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GENETIC TOXICOLOGY OF BENZENE, TOLUENE, XYLENES AND PHENOLS

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Introduction

The first recorded use of aromatic solvents in industry was in the early nineteenth century when coal tar naphtha, a by-product of coal gas manufacture, was used as a solvent for rubber. Benzene, toluene and xylene and other alkyl benzenes were the major constituents of coal tar naphtha and the value of these and other aromatic hydrocarbons both as solvents and as starting materials in many industrial processes was soon realised. Early in the twentieth century petroleum (crude oil) was found to be another rich source of aromatics and, with the impetus provided by two world wars, vast and diverse applications were found for these valuable chemicals. Not surprisingly, an extensive literature describes more than half a century of investigations into the toxicology and biochemistry of benzene, and to a lesser extent, toluene and the xylenes, but, as will be discussed, a number of vital questions remain unanswered.

Phenol has been used on a large scale in the manufacture of plastics, and condensation with formaldehyde produces a three-dimensional polymer which is an essential component of phenol—formaldehyde resins such as "Bakelite". Other industrial uses of phenol include the manufacture of dyestuffs and explosives and it is still widely used in the drug industry. Most of the other phenols have minor industrial value. The naphthols are important intermediates in the preparation of dyes and perfumes; β-naphthol is also used for making antioxidants used in rubber manufacture. Both catechol and the cresols have been used in the preparation of antiseptics and hydroquinone, apart from minor use as a food preservative, is an important industrial and photographic chemical.

In addition to reviewing the mutagenicity of the chemicals, some reference is made to their biotransformation and toxicity which should be considered when designing safe handling procedures.

Physical and chemical properties

A detailed discussion of physical and chemical properties of the compounds is beyond the scope of this review. The chemical structures and physical constants are shown in Table 1. Benzene, toluene and the xylenes are clear colourless aromatic hydrocarbons of characteristic odour, very sparingly soluble in water but generally miscible with other organic solvents such as diethyl ether, acetone, ethanol and chloroform. Commercial xylene is essentially a mixture of ethylbenzene, *para*, *meta*- and *ortho*-xylene. Phenol and the phenolic derivatives reviewed are generally water-soluble — some are hygroscopic — and readily soluble in ethanol and diethyl ether. The hydroxyl group of phenol increases the general reactivity of the benzene nucleus and the halogenation and nitration are readily achieved. The polyhydric phenols such as quinol and pyrogallol are powerful reducing agents.

Biotransformation

The most important route of absorption of aromatic solvents is by inhalation of vapours and mists. Because of their lipid solubility they are probably trans-

TABLE 1

CHEMICAL S

Compound
Benzene
Toluene
ortho-Xylene
meta-Xylene
para-Xylene
Phenol
ortho-Cresol
meta-Cresol
para-Cresol
Catechol

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TABLE I

CHEMICAL STRUCTURE AND PHYSICAL CONSTANTS

Compound	Synonyms	Molecular weight	Melting point (°C)	Boiling point (°C)	Density at 20°C
Benzene 	Benzole Cyclohexamine Coal naphtha	78.11	5.5	80.1	0.8794
Toluene 	Methylbenzene Toluol Phenylmethane	92.13	−95.0	110.4	0.8660
ortho-Xylene 	*o*-Xylol 1,2-Dimethylbenzene	106.2	−25.0	144.4	0.8800
meta-Xylene 	*m*-Xylol 1,3-Dimethylbenzene	106.2	−47.9	139.0	0.8640
para-Xylene 	*p*-Xylol 1,4-Dimethylbenzene	106.2	14.0	138.3	0.8611
Phenol 	Hydroxybenzene	94.11	40.6	181.9	1.072
ortho-Cresol 	2-Methylphenol 2-Cresol *o*-Hydroxytoluene *o*-Oxytoluene	108.1	30.8	190.8	1.047
meta-Cresol 	3-Methylphenol 3-Cresol *m*-Hydroxytoluene *m*-Oxytoluene	108.1	10.9	202.8	1.034
para-Cresol 	4-Methylphenol 4-Cresol *p*-Hydroxytoluene *p*-Oxytoluene	108.1	35.5	201.8	1.034
Catechol 	Pyrocatechol 1,2-Benzenediol 2-Hydroxyphenol 1,2-Dihydroxybenzene	110.1	10.50	240.0	1.371

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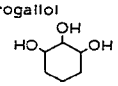
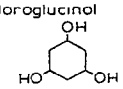
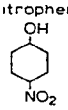
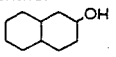
TABLE 1 (continued)

Compound	Synonyms	Molecular weight	Melting point (°C)	Boiling point (°C)	Density at 20°C
Resorcinol 	1,3-Benzenediol 1,3-Dihydroxybenzene 3-Hydroxyphenol	110.1	110.0	276.5	1.285
Quinol 	Hydroquinone 1,4-Benzenediol 1,4-Dihydroxybenzene	110.1	170,5	286.2	1.358
Pyrogallol 	1,2,3-Benzenetriol 1,2,3-Trihydroxybenzene Pyrogallic acid	1.26.1	134.0	309.0	1.453
Phloroglucinol 	1,3,5-Benzenetriol 1,3,5-Trihydroxybenzene	126.1	218.0	—	—
ortho-Nitrophenol 	2-Nitrophenol	139.1	45.0	214.5	1.657
meta-Nitrophenol 	3-Nitrophenol	139.1	97.0	194.0	1.485
para-Nitrophenol 	4-Nitrophenol	139.1	114.0	279.0	1.270
2,4,6-Trinitrophenol 	Picric acid Picronitric acid	229.11	121.8	Explodes above 300°C	1.763
α-Naphthol 	1-Naphthol 1-Hydroxynaphthalene	144.2	96.0	282.5	1.095 (at 4°C)
β-Naphthol 	2-Naphthol 2-Hydroxynaphthalene Isonaphthol	144.2	122.5	288.0	1.22

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ported in the blood absorbed on red-cell membranes and plasma lipoproteins and tend to accumulate in tissues in proportion to the latters fat content. Unchanged hydrocarbons are exhaled through the lungs at rates determined by their vapour pressures and their concentration in the blood, which in turn depends on the rate of absorption and metabolism. In general, metabolic products are excreted as water-soluble conjugates with glycine and glucuronic acid or as ethereal sulphates. Benzene, toluene and the xylenes are poorly absorbed through the skin [31] unlike phenol which is rapidly absorbed and which can, after extensive dermal exposure, prove fatal [75]. Most of the other phenols reviewed in this paper, particularly resorcinol, quinol, pyrogallol and the naphthols, are also rapidly absorbed through the skin.

Benzene and the phenols

The principal mammalian metabolites of benzene are phenol, catechol and hydroquinone (quinol) which are excreted in the urine as conjugates of glucuronide and ethereal sulphates (Fig. 1). After oral administration of [^{14}C]benzene to rabbits 43% is exhaled as unchanged benzene and 34% is excreted in the urine as conjugates of phenol (23.5%), hydroquinone (4.8%), catechol (2.7%) and hydroxy hydroquinone (0.3%) [60,61]. Other minor metabolites recovered from the urine are *trans—trans*-muconic acid (1.3%) and 1-phenyl mercapturic acid (0.5%) [60,61].

The mechanism of benzene metabolism has been the subject of a number of recent studies and is extensively reviewed by Snyder and Kocsis [81]. Gonasun et al. [33] demonstrated species differences in the rate of benzene metabolism by liver microsomes. Pre-treatment of mice with benzene stimulated benzene metabolism in vitro. Results suggested that benzene metabolism is mediated by mixed function oxidases and the rate of metabolism is significantly influenced by the binding of benzene to cytochrome P450. Saito et al. [74] confirmed that benzene pre-treatment stimulated rat-liver microsomes but that pre-treatment with phenolic metabolites did not. Benzene also induced a proliferation of smooth endoplasmic reticulum.

As phenol is the major benzene metabolite a number of studies have examined its fate after administration to animals, and qualitative and quantitative species differences in its metabolism have been demonstrated [12,13,30]. In a study of 19 species including man, in which [^{14}C]phenol was given orally [12,13], the cat excreted almost the whole dose (>90%) as sulphates of quinol and phenol with only small amounts of phenyl glucuronides in the urine. The pig excreted more than 90% of the dose of [^{14}C]phenol as phenyl glucuronide. As in the cat, both metabolic pathways were present in the pig, but only a small proportion of the administered dose was excreted as phenyl sulphate. The rhesus monkey, fruit bat and chicken excreted phenyl conjugates of sulphate and glucuronides but no quinol. 8 other species, including man, mouse and rat, excreted sulphate and glucuronic acid conjugates of both phenol and quinol.

One major difference between the metabolism of phenol and that of benzene is the absence of urinary muconic acid after the ingestion of phenol and this is thought to be due to 1,2-dihydrobenzene-1,2,-diol formation from benzene with subsequent transformation to muconic acids [61,37]. An arene oxide intermediate has been postulated [37] and the conversion of this into the *trans-*

Density at 20°C
1.285
1.358
1.453
1.657
1.485
1.270
1.763
1.095 (at 4°C)
1.22

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Fig. 1. Probable routes of the biotransformation of benzene in mammals.

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Fig. 2. Me

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dihydrodiol by an epoxide hydrase has been described [59].

The dihydric phenols, catechol, resorcinol and quinol are metabolised through similar pathways to phenol and excreted in the urine as monoethereal sulphates and monoglucuronides [28,29]. Pyrogallol, phloroglucinol and the naphthols also undergo similar biotransformations [89]. Part of an administered dose of 2,4,6-trinitrophenol is excreted unchanged and part is metabolised to picramic acid (2-amino-4,6-dinitrophenol) before excretion [89]. The three mononitrophenol isomers undergo biotransformations characteristic of both the hydroxyl and nitro groups and conjugate with sulphuric and glucuronic acids. They undergo reduction of the nitro group to a small extent giving *o*-, *m*- and *p*-aminophenols with subsequent hydroxylation to nitroquinol and nitrocatechol [69]. The cresols, too, conjugate with sulphate and glucuronide and are also hydroxylated to a lesser degree to dihydroxytoluenes [10].

Toluene

About 50% of an absorbed dose of toluene is exhaled unchanged. Trace amounts of toluene are excreted in the urine. The major route of metabolism is through oxidation to benzoic acid and excretion as hippuric acid and benzoylglucuronic acid (Fig. 2) [89,40,17]. Small quantities of *o*-, *m*- and *p*-cresyl sulphates and glucuronides are also excreted as a result of ring hydroxylation [17].

Xylenes

Absorbed xylenes are exhaled at a much lower rate than either benzene or

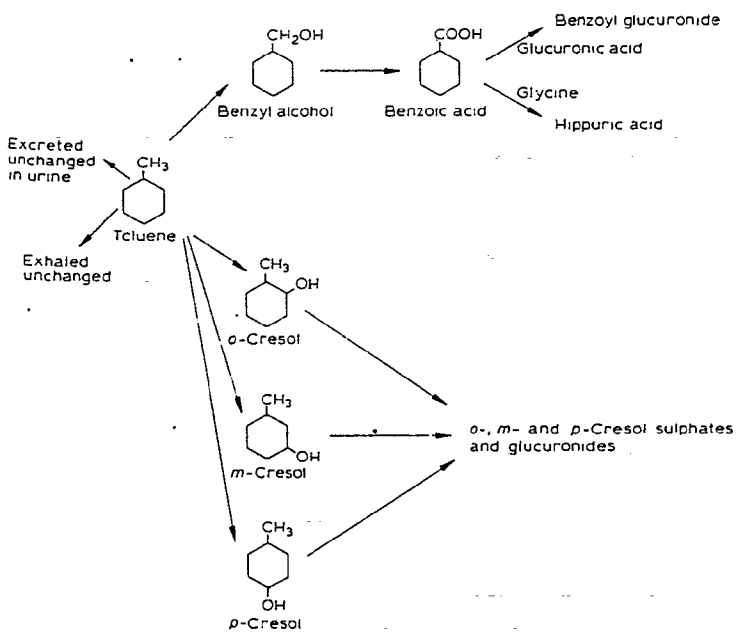

Fig. 2. Metabolism of toluene in mammals.

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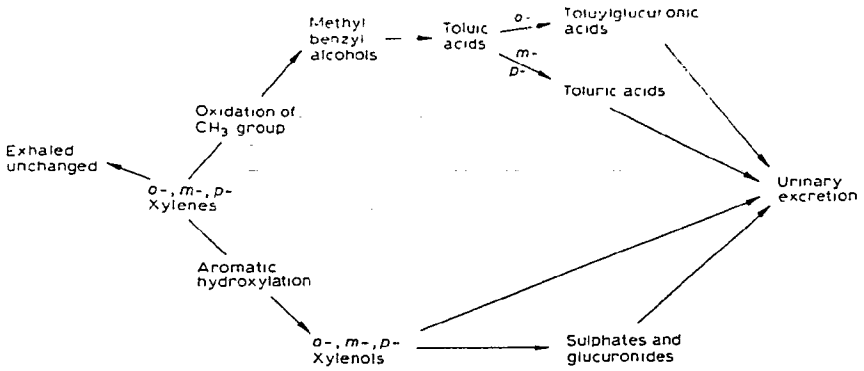

Fig. 3. Metabolism of *o*-, *m*- and *p*-xylenes.

toluene. One of the two methyl groups is oxidised resulting, with the *meta*- and *para*-isomers, in the corresponding toluic acids which are conjugated with glucuronic acid to toluric acids before excretion (Fig. 3). *o*-Xylene is almost completely oxidised to *o*-tolylglucuronic acid. In a minor pathway, the aromatic nucleus is hydroxylated to give the corresponding xylenols (dimethylphenols) which are excreted unchanged or as sulphate and glucuronic acid conjugates [89,40,17].

Toxicology

Acute toxicity

The acute toxic effects of the three aromatic solvents are very similar. They are dermal irritants and cause erythema but it is unlikely that severe systemic intoxication could result from percutaneous absorption through intact skin [31]. The major systemic effect of inhalation of high concentrations of benzene is in the central nervous system (CNS). Symptoms of excitation are followed by depression and finally, in some cases, by death due to respiratory failure [75]. Acute poisoning by high concentrations of toluene is uncommon but like benzene both toluene and xylene can produce effects on the central nervous system.

Phenol is much more toxic than the aromatic solvents and absorption through the skin is very rapid and when extensive can be fatal. The main effect is depression of CNS function and symptoms of intoxication frequently occur within 30 min of dermal exposure [75]. The acute systemic toxicity of the cresols is similar to, but less severe, than that of phenol and they are also very corrosive, producing chemical burns and dermatitis [75]. Little is known about the toxicity of the nitrophenols but they are reported to induce liver and kidney damage in experimental animals [75].

The dihydroxy- and trihydroxy-phenols are readily absorbed through the skin and are likely to cause skin disorders. Catechol, resorcinol and quinol may also affect the central nervous system, and quinol is considered to be more toxic than phenol [75]. Haemolytic anaemia and kidney and liver damage have been

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associated with exposure to pyrogallol [75]. The α- and β-naphthols are irritant to skin and mucous membranes, may cause kidney damage, and, like most of the phenols, can cause severe injury to the lens and cornea [75,26].

Chronic toxicity

The effects on health of chronic exposure to benzene have been dealt with extensively in two recent reviews [57,81]. The most severe long-term hazard is to the haemopoietic system. The first symptoms are accompanied by, and probably a result of, reduction in the number of red and white cells of the blood and deficiencies in blood coagulation. The bone marrow shows evidence of myelotoxicity, is often hypoplastic but in some cases proliferation of the most immature red cell and leucocyte precursers occurs. Continued exposure often results in an irreversible progression into chronic pancytopenia though cessation of contact with benzene can lead to recovery of an apparently normal blood picture. A more acute form of pancytopenia develops in some cases and, occasionally, acute or chronic leukaemia has been reported.

An association between long-term exposure to high concentrations of benzene and leukaemia has been described by many workers and includes cases of sub-acute myeloblastic [87,24,47], aleukaemic [73] and chronic myeloid leukaemia [76,87], and also, with less convincing evidence, lymphatic leukaemia [32] and Hodgkin's disease [2]. Symptoms of leukaemia are frequently preceded by aplastic anaemia and it has been suggested [73] that many benzene haemopathies diagnosed on the basis of peripheral blood picture as pancytopenia might, in fact, have been aleukaemic leukaemia.

The extent of the problem is difficult to assess. A recent review documents 250 cases in which leukaemia was diagnosed in subjects who had a record of chronic exposure to benzene [6]. As the NRC review [57] indicates, it is not possible, from the available literature, to estimate the incidence of leukaemia in the industrial population, or even the proportion of the industrial population chronically exposed to benzene (though the National Institute for Occupational Safety and Health estimates that 2 million workers in the USA have a potential exposure to benzene [57]). Only rarely can the extent and duration of benzene exposure be calculated from published reports on leukaemia and in almost all recorded cases the affected individuals were exposed to high concentrations of other chemical agents in addition to benzene.

Elucidation of the benzene/leukaemia question has been severely hindered by the lack of a suitable animal model. It is possible to reproduce several of the haematological disorders found in man [38] but attempts to induce leukaemia by exposure of laboratory animals to benzene have been uniformly unsuccessful [88]. This may be due to a unique factor in human metabolism, or a design flaw in the few experiments carried out in laboratory animals to date. It has been suggested [57] that benzene leukaemia occurs only in genetically sensitive individuals or only when benzene exposure occurs in conjunction with some other chemical, physical or viral agent.

Absorption of toluene over long periods may cause general malaise and minor CNS effects [31] but does not appear to induce the severe haemopoietic injury associated with chronic benzene exposure [48]. Fabre et al. [22] carried out extensive animal studies and were unable to find evidence of bone-marrow

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damage in a variety of species after chronic inhalation exposure to toluene. Toluene is used in several industries as a "safe" replacement for benzene [25].

Exposure to mixed xylenes induces effects similar to those of toluene and although minor haematological disturbance have been reported in certain species [31] severe myelotoxicity is not a feature of prolonged xylene absorption [31,25].

There is a paucity of information in the literature on the long-term systemic effects of phenols. Prolonged exposure to low concentrations of phenol vapour or mists results in digestive and nervous system disorders with, eventually in some cases, kidney and liver damage [75]. The phenol derivatives appear to produce similar effects. A study of industrial exposure to the dihydroxyphenols [26] showed that workers were exposed to low concentrations of resorcinol with no apparent ill-effect though chronic exposure to hydroxyquinone has been associated with corneal injury. Mitchell [52] reported the failure of the benzene metabolites phenol, catechol and hydroquinone to induce aplastic anaemia in rats, suggesting that these were not responsible for the myelotoxic effects of benzene.

Carcinogenicity

Limited carcinogenicity data have been generated on the compounds under review. The use of benzene as a solvent for hydrocarbon carcinogens in skin-painting studies suggests that it is not itself carcinogenic by this route. In one experiment [58] a control group of mice were skin-painted with benzene twice weekly for 32 months and one tumour (type unspecified) was found in a group of 25 animals. In a similar study, Shabad et al. [77] applied distilled benzene to the skin of mice for 5 months without inducing skin tumours.

A single application of phenol to mouse-skin stimulated cell mitosis, and repeated application of a 20% solution resulted in a high incidence of papillomas [72]. These studies were extended [8] and showed that after treatment of mouse skin with an initiator (0.3% 7,12-dimethylbenz[*a*]anthracene in acetone) applications of a 20% solution of phenol, *o*-, *m*- or *p*-cresol promoted papilloma formation. Benzene and β-naphthol did not behave as promoters. The application of 10 or 20% of phenol alone induced both papillomas and epithelial carcinomas. *o*- and *p*-Nitrophenols failed to induce skin tumours.

Boyland et al. [9] induced carcinoma in rat bladders implanted with cholesterol pellets containing hydroquinone and suggested that this substance might contribute towards cancer in cigarette smokers.

Toluene is reported to be an enhancing agent in 7,12-dimethylbenz-[*a*]-anthracene induced skin carcinogenesis [27] and xylene, like other skin irritants, increased the yield of tumours when applied to the skin of mice prior to subcutaneous injections of urethane [67].

The skin carcinogenicity of these compounds appears to be associated with their irritancy and the subsequent skin hyperplasia. No reference to systemic carcinogenicity was found for any of the chemicals being reviewed (except for the unique benzene/leukaemia cases).

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Mutagenesis

Two specific findings by early workers in the field of cytogenetics have greatly influenced the direction which subsequent research has taken on the genetic effects of the chemicals under review. Since the discovery that phenols were mitotic poisons, the subject has been studied in great depth in a variety of plant systems at the expense of experiments in micro-organisms and mammals. Similarly the benzene/leukaemia question has stimulated extensive investigations of the clastogenic properties of the hydrocarbon solvents with only limited concern for their ability to induce gene or point mutations.

Benzene

Microbial systems

Only a single report has been published of a study of the effect of benzene in microbial mutation systems. However, this was performed in detail by Lyon [45,46] using sensitive, plasmid-containing strains of *Salmonella typhimurium*. The strains used, developed by Ames and co-workers [50] were TA 98 (reverted by frameshift mutagens) and TA 100 (reverted by base-substitution mutagens). Benzene was tested in a plate incorporation assay both directly and in the presence of S9 microsomal fractions derived from rat liver. Benzene was incorporated at concentrations of 0.1 to 1.0 μl per plate and microsomal fractions at concentrations ranging from 1 to 50 μl per plate. S-9 fractions were prepared from liver homogenates from both phenobarbital and 3-methylcholanthrene (MCA) induced rats. In a further series of studies using the TA 100 strain, 1,1,1-trichloro-2,3-epoxypropane was introduced into the reaction mixture in an attempt to block the biodegradation of the theoretical epoxide metabolite [37]. Further variations on the in vitro microbial assay were carried out including the use of a 1-h pre-incubation of the reaction mixture before application to the agar plate and the novel and possibly very relevant approach of using the S-9 fraction prepared from bone marrow obtained from MCA-treated rats. The results of these in vitro assays were uniformly negative.

The author is grateful to Dr. M.M. Shahin of the University of Alberta, for permission to present his unpublished results which confirm the lack of mutagenic effect of benzene in the Salmonella test [78]. An extended range of tester strains was used including TA 1535, TA 1537 and TA 1538 in addition to TA 98 and TA 100. The results are shown in Table 2.

Lyon also performed a host-mediated assay in mice some of which were pretreated with phenobarbital. *Salmonella typhimurium* TA 1950 was the test organism. Mice were given two subcutaneous doses of 0.1 ml of benzene producing clinical deterioration. Micro-organisms harvested from benzene-treated animals showed no increase in mutation rate over the control value.

Chromosome studies in cultured cells

Koizumi et al. [39] studied the effects of benzene on DNA synthesis and chromosomes of cultured human lymphocytes and Hela cells. Concentrations ranging from 2.2×10^{-5} M to 2.2×10^{-3} M were added to human leucocytes at

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TABLE 2

MUTATION STUDIES WITH BENZENE IN *SALMONELLA TYPHIMURIUM* TESTER STRAINS

Revertant colonies per plate

Compound	Dose per plate	TA1535		TA 100		TA 1537		TA 1538		TA 98	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Control	—	32	19	50	79	65	36	25	44	30	51
2-Aminoanthracene	10 μg	29	248	78	1 233	75	344	28	660	34	1220
Ethyl methane sulphonate	10 μl	9000 *	12 000 *	10 500 *	16 000 *	7500 *	12 500 *	35	80	40	68
Benzene	20 μl	23	14	32	59	45	18	18	43	26	49
Benzene	50 μl	18	13	19	54	49	17	7	52	13	53
Benzene	100 μl	14	13	6	48	42	18	0	50	0	49
Benzene	200 μl	1	13	0	51	9	13	0	50	0	44
Benzene	400 μl	0	5	0	0	0	14	0	53	0	32
Benzene	600 μl	0	0	0	0	0	10	0	0	0	3

* Approximate values.

Rajdi M. Shahin (Unpublished results).

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the beginning of 72-h culture period. The combined incidence of chromatid gaps and breaks was significantly higher in cultures containing 1.1 or 2.2×10^{-3} M benzene than in untreated cultures. A reduction in tritium uptake in autoradiographs prepared from both leucocytes and Hela cells was attributed by the authors to inhibition of DNA synthesis by 24 h exposure to 1.1 or 2.2×10^{-3} M benzene. The increase in chromosome aberrations in cultured human leucocytes exposed to benzene was confirmed by Morimoto [54] who harvested cells after 53 h in culture, i.e. when the majority of cells had undergone only one mitosis. The synergistic effects of radiation (100 rad) and benzene on chromosomes was also investigated by the same worker. Only chromatid-type aberrations were recorded in cultures treated with 1×10^{-3} M benzene alone and irradiation of cultures increased the incidence of these features in an additive manner. No dicentrics and rings were found after benzene treatment. Exposure of cells to 100 rad induced an incidence of 0.100 rings or dicentrics per cell which was increased to 0.215 per cells when combined with 1×10^{-3} M benzene. These findings were investigated further [55] and it was suggested that the synergistic effect was a result of benzene-induced inhibition of repair of radiation-induced chromosome breakage.

Chromosome studies in laboratory animals

Chromatid deletions were demonstrated in metaphase chromosomes from bone-marrow cells of rats analysed 12 and 24 h after subcutaneous injections of 2 ml per kg undiluted benzene [63]. These findings were confirmed by Lyapkalo [44] in a study in which rats were given subcutaneous doses of 1 g/kg each day for 12 days. When rats were dosed with benzene or toluene or a mixture of both, Dobrokhotov [19] showed that the degree of chromosome damage induced by 0.2 g/kg/day of benzene was similar to that induced by 0.8 g/kg/day of toluene and that the effect was additive when both compounds were given.

Kissling and Speck [38] dosed rabbits subcutaneously with undiluted benzene at a dose of 0.2 ml per kg per day until sequential blood cell counts showed that the peripheral blood leucocytes were reduced to below $1000/mm^3$. At this point benzene dosing was temporarily stopped and bone-marrow cells were obtained for chromosome analysis. A high incidence of chromatid- and chromosome-type aberrations was found in all the rabbits analysed, persisting up to 60 days after the end of dosing with benzene.

A combined dominant lethal assay/bone marrow cytogenetic study has been reported [46] after intraperitoneal dosing of male rats with 0.5 ml benzene per kg body weight. No induced dominant lethality was detected. In the bone-marrow study, no consistent numerical chromosome changes were observed but both chromatid and chromosome aberrations were significantly increased over the control values up to 8 days after dosing. In cells analysed 70 days after dosing, aberrations were still increased over control animals but these differences were not statistically significant.

The same worker [46] also carried out a micronucleus assay in rats given intraperitoneal doses of 0.025, 0.05 and 0.25 ml/kg benzene on each of two successive days. Micronuclei counts carried out 6 h after the final dose showed that animals in the 0.05 and 0.25 ml/kg groups had significantly higher counts than had corresponding controls.

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Human chromosome studies

A number of studies have been carried out on the chromosomes of peripheral blood leucocytes or bone-marrow cells from workers with known, and often extensive, exposure to benzene. Such studies fall into two groups; chromosome investigations of patients with blood dyscrasias associated with benzene exposure — the so-called "benzene haemopathies" — and more wide-ranging studies of exposed working populations without clear evidence of clinical effects.

Pollini and Colombi [65] carried out chromosome analyses on bone-marrow cells cultured from cases of aplastic anaemia associated with benzene exposure. 70% of metaphases analysed showed numerical differences from the normal human karyotype, and chromatid fragments were detected in a proportion of the cells. A detailed cytogenetic study was carried out by Forni and Moreo [24] on a patient who developed leukaemia after 22 years continuous exposure to high concentrations of benzene. Sequential analyses were performed over a period of 15 months spanning the period during which the disease progressed from aplastic anaemia, with no immature cells in the peripheral blood, to a leukaemic condition characterised by peripheral blood myeloblasts. Initially, a high incidence of stable and unstable chromosome aberrations were present in blood lymphocytes. Later, before immature cells appeared in the blood, hyperdiploid cells were detected in the bone marrow, each with 47 chromosomes. The extra chromosomes differed between individual cells, generally belonging to the Denver groups C, F or G. When a frankly leukaemic blood picture emerged hyperdiploid cells from blood or bone marrow consistently contained an extra group C chromosome. The authors interpreted these findings with the hypothesis that the production of aberrations in bone marrow (i.e. haemopoietic stem cells) resulted in the development of clones with abnormal chromosome complements, one of which might, by some selective advantage phenomenon, develop into a leukaemic clone.

A similar study has been described by Sellyei and Kelemen [76] in Hungary. The patient developed pancytopenia after working for 18 months in atmospheric benzene concentrations varying between 200 and 1640 mg/m^3. 7 years after apparent remission of the anaemia the patient presented clinical signs of leukaemia. Karyotype analysis of 27 metaphases from bone marrow cells showed that all had an additional group D chromosome. 16 of the cells lacked one or more group G chromosomes and 12 cells had one or more additional group C chromosomes. 5 of the cells contained structural aberrations, including 2 dicentrics and a ring chromosome.

Both these cases have been described in some detail to illustrate the association between extensive exposure to benzene, aplastic anaemia, chromosome aberrations and leukaemia. In the first case, in particular, benzene appears to be the only solvent used to any extent by the patient over a period of 22 years.

Forni et al. [23] extended this line of investigation to 25 subjects with a clinical history of haemopathies thought to be due to benzene exposure and compared their cytogenetic pictures with those from 25 matched controls. The analyses were performed up to 18 years after clinical and haematological symtoms had, in most cases, disappeared. Both unstable and stable chromosome aberrations were increased as compared with the matched controls (1.89% C_u

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aberrations compared with 0.49% in the controls and 1.22% C_s aberrations compared with 0.04%). The stable chromosome changes, in particular, persisted after recovery from the haemopathy.

Two pregnant women, both with benzene-induced haemopathies were among those surveyed in this study [23]. During the period of observation, when chromosome abnormalities were present in her peripheral blood and bone marrow, one of the patients delivered two apparently normal infants. A cytogenetic study was carried out on one child and did not reveal any chromosome abnormalities.

In 1965, Tough and Court Brown reported their findings from a chromosome study of workers exposed to ambient benzene for periods varying from 1 to 18 years [85]. They reported a small but significant increase in stable and unstable chromosome aberrations compared with both on-site controls, and randomly selected, non-exposed males in the same age-group. This study was extended to three factories in which workers were exposed to benzene [86]. In two of the factories, where exposure to benzene varied from 25 to 150 ppm, the incidence of cells with unstable aberrations (C_s) was higher than that in the general population, but in the third group, who were exposed to approximately 12 ppm for an average period of 13 years, no differences were observed. An increase in the incidence of both C_u and C_s cells was correlated with increasing age. The authors were unable to demonstrate a direct relationship between benzene exposure and chromosome damage and concluded that though the increase in aberrations found in workers from two factories might be due to benzene or other environmental factors, the age of the workers might also have influenced the results.

Another report from Forni et al. [25] presents the results of chromosome examinations carried out on blood lymphocytes of 34 workers employed in a rotagravure plant and compared them with 34 matched controls. 10 of the workers were exposed to benzene prior to 1953, and then, when benzene was eliminated from the process, to toluene. 24 workers were exposed to toluene only. The individuals with a history of benzene exposure had a significantly higher incidence of C_u and C_s cells than had the matching controls and higher than in workers exposed to toluene only.

In a study carried out in Germany on 9 seemingly healthy refinery workers exposed to "low levels" of benzene the incidence of chromosome aberrations was significantly higher that of matching controls, but even so, was at the upper limit of the normal range [35].

Interpretation of studies carried out on workers exposed to benzene is complicated by the lack of quantitative data on the concentrations of benzene involved. Figures of 125 to 532 ppm are referred to by Forni et al. [25] and all cases suggest extremely high concentrations, probably in the hundreds of ppm. Even a detailed examination of the published data does not allow a close correlation between occupational exposure to benzene and persistence of chromosome aberrations [57] except where clinical syndromes were present, when an association between benzene-induced haemopathy and chromosome aberrations is clearly demonstrated.

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Toluene

The author has been unable to trace any reference in the literature to microbial mutation testing of toluene. However, the strongly lipophilic solvent has been used as a means of reducing the permeability of bacterial cell walls [51, 56,82]. Toluene-treated *Escherichia coli* are permeable to low molecular weight compounds such as deoxynucleoside triphosphates and retain many physiological functions [56]. This permits the study of both DNA-replication synthesis and repair synthesis. Such a property also suggests that highly lipophilic solvents might influence the penetration of test compounds into bacteria if used as solvents in microbial mutation tests. In toluene-treated *Bacillus subtilis* protein synthesis was inhibited and the absence of initiation of DNA synthesis is described [90].

Conflicting results have been reported on the effects of toluene on mammalian chromosomes. Forni et al., in their studies of workers exposed to benzene or toluene were unable to detect a significant increase in chromosome aberrations in workers exposed to toluene for periods up to 15 years [25]. Atmospheric concentrations of toluene during the period were generally close to the maximum alowable concentration of 200 ppm though, on occasions, workers were exposed to much higher concentrations.

Lyapkalo [44] injected rats with 1 g of toluene per kg body weight each day for 12 days and then studied chromosome damage in bone marrow cells. Chromatid gaps and breaks were detected in 11.5% of cells analysed (compared with 57.2% in benzene-treated rats and 3.9% in untreated animals). The persistence of chromosome aberrations after inhalation exposure of rats to toluene (160 ± 42 mg/m^3) was described by Dobrokhotov and Enikoev [20]. Chromosome damage was detected in bone-marrow cells earlier and to a greater degree than in peripheral blood leucocytes. A month after cessation of exposure, chromosome aberrations were still present although blood cell counts had returned to normal. When benzene and toluene were given together the observed chromosome damage was the sum of the effects of both compounds [19,20]. Dobrokhotov [19] estimated that 0.8 g/kg/day of toluene induced the same frequency of chromosome damage in rats as 0.2 g/kg/day of benzene.

In the animal experiments reported, exceedingly high concentrations of toluene were required to induce chromosome aberrations and, considering Forni's findings, it seems unlikely that industrial exposure within the current TLV limit (100 ppm) will cause detectable chromosome damage.

Xylene

A literature search has uncovered no reference to mutagenicity studies on xylene in any test system. When tested in a mixture with an emulsifier on reproductive capacity in rats, no reduction in litter size or survival of newborn rats was observed and no increase in foetal malformation was recorded [71].

Phenol

Demerec et al. [18] showed that phenol was mutagenic when applied to cultures of *E. coli*, B/Sd-4 and Hadorn and Niggli [34] demonstrated the mutagenicity of phenol in Drosophila after exposing isolated gonads in vitro and then implanting them into host larvae. Using this method, visible, recessive-

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lethal and lethal mutations were observed in imagines derived from treated larvae.

Levan and Tjio [41] observed C-mitosis in root tips of *Allium cepa* exposed to phenol but chromosome fragmentation was very rare.

No in vivo mutagenicity studies have been reported with phenol.

Cresols

In root-tip chromosomes in *Allium cepa* the cresol isomers were shown to induce C-mitosis but only marginal evidence of chromosome fragmentation [41]. The effect of cresols on *Allium cepa* was examined in greater detail by Sharma and Ghosh [80]. Many of the effects they described were at lethal concentrations, but at sublethal concentrations abnormal metaphases and anaphases were demonstrated in recovering root tip mitoses. Of the three isomers, *m*-cresol induced the most pronounced chromosome aberrations with anaphase bridges and fragments. The authors attributed this observation to the position of the hydroxyl group on the toluene ring and suggested that only when the hydroxyl group was in the *meta* position could chromosome breakage be induced. The isomers induced mitotic spindle effects such as C-mitosis and polyploidy to a similar degree, regardless of the position of the hydroxyl group.

Hydroquinone (quinol)

Quinol, tested in a logarithmic phase culture of *Micrococcus pyogenes* var. aureus, strain FDS 209 in nutrient broth, failed to induce forward mutation to penicillin- or streptomycin-resistance [15]. When tested as a constituent of tobacco smoke in a bacterial DNA-repair assay quinol inhibited growth of DNA polymerase-deficient *E. coli* suggesting an ability to induce repairable DNA damage in bacteria [7].

The compound failed to induce chromosome aberrations in root-tip meristems of *Vicia faba* [43], and did not affect the ploidy of nodal meristems of *Callisia fragrans* [70], but in root-tip mitoses of *Allium cepa* a dose-related increase in chromosome fragmentation has been described [42] as well as the induction of anaphase translocation bridges [41]. Fragmentation, micronuclei and bridges have been reported in mitotic divisions of branchlets of *Chara zeylanica* grown in the dark [14]. Sharma and Chatterjee [79] studied the influence of oxygen on aberrations induced in root chromosomes of germinated bulbs of *Allium sativum* and germinated seeds of *Vicia faba*, *Nigella sativa* and *Trigonella foenum-graecum* by solutions of quinol ranging from 0.001 M to 0.1 M. Experiments were replicated in normal atmospheres and atmospheres free of oxygen. Chromosome breakage was detected in all the plants treated in normal atmospheres and was either absent or much reduced in anaerobic conditions.

Parmentier and Dustin [62] have described a colchicine-like accumulation of metaphases in the small intestine of mice injected with 125 mg/kg quinone. This occurred within 2 h of the injection and was followed by pyknotic nuclei and chromosome disintegration.

Resorcinol

Resorcinol failed to induce mutations in *Salmonella typhimurium* TA 98,

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TA 100, TA 1535 or TA 1537 in the presence or absence of rat-liver microsomes [49], but has been shown to induce chromosome breakage in plant-cell metaphases or anaphases [41,14,68,3].

Catechol

Like most of the phenols, catechol induced both spindle-effects and anaphase fragments [41] as well as C-mitosis and a variety of metaphase aberrations [80] in chromosomes of *Allium cepa*. No other genetic studies have been recorded.

Pyrogallol

Pyrogallol produced repairable DNA damage when tested against polymerase-deficient and repair-competent *E. coli* [7], but failed to induce lambda-phage formation in *E. coli* X12 [36]. The effects of pyrogallol on plant chromosomes has been studied extensively inducing chromosome damage in *Allium cepa* [41,42], *Chara zeylanica* [14], *Faba vulgaris* [21] and *Tulbaghia violacea* [68]. Therman-Suomalainen [84] showed that chromosome breakage occurred almost exclusively at the secondary constrictions in preparations from *Podophyllum emodi*, *Pisum arvense* and *Vicia sativa* exposed to pyrogallol. Riley and Hoff [68] studied the effect of the compound on root tips of *Tulbaghia violacea* and detected a variety of aberrations in metaphase chromosomes which were not restricted to the secondary constrictions.

The only recorded study using mammalian chromosomes was reported by Mitra and Manna [53]. Chromosome breakage was studied in mouse bone-marrow cells collected 24 h after intraperitoneal injections of 0.25 ml of 0.01, 0.02 or 0.03 M solutions of pyrogallol. No increase in chromosome aberrations was detected at the lower dose but both 0.02 and 0.03 M solutions increased the incidence of chromatid breaks. The distribution of breaks between chromosomes and along chromatids was of a random nature.

Phloroglucinol

In addition to its plant-chromosome breaking activity [41,68] phloroglucinol has been shown to increase the incidence of mutation to viomycin-resistance in *Rhizobium trifolia* [11]. The authors consider that viomycin-sensitivity is controlled by a specific plasmid the activity of which phloroglucinol inhibits.

Nitrophenols

All three isomers gave negative results when tested for their ability to induce steptomycin-independence in streptomycin-dependent *E. coli* Sd-4-73 [83]. *p*-Nitrophenol also failed to induce mutations in Salmonella both with and without microsomal activation [49]. Adler et al. [1] used the difference in growth inhibition of wild-type *Proteus mirabilis* and the corresponding repair-deficient strain as an indication of DNA damage. *p*-Nitrophenol showed evidence of DNA damage. Propping et al. [66] described the results of in vitro and host-mediated assays in which *S. typhimurium* G46 and *Serratia marcescens* α21 were used as test organisms. *p*-Nitrophenol gave negative results in both cases.

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Low concentrations (0.005%) of *o*- and *m*- and *p*-nitrophenol inhibited mitosis in root tips of *Allium cepa* but only the *para*-isomer induced detectable chromosome fragmentations [80]. Amer and Ali [4] studied the effects of *o*- and *p*-nitrophenol on the lateral root mitoses of *Vicia faba* seedlings. At concentrations ranging from 0.025% to 0.1% the mitotic index was reduced. Anaphase bridges were induced by both isomers but, as reported by Levan and Tjio with *Allium cepa* [41], chromosome fragmentation was not detected.

Poirier et al. [64] used *p*-nitrophenol as a non-tumour promoting analogue of phenol in a study of the inhibition of DNA-repair synthesis induced by *N*-acetoxy-2-acetylaminofluorene in human diploid fibroblasts (W1-38). *p*-Nitrophenol completely inhibited both repair synthesis and normal DNA-replication synthesis at a concentration of 10^3 μM.

2,4,6-Trinitrophenol

Streptomycin-independent mutants were induced in streptomycin-requiring *E. coli* B/Sd-4 after pre-incubation in the presence of 0.18% trinitrophenol for 3 h before plating on streptomycin free agar [18]. In contrast, Auerbach and Robson [5] failed to demonstrate sex-linked lethals in Drosophila after bathing the eggs in aqueous solutions of 2,4,5-trinitrophenol.

α- and β-Naphthol

In experiments with *Allium cepa* both naphthols affected mitotic spindle function, but chromosome fragmentation was extremely rare [41]. Amer and Ali [4] found that the two isomers differed in their effects on root tip mitoses of *Vicia faba*. Both isomers induced chromosome lagging at anaphase, but anaphase bridges were found only after treatment with β-naphthol. α-Naphthol produced a colchicine-like accumulation of metaphases.

McCann et al. [49] tested α-naphthol in a series of Salmonella strains both with and without microsomal activation and obtained negative results.

Discussion and conclusions

During the research for this review it became apparent that there were a number of areas where genetic toxicology data were extremely sparse and the data that were available often presented conflicting evidence on the genetic effects of the compounds.

Most of the phenols are mitotic spindle poisons in plant tissues. Phenol is a microbial mutagen in *E. coli* Sd-4 while *p*-nitrophenol, α-naphthol and resorcinol were negative when tested in Salmonellae. A systematic study of the mutagenic effects of the phenols — particularly those that are benzene metabolites in man — might shed some light on the genetic hazard posed by these compounds and also on the influence of isomerism and different functional groups on mutagenicity. Considering their severe effects on plant chromosomes it is surprising that their clastogenic properties have not been studied in vivo.

In the past, both toluene and mixed xylenes have been recommended as "safe" alternatives to benzene in certain industries. Neither produce clinical hazard to the same degree as benzene. The limited evidence so far available

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suggests that the industrial use of toluene is unlikely to induce chromosome damage in exposed workers [25].

In spite of a vast research effort on the subject, the major problem area remains the association between benzene exposure, myelotoxicity, chromosome damage and leukaemia. That prolonged exposure to high concentrations of benzene results in myelotoxicity is beyond doubt but the great differences in individual susceptibility to this hazard remain unexplained. Similarly, there is convincing evidence for the production of both structural and numerical chromosome changes in experimental animals given large doses of benzene. The evidence for chromosome damage in symptom-free workers after exposure to benzene is less convincing but the interpretation of the results of such studies is complicated by the lack of accurate measurements of benzene exposure. Perhaps a well-designed, adequately controlled comparison between a working population with a known benzene exposure and a matched population without any evidence of benzene exposure might provide the answer. It is tempting to suggest that, with recent improvements in industrial hygiene, working populations are no longer exposed to high enough concentrations to provide positive material for such a study.

Extensive chromosome changes have been detected in patients suffering from various facets of benzene-induced myelotoxicity but the precise relationship between the haemopathies and chromosome damage has yet to be resolved.

Almost all cases of "benzene leukaemia" were extensively exposed to other chemicals though benzene was common to them all. It appears likely that other environmental factors in addition to benzene exposure are involved in the development of leukaemia and a co-leukaemic role for benzene would explain the failure to reproduce benzene leukaemia in experimental animals.

Valuable information might be gained from studies of the macromolecular interactions of benzene. Does benzene or one of its metabolites bind to mammalian DNA or RNA, and if so, is the nature of the binding in haemopoietic cells different from that with liver macromolecules? If no DNA binding can be demonstrated, is a mitotic spindle lesion responsible for the structural and numerical chromosome changes (considering the effect of phenols in plant mitoses)? It seems likely that the metabolite responsible for the myelotoxicity is generated in the liver as Mitchel [52] demonstrated that the severity of the anaemia was greatly reduced in partially (75%) hepatectomised rats. If this metabolite is an arene oxide why has this never been identified in vivo? Presumably Mitchel's findings indicate transportation of the active metabolite to the haemopoietic stem cells. Benzene has been shown to reduce DNA synthesis; it has been suggested that it might inhibit DNA repair [54]; it would be useful to know if benzene induces DNA strand-breakage or DNA repair in vivo.

Although this paper began as a review of the genetic toxicology of benzene and the phenols, and of toluene and the xylenes, it appears to have posed more questions than it has succeeded in answering and to have identified some of the unresolved problems in this very important group of chemicals.

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