfocytow krwi obwodowey u osob z przebytym przewleklym zatruciem benzenem, *Med Pr,* **22**, 187.

Liou, S.H., J.C. Lung, Y.H. Chen, T. Yang, L.L. Hsieh, C.J. Chen, and T.N. Wu (1999) Increased chromosome-type chromosome aberration frequencies as biomarkers of cancer risk in a blackfoot endemic area, *Cancer Research,* **59**, 1481–4.

Liu, L., Q. Zhang, J. Feng, L. Deng, N. Zeng, A. Yang, and W. Zhang (1996) The study of DNA oxidative damage in benzene-exposed workers, *Mutat Res*, **370**, 145–50.

Look, A.T. (1997) Oncogenic transcription factors in the human acute leukemias, *Science*, **278**, 1059–64.

Lumley, M., H. Barker, and J.A. Murray (1990) Benzene in petrol [letter; comment], *Lancet*, **336**, 1318–9.

Mahendra, P., E.M. Richards, P. Sinclair, E. Nacheva, and R.E. Marcus (1996) t(9;13)(q34;q12) chromosomal translocation persisting 4 years post autologous bone marrow transplantation for secondary AML despite morphological remission, *Clin Lab Haematol*, **18**, 121–2.

Major, J., M. Jakab, G. Kiss, and A. Tompa (1994) Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene, *Environ Mol Mutagen*, **23**, 137–42.

Marcon, F., A. Zijno, R. Crebelli, A. Carere, T. Veidebaum, K. Peltonen, M. Schuler, R. Parks, and D.A. Eastmond (1999) Chromosome damage and aneuploidy detected by interphase multicolor FISH in benzene exposed shale oil workers, *Mutation Research,* **445**: 155–66.

Maugeri, S., R. Colombi, G. Pollini, E. Strosselli and R. Corsico (1965) (Features and development of benzol hemopathy), *Med Lav*, **56**, 544–60.

Mitelman, F., L. Brandt, and P.G. Nilsson (1978) Relation among occupational exposure to potential mutagenic/carcinogenic agents, clinical findings, and bone marrow chromosomes in acute nonlymphocytic leukemia, *Blood*, **52**, 1229–37.

Mitelman, F., P.G. Nilsson, L. Brandt, G. Alimena, R. Gastaldi, and B. Dallapiccola (1981) Chromosome pattern, occupation, and clinical features in patients with acute nonlymphocytic leukemia, *Cancer Genet Cytogenet*, **4**, 197–214.

Morrison, R.T. and R.N. Boyd (1966) *Benzene, Organic Chemistry,* Allyn and Bacon, Inc., Boston.

Muzyka, V., S. Veimer, and N. Shmidt (1998) Particle-bound benzene from diesel engine exhaust, *Scand. J. Work. Environ. Health.* **24**, 481–5.

Nise, G., B. Hogstedt, I. Bratt, and S. Skerfving (1991) Cytogenetic effects in rotogravure printers exposed to toluene (and benzene), *Mutat Res*, **261**, 217–23.

Nowell, P.C. and D.A. Hungerford (1960) Chromosome studies on normal and leukemic human leukocytes, *J Nat Cancer Inst,* **25**, 85–109.

OSHA (1987) Final rule on occupational exposure to benzene, *Federal Register*, **54**, 34660–34762.

Pedersen-Bjergaard, J., M. Pedersen, D. Roulston, and P. Philip (1995) Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia, *Blood*, **86**, 3542–52.

Pedersen-Bjergaard, J. and P. Philip (1991) Two different classes of therapy-related and de-novo acute myeloid leukemia?, *Cancer Genet Cytogenet*, **55**, 119–24.

Pedersen-Bjergaard, J. and J.D. Rowley (1994) The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation, *Blood*, **83**, 2780–6.

Picciano, D. (1979) Cytogenetic study of workers exposed to benzene, *Environ Res*, **19**, 33–8.

Pollini, G. and G.P. Biscaldi (1977) (Studies on the karyotype of lymphocytes in subjects with benzene hemopathy 12 years after poisoning), *Medicina del Lavoro*, **68**, 308–12.

Pollini, G., G.P. Biscaldi, and G.F. De Stefano (1976) (Increase of Feulgen's reaction response in peripheral blood lymphocytes in benzene-poisoned subjects 10 years after intoxication. Preliminary data), *Med Lav*, **67**, 506–10.

Pollini, G., G.P. Biscaldi, and G. Robustelli della Cuna (1969) (Chromosome changes of the lymphocytes discovered after five years in subjects with benzolic hemopathy), *Med Lav,* **60**, 743–58.

Pollini, G. and R. Colombi (1964a) Il danno cromosomico midollare nell'anemia aplastica benzolica, *Med Lav,* **55**, 241.

Pollini, G. and R. Colombi (1964b) Il danno cromosomico dei linfociti nell'emopatia benzenica, *Med Lav*, **55**, 641.

Pollini, G., E. Strosselli, and R. Columbi (1964c) Sui rapporti fra alterazioni cromosomiche delle cellule emiche e gravita' dell'emopatia benzenica, *Med Lav*, **55**, 735–51.

Popp, W., C. Vahrenholz, S. Yaman, C. Muller, G. Muller, W. Schmieding, K. Norpoth, and R. Fahnert (1992) Investigations of the frequency of DNA strand breakage and cross-linking and of sister chromatid exchange frequency in the lymphocytes of female workers exposed to benzene and toluene, *Carcinogenesis*, **13**, 57–61.

Pui, C.H., R.C. Ribeiro, M.L. Hancock, G.K. Rivera, W.E. Evans, S.C. Raimondi, D.R. Head, F.G. Behm, M.H. Mahmoud, J.T. Sandlund et al. (1991) Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia, *N Engl J Med*, **325**, 1682–7.

Richardson, S., R. Zittoun, S. Bastuji-Garin, V. Lasserre, C. Guihenneuc, M. Cadiou, F. Viguie, and I. Laffont-Faust (1992) Occupational risk factors for acute leukaemia: a case-control study, *Int J Epidemiol*, **21**, 1063–73.

Rieger, R., A. Michaelis and M.M. Green (1991) *Glossary of Genetics and Cytogenetics, Classical and Molecular*, Springer-Verlag, New York.

Robustelli della Cuna, G., A. Favino, G.P. Biscaldi, and G. Pollini (1972) [Transformation into acute leukemia of a case of involutive myelopathy due to benzene poisoning], *Haematologica*, **57**, 65–89.

Rooney, D.E. and B.H. Czepulkowski (1992) *Human Cytogenetics: A Practical Approach,* in: D. Rickwood and

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B.D. Hames (Eds.), Oxford University Press, New York, pp. 30–49.

Rowley, J.D. (1973) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining, *Nature*, **243**, 290–3.

Rowley, J.D. (1998) The critical role of chromosome translocations in human leukemias, *Annu Rev Genet*, **32**, 495–519.

Rozman, C., M. Ribas-Mundo, S. Woessner, and F. Conesa-Landinez (1965) Estudio clinico-hematologico de 43 casos de anemia aplastica, con particular atencion a las de origen benzolico (20 casos), *Medicina Clinica*, **44**, 10–22.

Rozman, C., S. Woessner, and J. Saez-Serrania (1968) Acute erythromyelosis after benzene poisoning, *Acta Haemato*l, **40**, 234–7.

Rupa, D.S., L. Hasegawa, and D.A. Eastmond (1995) Detection of chromosomal breakage in the 1cen-1q12 region of interphase human lymphocytes using multicolor fluorescence *in situ* hybridization with tandem DNA probes, *Cancer Res*, **55**, 640–5.

Saita, G. (1973) Benzene induced hypoplastic anaemias and leukemias, in: R.H. Girdwood (Ed.), *Blood Disorders Due to Drugs and Other Agents,* Elsevier, Amsterdam, pp. 127–146.

Sandberg, A.A. (1990) *The Chromosomes in Human Cancer and Leukemia,* Elsevier, New York.

Santesson, C.G. (1897) Uber chronische vergiftung mit steinkohlenteerbenzin: vier todesfalle, *Arch Hyg Berl*, **31**, 336–76.

Santos-Mello, R. and B. Cavalcante (1992) Cytogenetic studies on gas station attendants, *Mutat Res*, **280**, 285–90.

Sarto, F., I. Cominato, A.M. Pinton, P.G. Brovedani, E. Merler, M. Peruzzi, V. Bianchi, and A.G. Levis (1984) A cytogenetic study on workers exposed to low concentrations of benzene, *Carcinogenesis*, **5**, 827–32.

Sasiadek, M. (1984) (Chromosome changes in workers after long-term exposure to benzene and its derivatives), *Pol Tyg Lek*, **39**, 1327–9.

Sasiadek, M. (1992) Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene, *Environ Health Perspect*, **97**, 255–7.

Sasiadek, M. and J. Jagielski (1990) Genotoxic effects observed in workers occupationally exposed to organic solvents, *Pol J Occup Med*, **3**, 103–8.

Sasiadek, M., J. Jagielski, and R. Smolik (1989) Localization of breakpoints in the karyotype of workers professionally exposed to benzene, *Mutat Res*, **224**, 235–40.

Schinzel, A. and W. Schmid (1976) Lymphocyte chromosome studies in humans exposed to chemical mutagens. The validity of the method in 67 patients under cytostatic therapy, *Mutat Res*, **40**, 139–66.

Seiji, K., C. Jin, T. Watanabe, H. Nakatsuka, and M. Ikeda (1990) Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits, *Int Arch Occup Environ Health*, **62**, 171–6.

Selling, L. (1916) Benzol as a leucotoxine. Studies on the degeneration of the blood and hematopoietic organs, *Johns Hopkins Hosp Rep*, **17**, 83–142.

Sellyei, M. and E. Kelemen (1971) Chromosome study in a case of granulocytic leukaemia with 'Pelgerisation' 7 years after benzene pancytopenia, *Eur J Cancer*, **7**, 83–5.

Siena, S., G. Sammarelli, M.G. Grimoldi, R. Schiavo, A. Nozza, M. Roncalli, C. Mecucci, A. Santoro, and C. Carlo-Stella (1999) New reciprocal translocation t(5;10)(q33;q22) associated with atypical chronic myeloid leukemia, *Haematologica*, **84**, 369–72.

Silva, J.M. and R. Santos-Mello (1996) Chromosomal aberrations in lymphocytes from car painters, *Mutat Res*, **368**, 21–5.

Smerhovsky, Z., K. Landa, P. Rössner, M. Brabec, Z. Zudova, N. Hola, Z. Pokorna, J. Mareckova, and D. Hurychova (2001) Risk of cancer in an occupationally exposed cohort with increased level of chromosomal aberrations, *Environ Health Perspect*, **109**, 41–5.

Smith, M.A., R.P. McCaffrey, and J.E. Karp (1996) The secondary leukemias: challenges and research directions, *J Natl Cancer Inst,* **88**, 407–18.

Smith, M.T. (1996) Overview of benzene-induced aplastic anaemia, *Eur J Haematol Suppl*, **60**, 107–10.

Smith, M.T. and L. Zhang (1998) Biomarkers of leukemia risk: benzene as a model, *Environ Health Perspec,* **106** Suppl 4, 937–46.

Smith, M.T., L. Zhang, Y. Wang, R.B. Hayes, G. Li, J. Wiemels, M. Dosemeci, N. Titenko-Holland, L. Xi, P. Kolachana, S. Yin, and N. Rothman (1998) Increased translocations and aneusomy in chromosomes 8 and 21 among workers exposed to benzene, *Cancer Res*, **58**, 2176–81.

Solé, F., M.R. Caballin, M.D. Coll, S. Woessner, and J. Egozcue (1990) Acute lymphoblastic leukemia with t(4;11) in a patient previously exposed to a carcinogen, *Cancer Genet Cytogenet*, **49**, 133–6.

Sorsa, M., A. Ojajarvi, and S. Salomaa (1990) Cytogenetic surveillance of workers exposed to genotoxic chemicals: preliminary experiences from a prospective cancer study in a cytogenetic cohort, *Teratogenesis, Carcinogenesis, and Mutagenesis,* **10**, 215–21.

Sorsa, M., J. Wilbourn, and H. Vainio (1992) Human cytogenetic damage as a predictor of cancer risk, *IARC Sci Publ,* 543–54.

Surrallés, J., K. Autio, L. Nylund, H. Jarventaus, H. Norppa, T. Veidebaum, M. Sorsa, and K. Peltonen (1997) Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene-exposed workers, *Carcinogenesis*, **18**, 817–23.

Tasaka, T., M. Nagai, K. Sasaki, M. Murata, T. Taoka, K. Ikeda, T. Tanaka, T. Abe, J. Takahara, and S. Irino (1992) Translocation (3;21) (q26.2;q22.1) found in a patient with myelodysplastic syndrome and long-term exposure to organic solvents, *Br J Haematol*, **82**, 473–5.

Tjio, J.H. and A. Levan (1956) The chromosome number of man, *Hereditas*, **42**, 1–6.

Tompa, A., J. Major, and M.G. Jakab (1994) Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related de-

crease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes, *Mutat Res*, **304**, 159–65.

Tough, I.M. and W.M. Court Brown (1965) Chromosome aberrations and exposure to ambient benzene, *Lancet*, **7387**, 684–685.

Tough, I.M., P.G. Smith, W.M. Court Brown, and D.G. Harnden (1970) Chromosome studies on workers exposed to atmospheric benzene: the possible influence of age, *Eur J Cancer*, **6**, 49–55.

Tucker, J.D. and J.R. Senft (1994) Analysis of naturally occurring and radiation-induced breakpoint locations in human chromosomes 1, 2 and 4, *Radiation Research*, **140**, 31–6.

Tunca, B.T. and U. Egeli (1994) Analysis of chromosomal aberrations in shoe workers exposed long term to benzene, *Occup Environ Med*, **51**, 50–3.

Tunca, B.T. and U. Egeli (1996) Cytogenetic findings on shoe workers exposed long-term to benzene, *Environ Health Perspect,* **104** Suppl 6, 1313–7.

Van den Berghe, H., A. Louwagie, A. Broeckaert-Van Orshoven, G. David, and R. Verwilghen (1979) Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene, *Blood*, **53**, 558–66.

Vigliani, E.C. and A. Forni (1969) Benzene, chromosome changes and leukemia, *J Occup Med,* **11**, 148–9.

Vigliani, E.C. and G. Saita (1964) Benzene and leukemia, *N Engl J Med,* **271**, 872–6.

Vineis, P., G.C. Avanzi, B. Giovinazzo, G. Ponzio, G.R. Cambrin, and G. Ciccone (1990) Cytogenetics and occupational exposure to solvents: a pilot study on leukemias and myelodysplastic disorders, *Tumori*, **76**, 350–2.

Watanabe, T., A. Endo, Y. Kato, S. Shima, T. Watanabe, and M. Ikeda (1980) Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers, *Int Arch Occup Environ Health*, **46**, 31–41.

Weiskotten, H.G., G.B.F. Gibbs, E.O. Boggs and E.R. Templeton (1920) The action of benzol. VI. Benzol vapor leucopenia (rabbit), *J Med Res*, **41**, 425–38.

Weiskotten, H.G., S.C. Shwartz, and H.S. Steinsland (1916) The action of benzol on blood and blood forming tissues, *J Med Res*, 63–9.

Werner, N. (1986) (Initial results from the study of chromosome examination in peripheral lymphocytes of workers engaged in benzene production), *La Santé Publique*, **29**, 55–61.

Xu, H., X. Zhang and B. Xue (1995) (Studies on comprehensive evaluation of toxic hazard in low-concentration benzene pollution), *Chung-Hua Yu Fang i Hsueh Tsa Chih* (*Chin J Prevent Med*), **29**, 264–6.

Xu, X., J.K. Wiencke, T. Niu, M. Wang, H. Watanabe, K.T. Kelsey, and D.C. Christiani (1998) Benzene exposure, glutathione *S*-transferase theta homozygous deletion, and sister chromatid exchanges, *Am J Ind Med*, **33**, 157–63.

Yardley-Jones, A., D. Anderson, P.C. Jenkinson, D.P. Lovell, S.D. Blowers, and M.J. Davies (1988) Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene, *Br J Ind Med*, **45**, 694–700.

Yardley-Jones, A., D. Anderson, D.P. Lovell, and P.C. Jenkinson (1990) Analysis of chromosomal aberrations in workers exposed to low-level benzene, *Br J Ind Med,* **47**, 48–51.

Zhang, B. (1996a) [Investigation of health status in workers exposed to low-level benzene], Chung-Hua Yu Fang i Hsueh Tsa Chih [Chin J Prevent Med], 30, 164–6.

Zhang, L., N. Rothman, Y. Wang, W. Guo, R.B. Hayes, G. Li, S. Yin, and M.T. Smith (2000) Spectrum of chromosomal changes detected by fluorescence *in situ* hybridization in workers exposed to benzene, in: AACR (Ed.), 91st Annual Meeting of American Association for Cancer Reaserch, Confernece Proceedings, San Francisco.

Zhang, L., N. Rothman, Y. Wang, R.B. Hayes, W. Bechtold, P. Venkatesh, S. Yin, Y. Wang, M. Dosemeci, G. Li, W. Lu, and M.T. Smith (1996b) Interphase cytogenetics of workers exposed to benzene, *Environ Health Perspect,* **104** Suppl 6, 1325–9.

Zhang, L., N. Rothman, Y. Wang, R.B. Hayes, G. Li, M. Dosemeci, S. Yin, P. Kolachana, N. Titenko-Holland, and M.T. Smith (1998a) Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene, *Carcinogenesis*, **19**, 1955–61.

Zhang, L., N. Rothman, Y. Wang, R.B. Hayes, S. Yin, N. Titenko-Holland, M. Dosemeci, Y. Wang, P. Kolachana, L. Xi, G. Li, and M.T. Smith (1999) Benzene increases aneuploidy in the lymphocytes of exposed workers: A comparison of data obtained by fluorescence *in situ* hybridization in interphase and metaphase cells, *Environ Mol Mutagen*, **34**, 260–268.

Zhang, L., Y. Wang, N. Shang, and M.T. Smith (1998b) Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes, *Leuk Res*, **22**, 105–13.

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