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Genotoxicity of Benzene and Soluble Benzene Substituted Organic Compounds in Mammals - A Review

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ABSTRACT

Benzene, a common industrial solvent and a component of gasoline, is known to be highly toxic due to its carcinogenic activity. Benzene as such is an insoluble liquid which undergoes several mechanisms inside a living cell to introduce functional group to the benzene ring to make it more soluble. In this process several benzene metabolites are formed in vivo which in turn becomes genotoxic. It should be noted that even the secondary metabolite of benzene becomes genotoxic inside the cell. The term genotoxic refers to the adverse effect that happens in the cell that damages genetic information and finally leading to cell damage. This is initiated by benzene and its metabolites directly reacting with DNA and, benzene and its metabolites reacting with by products of the cell during a mechanism pathway. Apart from benzene, there are several benzene substituted organic compounds that are used in many industries including pharmaceutical industries and human beings are daily exposed it. Genotoxicity of 19 commonly used soluble benzene substituted organic compounds are also discussed.

Key words: Benzene, DNA Damage, Genotoxicity, Mammals, Soluble benzene substituted organic compounds, Toxicity.

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INTRODUCTION

Benzene, a common industrial chemical and component of gasoline, as such has been reported to be highly toxic and many laboratories and industries have replaced benzene with other organic solvents. The occupational exposure limit in the United Kingdom (UK) and the United States (US) was 10 ppm based on the association of benzene exposure with anemia. Recently, it was lowered to 5ppm and 1ppm respectively as a reflection for the risk of neoplasia (the process of tumor formation). The American Conference of Governmental Industrial Hygienists (ACGIH) have considered benzene as A1 carcinogen and decreased the threshold limit value (TLV) to be less than 0.1ppm [1].

Benzene structure represents a six carbon ring with three double bonds, represented by hexagon, proposed by Friedrich August Kekule in 1872. Each carbon atom ($1s^2 2s^2 2p_x^1 2p_y^1$) has to join with one hydrogen atom ($1s^1$) and two carbon atoms. In such process, it does not have enough unpaired electrons to form the required number of bonds and hence it promotes one of the $2s^2$ pair into the empty $2p_z$ orbital forming sp^2 hybrids. This sp^2 hybrid forms sigma bonds with two other carbon and one hydrogen atom. The p electron of each carbon atom is overlapping with those on both sides of it. This extensive sideways overlap produces a system of pi bonds which are spread out over the whole carbon ring and termed to be 'delocalised'. The 6 delocalised electrons go in three molecular orbitals- two in each forming a stable molecule [2].

Now, having understood the structural stability of benzene, it is important to understand the reason behind benzene toxicity in living organisms. Any living organisms eliminate an uptaken chemical compound by breaking down into soluble compounds. In human body, this happens in the liver by introducing functional groups on to the benzene ring to make it more soluble. It is for the same reason; benzene is classified as a non-mutagen in the Ames test. The metabolism of benzene has been reported to yield glucuronide and sulphate conjugates of phenol, quinol, catecol, L-phenylmercapturic acid, mucoaldehyde and trans, trans-muconic acid by ring sucission [1]. In addition, the metabolic mechanisms of benzene involve the formation of phenol metabolites by peroxidase in bone marrow which again generates reactive quinols [3]. Quinol is oxidized to p-benzoquinone, which binds to vital cellular components or undergoes redox cycling to generate oxygen radicals [1, 4].

Further, there is a report on the relationship between the chemical constitution of organic compounds and their toxicity to insects [4]. It was shown that

- The substitution of certain radicals in the benzene ring affects toxicity
- The toxic action depends not only upon the radicals but also on the substituted numbers
- In certain cases toxicity depend on the substituted radical's relative position.

Quantitative structure-toxicity relationships (QSTR) was derived for an extensive series of halogenated benzene, anilines, phenols, nitrobenzenes, toluenes and other substituted benzenes. A combination of molar refractivity and nucleophilic susceptibility of one of the meta ring carbons was found to predict the toxicity [5]. Hence, it can be inferred that structurally, the benzene substituted aromatic compounds increases the toxicity within the living organism.

The mechanisms of benzene and its substituted derivatives undergo formation of metabolites which causes genotoxic effect on the living organisms. Lots of genotoxic effects of benzene and its substituted organic compounds in living organisms including insects, earthworms, aquatic organisms, bufferflies, mammals etc are available in the literature. This review focuses mainly on the genotoxic effects of few commonly used soluble benzene substituted organic compounds in mammals. Genotoxicity is defined as the property of a chemical agent that damages the genetic information, and can be potentially mutagenic and carcinogenic. The DNA damage includes single- and double- stand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations and structural and numerical aberration [6].

METABOLISM OF BENZENE

Figure 1 displays the metabolism of benzene [7] involving several key enzymes. Benzene oxide and oxepin are formed in the liver by cytochrome P450 2E1 (CYP2E1). The oxide is converted non-enzymatically to phenol, which in turn, may be metabolized by CYP2E1 to di- and tri-hydroxybenzenes. Myeloperoxidase (MPO) can convert the intermediates to highly reactive and toxic free radical semiquinones and quinones. Quinone oxidoreductase (NQO1) reduces benzoquinones to hydroquinone and catechol, resulting in detoxification. Glutathione-S-transferases (GSTM1, GSTT1) are also involved in detoxification, by converting the oxide to non-toxic S- phenylmercapturic acid. In other hand, benzene oxepin can be converted by means of alcohol and aldehyde dehydrogenases (ALDH) to the toxic metabolite trans, trans-muconaldehyde. The toxic process for benzene begins with the production of CYP2E1-mediated phenols in the liver. Subsequently, these benzene mono-, di-, and tri-hydroxy compounds are believed to move to the bone marrow where MPO converts the phenols to several quinones, which are the ultimate toxic agents. However, NQO1 present in the bone marrow as well as most other tissues of the body is protective in that this enzyme is able to convert quinone compounds to the less toxic hydroquinone. Most benzene metabolites are excreted in the urine within 48 hours after environmental exposure.

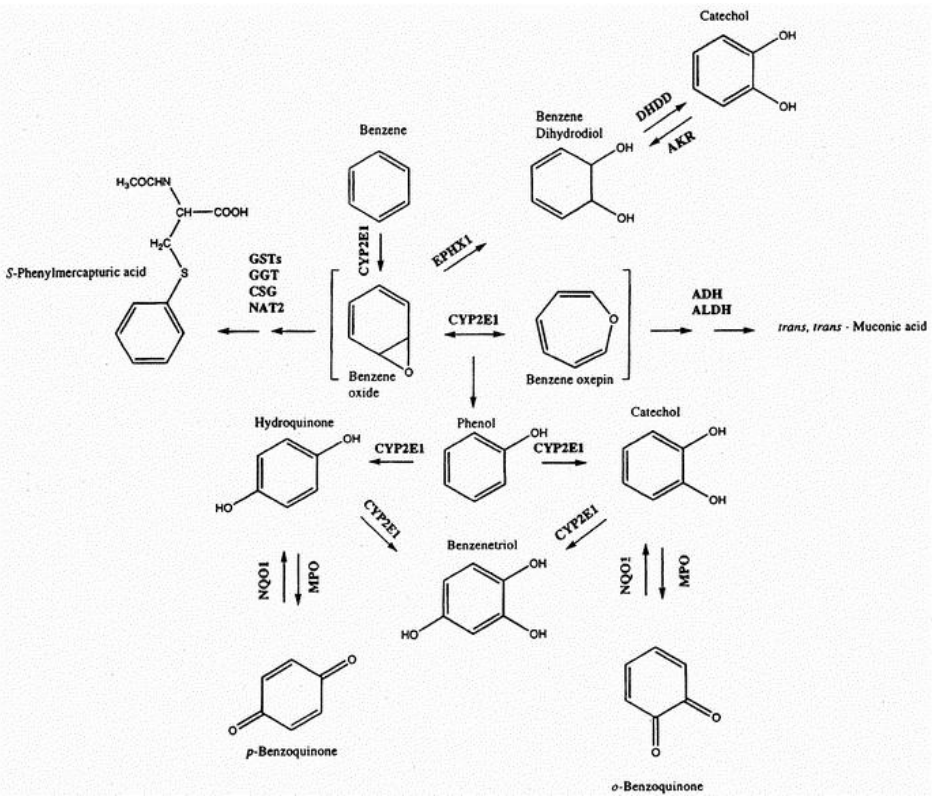

Fig 1: Metabolic pathway of benzene [7].

Note: γ-glutamyltransferase (GGT), cysteinylglycinase (CSG), N- acetyltransferase-2 (NAT2), microsomal epoxide hydrolase (EPHX1), dihydrodiol dehydrogenase dimeric form (DHDD), aldoketoreductases (AKR), alcohol dehydrogenases (ADH)

GENOTOXICITY OF BENZENE

Epidemiology

In an occupational exposure study of benzene involving 49 workers, chromosomal aberrations were significantly higher in the test group than the controls at the concentration of 3-68.7 mg/m3 in the work environment [8]. Similar results were observed in an exposure study involving female workers in shoe industry. An increased dicentric chromosome as a result of chromosomal aberration was observed in test groups of this study [9]. In another study, 33 men was found to posses oxidative DNA adduct (8-hydroxydeoxyguanosine) in their urine sample. Added to this, single-strand breaks were noticed in DNA of leukocytes on low level exposure to benzene (0.13 ppm) [10]. Although several exposure study reveals the chromosomal aberration over benzene exposure in human beings, the metabolite and mechanisms behind this is yet to be identified [11].

As mentioned earlier, benzene being insoluble forms benzene metabolites by introducing functional groups to the benzene ring. Benzene toxicity in human body happens in all the four processes involving ingestion, digestion, absorption-assimilation and elimination and the processes benzene toxicity is the result of:

- Benzene and its metabolites directly reacting with DNA
- Benzene reacting with the byproducts of the cell during a pathway

DNA Damage due to Benzene Metabolites

Benzene metabolites para-benzoquinone and hydroquinone was found to yield deoxycytidine and deoxyguanosine respectively along with single base substitution in GC pairs in DNA of treated plasmid in the human kidney cell line, Ad292 [12]. The single base substitutions were predominantly GC→TA transversions and GC→AT transitions found in fibroblast cells [13].

Genotoxicity due to benzene metabolites

Benzene was found to interact with the byproducts of the cells causing genotoxicity. A variety of inflammatory cells was known to produce peroxynitrite, the reaction product of nitric oxide and superoxide during immune activation. Benzene on exposure with peroxynitrite forms phenol, nitrophenols and nitrobenzene [14] which could lead to carcinogenesis. Phenol is converted to 4-4’-diphenoquinone using peroxidase in the presence of hydrogen peroxide. Similarly 1, 4-benzoquinone is produced as a result of hydroquinone reaction with peroxidase and hydrogen peroxide. These two compounds have been shown to damage DNA. Thus secondary metabolites of benzene also play a role in genotoxicity in humans and other living organisms [15]. Catechol is a major primary and secondary metabolite of benzene resulting in Chromosomal aberrations along with sister chromatid exchange and aneuploidy in mammalian cell culture [16]. The

metabolites phenol, catechol, and 1,4-benzoquinone were known to increase micronuclei formation in human lymphocytes [17].

BENZENE SUBSTITUTED ORGANIC COMPOUNDS

The following paragraph will discuss on the genotoxic effect of commonly used soluble benzene substituted organic compounds in mammals. Since the list considered for this review is high, main focus is done on the compounds' synthesis, uses and major genotoxic effect performed over mammals. Few negative genotoxicity effects of soluble benzene substituted organic compounds have also been considered in this review.

1. Aniline [IUPAC: Phenyl amine]

Aniline is a toxic organic compound, used as a base to make dyes, drugs, explosives, plastics, and photographic and rubber chemicals. The dye, chemical and rubber manufacturing industries were major sources of occupational exposure to aniline. Aniline was the key intermediate in the developing textile dye industry in Europe during the 1800s [18]. Aniline was not carcinogenic in experimental animals [19], but found to produce selective toxicity in spleen by release of free iron, however, the mechanism involved in the free iron release and Aniline-DNA interaction is not known [20]. It should also be noted that 4-aminobiphenyl, 2-naphthylamine and benzidine in aniline dyes were shown to be carcinogenic [19].

2. Toulene [IUPAC: Methyl benzene]

Toulene, mono substituted benzene derivative and also an aromatic hydrocarbon is used as industrial feed stock and as a solvent. Toulene was reported genotoxic in terrestrial environment due to greater extent of DNA damage it induces upon interaction [21]. Recent investigations have shown that toluene may induce male reproductive dysfunctions and carcinogenicity. It was found that 8-oxy-7,8-dihydroxy-2'-deoxyguanosine was formed in testes of the male rats induced by toluene via DNA damage induced by toluene metabolites [22].

3. Phenol [IUPAC: Phenol]

Phenol, an aromatic organic compound is both a manufactured chemical and natural substance. Phenol, although one of the compounds used during the extraction and purification of DNA from the living cell [23], it is known to interaction with DNA in vivo and cause DNA damage. During the extraction process, phonol can oxidize the nucleobases, especially guanine forming 8-hydroxyguanine (8-OHGua). 8-OHGua has been reported to be a key biomarker relevant to carcinogenesis and cellular oxidative stress, important in tumor promotion.

4. Anisole [IUPAC: Methoxybenzene]

Anisole is a colourless liquid, used as precursor in perfumes, insect pheromone and pharmaceutical industries [24]. Anisole is relatively non-toxic with lethal dose value of 3700

mg/kg in rats. There is no report on the purine reaction with anisole till date, however, anisole derivatives have been found to react with purine derivatives. On the other hand, nitrite-butylated hydroxylanisole (BHA) was tested by Natake et al [25], for its DNA-damaging and mutagenic activity. The active DNA damaging product in nitrate-BHA system was determined to be 2-tert-butyl-quinone which gave positive rec-assay test (- a test performed for the detection of DNA damaging agents [26] and negative Ames test (- a test performed to assess the mutagenic potential of a chemical compound [27].

5. Bromobenzene [IUPAC: Bromobenzene]

Bromobenzene is formed by electrophilic substitution of bromine with benzene. Bromobenzene is considered to be a toxic substance as it causes liver, kidney and nervous system damage**.** The distribution of bromobenzene following intraperitoneal injection bromobenzene was studied in male Sprague-Dawley rats. Significant levels of bromobenzene was found in fat, liver, kidney, brain, heart, lung, stomach, blood plasma even after 24 hours of injection [28]. 8-hydroxy-2'-deoxyguanosine and double strand breakage was noticed in rat liver slices after its interaction with bromobenzene [29] ultimately leading to genotoxicity. An in vitro model using a suspension of rabbit renal proximal tubules showed nephrotoxicity of bromobenzene due to its metabolite, 2-bromohydroquinone [30].

6. Chlorobenzene [IUPAC: Chlorobenzene]

Chlorobenzene is a synthetic aromatic organic compound formed by chlorination of benzene. This chemical compound is used as an intermediate in the production of commodities such as herbicides, dyes and rubber. QSAR for non-specific toxicity of chlorobenzene has been reported in air/water bound organisms [31] and also in soil bound organisms, specifically earthworms [32]. The toxic effects of chlorobenzene over mammals have not been reported. However, the physiochemical properties of chlorobenzene that elicit toxic response have been evaluated. It was found that the chlorinated compounds posses greater toxicity. Furthermore, it has been showed that the more symmetrical the compound, greater the toxicity [33].

7. Nitrobenzene [IUPAC: Nitrobenzene]

Nitrobenzene is formed as a result of nitration process which involves a formation of nitronium ion (NO_2^+), followed by an electrophilic substitution reaction with benzene. In industries, it is used in the production of aniline, which is a precursor in the production of dyes, drugs, explosives, plastics, and photographic and rubber chemicals. Raleigh et al., have reported biological inactivation due to nitrobenzene radiosensitizers. They found that the 5'-purine nucleotides were sensitized whereas the 3'-purine and –pyramydine nucleotides were not and this increases with the increase in nitrobenzene electron affinity. This could be directly related to the change in cellular molecules ultimately leading to biological inactivity [34]. Nitrobenzene exposure was also reported to induce hydroxylation of thymine nucleobase [35].

Nitrobenzene exposure in man or experimental animals is most often associated with methemoglobinemia. Histopathologic changes also are observed in the hemato-lymphoreticular system, central nervous system, and liver. In addition, lesions have been reported in adrenals and

testes [36]. Oral ingestion of nitrobenzene was found to be fatal in a rare self-poisoning clinical case due to methemoglobinemia. 48 hours after the ingestion, the nitrobenzene concentration in the blood was found to be 3.2 µg/ml [37] Methemoglobinemia is a condition in which the iron within the haemoglobin is oxidized from the ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state. This result in the inability to transport oxygen and causes a brownish discolouration of the blood. Nitrosobenzene, a nitrobenzene metabolite have been reported to induce NADH plus Cu(II)-mediated DNA cleavage frequently at thymine and cytosine residues resulting in carcinogenicity and reproductive toxicity [38].

8. o-Chlorophenol [IUPAC: 2-Chlorophenol]

o-Chlorophenol, a phenol derivative, formed by electrophilic halogination of phenol with chlorine, commonly used as a disinfectant. Being a disinfectant, o-Chlorophenol is one of the major pollutants found in the environment. In a comparative toxicity test among monochlorophenol, dichlorophenols and pentachlorophenols, o- and p- chlorophenol were reported to be more toxic than dichlorophenol and pentachlortophenols in mice [39]. Although o-chlorophenol is considered to be a toxic pollutant, there is no report on the effect of o-chlorophenol in human beings. However, it has been suggested [40] that higher chlorinated phenols interfere with the oxidative phosphorylation, a metabolic pathway that uses the energy released by the oxidation of nutrients to produce adenosine triphosphate inside the mitochondria of a cell. Since o-chlorophenol is known interact with DNA forming its adducts, nucleic acid sensor based on polyaniline (PANI) have been developed for insecticide detection [41].

9. p-Cresol [IUPAC: 4-Methylphenol]

Cresol is a natural (from coal tar) as well as a synthetic compound (methylation of phenol or hydrolysis of chlorotoulene) has a methyl group substituted onto the ring of phenol. Cresols are used as precursors or synthetic intermediates in industries involved in the manufacture of plastics, pesticides, pharmaceuticals and dyes. Short term and long term exposure to cresol have been proven to be harmful in living organisms. Cresol, being lipophilic strongly binds to plasma protein. Out of the three cresol isomers (o-, m- and p- cresol), p-cresol was found to be more toxic by rapidly depleting glutathione levels, an antioxidant found inside a cell [42]. p-cresol was reported to form monogluthatione conjugates with structure resembling the quinone methide intermediates in rat liver slices [43]. p-cresol is metabolized through conjugation (sulphation and glucuronization) but unconjugated p-cresol have been found to be retained when the kidneys fail [44]. The conjugated p-cresol have been found to inhibit epithelial cell proliferation and tube formation leading to cardiovascular disease (CVD) [45]. This is a reason why the patients with chronic kidney disease (CKD) potentially develop CVD in a course of time.

10. Salicylic acid [IUPAC: 2-hydroxybenzoic acid]

Salicylic acid is type of phenolic acid and beta hydroxyl acid, is available naturally in plants performing in the actions involved in development, photosynthesis, transpiration, ion uptake and transport. This mono-hydroxybenzoic acid compound is known for its medical properties due to it anti-inflammatory properties and hence used to ease aches and pains, and reduce fever. Salicylic acid was found to protect oxidative damage in plants [46] in a short term exposure.

However on a long term effect study with carrot, it was not as effective in alleviating the oxidative stress as determined with the short-term effects in the literature. Oxidative stress refers to the imbalance created between the reactive oxygen species biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [47]. Salicylic acid is known to reduce signs of aging in the skin and is widely used in cosmetic. In a short term study on salicylic acid (2%) skin toxicity, it did not show any harmful effects over the skin [48]. However, its long term effect on the skin is not known. A study on the role of salicylic acid in fatal intoxications with special reference to accidental poisonings was performed. There exists a significant correlation between salicylic acid intoxication and the presence of an infection in the respiratory tract in the reported deaths in the period between 1980 and 1989 [49]. It should be noted that the salicylic acid present in tropical preparation cannot be fatal as the concentration used is not more than 5%.

11. Resorcinol [IUPAC: benzene-1,3-diol]

Resorcinol is 1, 3- isomer of benzenediol is formed naturally as well as synthetically. It is produced naturally by the distillation of Brazil wood extract, an extract from a species of Brazil timer wood. Synthetically, it is formed by melting 3-iodophenol, phenol-3-sulfonic acid or benzene-1,3- disulfonic acid with potassium carbonate: by the action of nitrous acid on 3-aminophenol or on 1,3-diaminobenzene. Resorcinol is used as an antiseptic and disinfectant for tropical applications. Pharmaceutical industries use this compound as chemical intermediate for the synthesis of many compounds with pharmaceutical applications. It is also used in the production of diazo dyes, plasticizers and as UV absorber in resins. In another study, pealing paste containing containing 40% of resorcinol was found to exhibit dizziness, pallor, cold sweat, tremors, collapse and violet-black urine upon application [50]. To correlate the adverse effect of resorcinol, a study was performed over rats where 0, 120, 360, 1000 and 3000 mg/L of resorcinol was delivered via drinking water. The orally administered resorcinol got readily absorbed in the gastrointestinal tract [51]. Decreased thyroid gland follicular colloid content was observed in male rats for 3000 mg/L of resorcinol intake. However, this decreased level was not considered adversed in the study and concluded as no-observed-adverse-effect level (NOAEL) for 3000mg/L resorcinol uptake and no-observed-effect level (NOEL) for $\leq$ 1000 mg/L. Added to this, no offspring toxicity was observed in the second generation [52]. Accidental resorcinol intake of a 30 weeks pregnant woman was reported to have the medical conditions such as unconsciousness, drowsiness, respiratory failure, tonic-clonic seizures and hypothermia, and the biochemical findings were leucocytosis, high bilirubin levels, severe metabolic acidosis and green-coloured urine, finally death of fetus [53]. On the other hand, multiple daily application of resorcinol in topical applications over months may result in inflamed and lesioned human skin and finally may result thyroid gland toxicity in humans [54], but not on rats tested with hair dyes containing resorcinol [55].

12. m-Nitrobenzoic acid [IUPAC: 3-Nitrobenzoic acid]

m-Nitrobenzoic acid is a synthetic compound prepared by nitration of benzoic acid. It is used as a precursor to 3-aminobenzoic acid, which is used in dye industries [56]. The toxicity effects of m-Nitrobenzoic acid is very less in the literature. The known fact is that m-Nitrobenzoic acid was found to give a more positive result in rec assay [57].

13. o-Toluidine [IUPAC: 2-methyl,1-amino benzene]

Toluidine with the chemical formula CH3C6H4NH2 have its chemical structure similar to aniline except the methyl group substituted onto the benzene ring. Unlike structure, its chemical properties also resemble aniline and are commonly used in dye industries. o-Toluidine exposure was found to have long term toxicity such as carcinogenicity [58]. 4-Amino-3-methylphenol, a mojor metabolite of o-toulidine was found to cause DNA damage in the presence of Cu(II) with thymine and cytosine residues being predominant cleavage sites. Other minor metabolite include o-Nitrosotoluene does not induce damage in the presence of metal but the addition of NADH was found to cause DNA damage very effectively. Simultaneously, o-Toluidine metabolites increased 8-oxo-7-, 8-dihydro-2'deoxyguanosine formation in calf thymus DNA in the presence of copper ions resulting in the expression of carcinogenicity [59].

14. Hydroquinone [IUPAC: Benzene-1,4-diol]

Hydroquinone, a symmetrical aromatic compound is a type of phenol with chemical formula $C_6H_4(OH)_2$. Hydroquinone is produced industrially by dialkylation of benzene with propene to 1,4-diisopropylbenzene which reacts with air and rearranges to give acetone and hydroquinone in acid and hydroxylation of phenol. In addition it is a major metabolite of benzene. This compound was used medially in tropical applicant for skin depigmentation. As mentioned earlier, hydroquinone, being symmetrical, it is highly toxic [33]. A 30 year old woman who had been using hydroquinone based skin bleaching creams for four years was reported to have the following medical conditions: 80/40 mm Hg supine with un-recordable diastolic pressure on standing, loss of deep tendon reflex and impairment of deep sensation in the lower limbs leading to diagnosis of peripheral neuropathy with autonomic neuropathy [60]. On the other hand, hydroquinone and catechol (major metabolites of benzene) was found to produce a genotoxic response in cultured human lymphocytes by increasing micro-nucleated cells over background. Further it was suggested that hydroquinone and catechol act together to disrupt the mitotic spindle and interfere with chromosome segregation inducing leukemia in humans [61]. Unlike o-Toluidine the presence of hydroquinone and copper ions was found to have an impact on DNA by inducing both the single and double strand breaks [62] and formation of 8-hydroxydeoxyguanosine resulting in genetic disorders and myelotoxicity [63].

15. Vanillin [IUPAC: 4-Hydroxy-3-methoxybenzaldehyde]

Vanillin is a phenolic aldehyde, having aldehyde, hydroxyl and ether functional groups is a natural as well as synthetic product. Natural vanillin is extracted from vanillin bean. Vanillin is majorly used as a flavoring agent in food and medicines and in rare cases used as an intermediate in the production of fine chemicals. Exposure to the vanillin has proven to have a positive effect by protecting DNA from oxidative attack [64]. There was a concentration dependant inhibition of the disappearance of super-coiled form of plasmid pBR322 upon exposure to gamma radiation. Presence of 0.5mM vanilline has dose modifying factor of 6.75 for 50% inactivation of super-coiled form in vitro. This could be due to scavenging of radicals generated during radiation apart from modulation of DNA repair. Similar results have been observed for human and mouse peripheral blood leucocytes and splenic lymphocytes [65].

16. Dinitrotoluen [IUPAC: 1-methyl-2,4-dinitro benzene]

Dinitrotoluene is formed by dinitration of toluene. Dinitrotoluene is used in the production of toluene diisocyanate in polymer industrie and in explosive industries for the production of trinitrotoluene. Unlike nitrobenzene, dinitrotoluene was found to induce methemoglobin in blood but however not proven to be fatal [66]. There was a concentration dependent increase in the induction of stress genes in recombinant cell lines generated from human liver carcinoma cells for 2,4- Dinitrotoluene. It was found that 2,4-DNT cause protein damage and/or perturbate protein biosynthesis there by altering DNA helical structure [67]. Ediberto et al., examined the potential of 2,4-DNT to damage DNA of primary rat hepatocytes and suggested that the damage of DNA to play a major role in carcinogenicity [68].

17. Thymol [IUPAC: 2-isopropyl-5-methylphenol]

Thymol is a natural compound extracted from *Thymus vulgaris* is used as preservative, anaesthetic and antiseptic. Unlike Vanillin, thymol significantly reduces the level of DNA damage induced in cell by hydrogen peroxide [69]. Thymol, a good scavengers of peroxyl radicals, was found to decrease peroxidation of phospholipid liposomes in the present of ferric ions and ascorbate [70].

18. Mesitylene [IUPAC: 1,3,5-trimethylbenzene]

Mesitylene is a symmetric derivative of benzene where three methyl substituents placed alternatively at the sides of the benzene ring. Mesitylene is prepared by equilibration of xylene or methyl alkylation over solid acid catalyst. Mesityle is used as a precursor to colorants, ligand in organometallic chemistry, develop photo-patternable silicons in electronics. Oral dose of mesitylene was administered to rats in order to understand its metabolism. The administered mesitylene was excreated as toxic 3,5-dimethylhippuric acid [71], 2,4,6- trimethylphenol [72] and traces of glucuronic and sulphuric conjugates [73]. In humans, upon mesitylene exposure, the urine sample was found to contain 3,5- dimethylbenzoic acid. Mesityle and its metabolites were found to retain in the lungs of the exposed study group [74]. . However, mesityle and its metabolites were considered toxic, its overall effect over mammals have to be explored.

19. Eugenol [IUPAC: 2-Methoxy-4-(2-propenyl)phenol]

Eugenol is an allyl chain-substituted guaiacol oily liquid with the molecular formula C10H12O2, naturally extracted from clove, nutmeg, cinnamon, basil and bay leaf. Due to its sweet aroma, eugenol is commonly used as an ingredient in perfumeries, flavorings and in medicine as antiseptic and anaesthetic. Eugenol is another benzene substituted compound which shows a negative genotoxic effect. A negative result was obtained for DNA damaging, mutagenic and chromosomal abbarational effect on exposure to eugenol [75].

CONCLUSION

Since the list of the compounds was huge, only the genotoxic effects on mammals have been reviewed. One of the common facts that can be noticed in all the genotoxic compound is that the major excretory organs namely lungs, kidneys and skin are most affected during metabolism. The reaction kinetics of the formation of benzene metabolites is not well understood. On the other hand out of 19 compounds considered for this review, vanillin, thymol and eugenol have been reported to protect DNA against damage. The mechanism behind this chemical structure towards DNA protecting and damaging properties also have to be identified in detail.

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