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Molecular Modelling Analysis of the Metabolism of Toluene

Fazlul Huq
School of Biomedical Sciences, Faculty of Health Sciences,
The University of Sydney, Australia

Abstract: Toluene has been widely used as an organic solvent, ingredient of thinners, as a coating in the leather industry and in the synthesis of a number of chemicals. It is a common cause of neurotoxicity in people that intentionally and repetitively breather high concentrations of toluene over a long period of time. Recent investigations have shown that toluene may induce reproductive dysfunctions and cancer. However, little is known about the molecular mechanisms by which toluene elicits its toxic effects on male reproductive organs and carcinogenicity. Following exposure in humans, toluene is readily transformed into several metabolites including benzyl alcohol, ortho-, meta- and para-cresols. The main metabolic pathway involves its oxidation to benzyl alcohol which is further oxidised to benzoic acid via benzaldehyde and excreted in the urine as hippuric acid. Ortho-, meta- and para-cresols are formed as minor metabolites through the formation of epoxides although there is evidence for direct hydroxylation of aromatic ring. Ortho-cresol is further hydroxylated to form MHQ which on oxidation produces MBQ. Molecular modelling analyses based on molecular mechanics, semi-empirical and DFT calculations show that the most toxic metabolite of toluene namely MBQ has the smallest LUMO-HOMO energy difference and hence it will be most reactive kinetically. The presence of electron-rich and electron-deficient sites indicates that the metabolite may undergo both electrophilic and nucleophilic attacks, the latter providing an explanation as to why it can cause oxidative damage to DNA.

Key words: Benzene, glutathione, toxicity, molecular modelling

Introduction

Toluene has been widely used as an organic solvent, ingredient of thinners, as a coating in the leather industry and in the synthesis of a number of chemicals (Fujji *et al*., 1999). It is also used as a blending agent in petrol to increase octane rating (Murata *et al*., 1999). Toluene being also a component of cigarette smoke, automobile exhausts and vapours from various commodities, humans may experience toluene exposure in the workplace and the living enviroments. Toluene is a common cause of neurotoxicity in people that intentionally and repetitively breathe high concentrations of toluene over a long period of time (Barnes, 1979). Due to its toxicity to the central nervous system, toluene exposure is regulated in many countries. An airborne concentration of 50 ppm as occupational exposure limit during the work shift is recommended (ACGIH, 2004). Recent investigations have shown that toluene may induce reproductive dysfunctions and cancer (Nakai *et al*., 1993). However, little is known about the molecular mechanism by which toluene elicits its toxic effects on male reproductive organs and carcinogenicity.

Following exposure in humans, toluene is readily transformed into several metabolites including benzyl alcohol, ortho-, meta- and para-cresols (Fustononi *et al*., 2005; Woiwode and Drysch, 1981). The main metabolic pathway involves its oxidation to benzyl alcohol which is catalysed by cytochrome P450 oxidase. Benzyl alcohol is oxidised to benzoic acid via benzaldehyde and excreted

in the urine as hippuric acid (N-benzylglycine) (Nakajima *et al*., 1993; Waxman and Walsh, 1983). Ortho-, meta- and para-cresols are formed as minor metabolites through the formation of epoxides although there is evidence for direct hydroxylation of the aromatic ring. Ortho-cresol is further hydroxylated to form methylhydroquinone (MHQ) (Bray, 1950) which on oxidation produces methylbenzoquinone (MBQ) (Nakashima and Kawarishi, 2003). MHQ and MBQ are both derivatives of hydroquinone (HQ) and 1,4-benzoquinone (BQ). HQ and BQ are metabolites of benzene which are well recognized human carcinogens (IARC, 1987). It has been reported that both HQ and BQ cause oxidative damage to DNA (Murata *et al*., 1999). Ortho-cresol itself is also known to have tumour promoting activity. As the minor metabolites of toluene have the ability to cause oxidative damage to DNA, it has been proposed that toluene may exhibit carcinogenicity and toxicity via oxidative DNA damage (Murata *et al*., 1999). Figure 1 gives a schematic representation for the formation of metabolites of toluene.

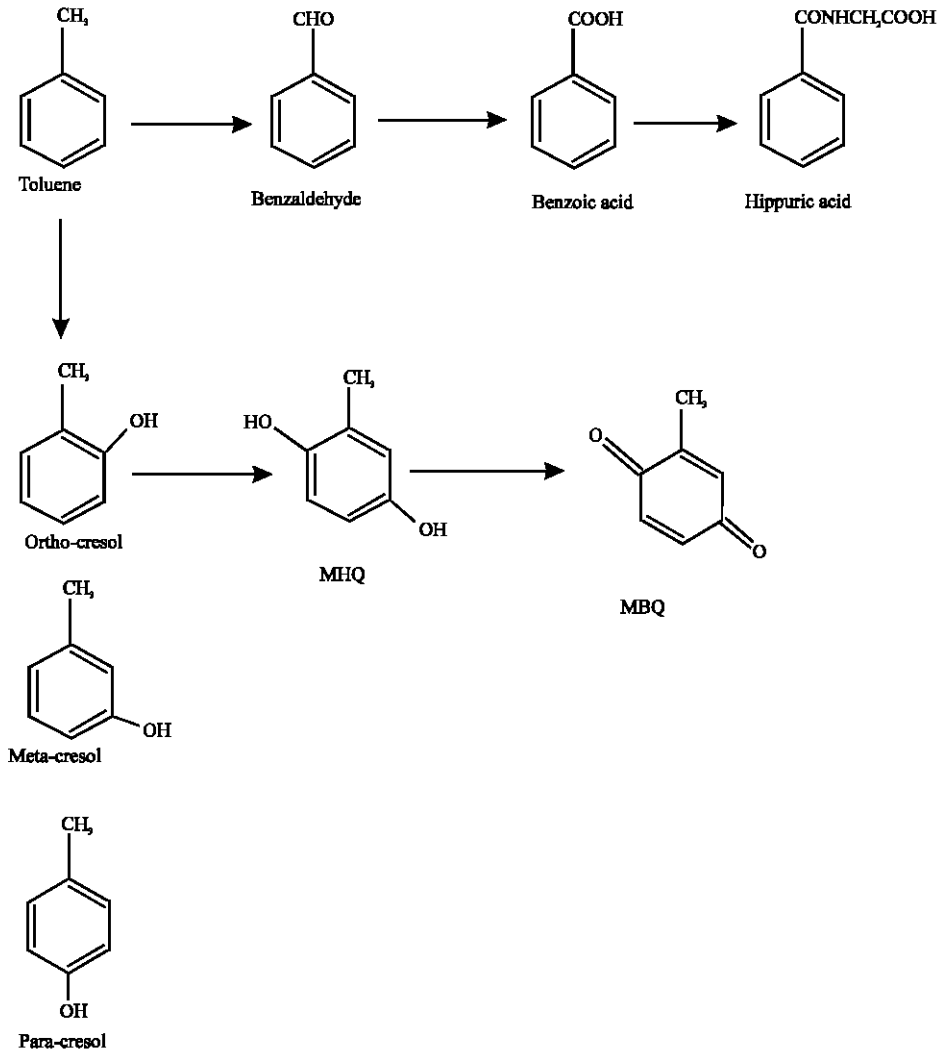


Fig. 1: Metabolic pathways of toluene (Adapted from Fujji *et al*., 1999)

In this study, molecular modelling analyses have been carried out using the program Spartan '02 (Spartan, 2002) to investigate the relative stability of toluene and its metabolites in order to obtain knowledge on the role of metabolic activation in the toxicity of toluene. The work was carried out in the School of Biomedical Sciences, The University of Sydney during the period October 2005 to February 2006.

Computational Methods

The geometries of toluene, benzyl alcohol, benzaldehyde, benzoic acid, hippuric acid, tolune-2,3-epoxide, ortho-cresol, meta-cresol, para-cresol, MHQ, MBQ, O_2 and H_2O have been optimised based on molecular mechanics (Fig. 1), semi-empirical and DFT calculations, using the program Spartan '02. Molecular mechanics calculations were carried out using MM+ force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried using the program Spartan '02 at B3LYP/6-31G* level. The order of calculations: molecular mechanics followed by semi-empirical followed by DFT minimized the chances of the structures being trapped in local minima rather reaching global minima. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. Some calculations were also carried out using the program HyperChem 7.0 to check reliability of the calculations. It was found that when the stated order was followed, structures corresponding to global minimum or close to that were reached in most cases. Although RMS gradient of 0.001 may not be sufficiently small for vibrational analysis, it is believed to be sufficiently low for calculations associated with electronic energy levels. For the optimised structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, surface area, volume, dipole moment, solvation energy, energies of HOMO and LUMO.

Results and Discussion

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, dipole moment, energies of HOMO and LUMO as per both PM3 and DFT calculations for toluene, benzyl alcohol, benzaldehyde, benzoic acid, hippuric acid, toluene-2,3-epoxide, ortho-cresol, meta-cresol, para-cresol, MHQ, MBQ, O_2 and H_2O. Figure 2-12 give the regions of negative electrostatic potential (greyish-white envelopes) in (a), HOMOs (where red indicates HOMOs with high electron density) in (b), LUMOs in (c) and surface charges (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimised structures of toluene, benzyl alcohol, benzaldehyde, benzoic acid, hippuric acid, toluene-2,3-epoxide, ortho-cresol, meta-cresol, para-cresol, MHQ and MBQ.

The solvation energies of toluene, benzyl alcohol, benzaldehyde, benzoic acid, hippuric acid, toluene-2,3-epoxide, ortho-cresol, meta-cresol, para-cresol, MHQ and MBQ, O_2 and H_2O from PM3 calculations in kcal mol^{-1} are respectively -2.66, -6.71, -4.43, -10.36, -15.85, -8.90, -5.38, -5.48, -5.46, -9.84, -4.95, -12.58 and -8.22 respectively. The metabolites benzoic acid, hippuric acid, MHQ and toluene-2,3-epoxide would be expected to be more soluble in water as they have greater solvation energy values. Similar conclusion can be drawn based on the values from DFT calculations.

Among the metabolites of toluene, MBQ is found to have the smallest LUMO-HOMO energy difference (3.84 eV from DFT calculations), indicating that the metabolite would be most reactive kinetically. This may explain why the compound is carcinogenic and can cause oxidative damage to DNA.

Table 1: Calculated thermodynamic and other parameters for toluene and its metabolites (DM stands for dipole moment)

Molecule	Calculation type	Total energy (kcal mol^{-1}/ atomic unit*)	ΔH_f (kcal mol^{-1})	Enthalpy (kcal mol^{-1})	Entropy (cal mol^{-1} K^{-1})	Solvation energy (kcal mol^{-1})
Toluene	PM3	18.81	21.47	129.18	140.12	-2.66
	DFT	-271.57		84.41	78.21	-0.79
Benzyl alcohol	PM3	-31.79	-25.08	87.30	82.25	-6.71
	DFT	-346.77		88.30	83.00	-4.99
Benzaldehyde	PM3	-15.07	-10.65	68.25	72.32	-4.43
	DFT	-345.58		73.09	79.49	-3.71
Benzoic acid	PM3	-74.98	-64.62	76.84	85.98	-10.36
	DFT	-420.82		77.03	86.42	-8.30
Hippuric acid	PM3	-115.62	-99.77	113.29	110.89	-15.85
	DFT	-628.83		115.17	106.05	-8.15
Toluene-2,3-epoxide	PM3	-79.51	88.40	72.21	81.50	-8.90
	DFT	-345.43		71.62	83.47	-9.25
Ortho-cresol	PM3	-35.65	-30.27	87.07	80.08	-5.38
	DFT	-346.79		87.82	81.88	-5.41
Meta-cresol	PM3	-36.49	-31.01	87.75	83.30	-5.48
	DFT	-346.79		87.76	84.16	-5.89
Para-cresol	PM3	-36.39	-30.93	87.78	82.93	-5.46
	DFT	-346.78		87.69	84.22	-5.81
MHQ	PM3	-85.11	-75.26	92.30	87.64	-9.84
	DFT	-422.01		90.99	87.91	-10.62
MBQ	PM3	-45.57	-40.63	76.42	88.24	-4.95
	DFT	-420.78		76.05	86.91	-3.89
O_2	PM3	-5.80	18.38	4.48	46.67	-12.58
	DFT					
H_2O	PM3	-61.64	-53.43	15.50	44.99	-8.22
	DFT	-76.42		15.06	45.14	-9.27

Molecule	Calculation type	Free energy (kcal mol^{-1})	DM (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
Toluene	PM3	87.41	0.62	-9.40	-0.37	9.03
	DFT	61.09	0.32	-6.40	0.15	6.55
Benzyl alcohol	PM3	62.78	1.61	-9.67	0.13	9.80
	DFT	63.56	1.53	-6.58	-0.10	6.48
Benzaldehyde	PM3	48.29	2.69	-10.05	-0.48	9.57
	DFT	49.39	3.30	-6.94	-1.71	5.23
Benzoic acid	PM3	51.21	4.62	-10.33	-0.53	9.80
	DFT	51.80	4.78	-7.21	-1.39	5.82
Hippuric acid	PM3	80.23	4.24	-10.39	-0.59	9.80
	DFT	83.55	2.67	-7.07	-1.50	5.57
Toluene-2,3-epoxide	PM3	47.91	2.02	-9.28	0.05	9.53
	DFT	46.73	2.02	-5.73	-0.08	5.65
Ortho-cresol	PM3	63.20	1.36	-9.06	0.28	9.34
	DFT	63.41	1.67	-5.83	0.16	5.99
Meta-cresol	PM3	62.92	0.93	-9.11	0.28	9.39
	DFT	62.66	1.06	-5.88	0.05	5.93
Para-cresol	PM3	63.06	1.19	-8.95	0.32	9.27
	DFT	62.58	1.32	-5.74	0.07	5.81
MHQ	PM3	66.18	0.31	-8.68	0.15	8.83
	DFT	64.78	0.36	-5.31	0.06	5.37
MBQ	PM3	50.11		-10.79	-1.65	9.14
	DFT	50.14	0.70	-7.23	-3.39	3.84
O_2	PM3	-9.44	0.00	-10.73	-0.98	9.75
	DFT	-8.75	0.00	-6.88	-4.88	2.00
H_2O	PM3	2.08	1.74	-12.32	4.06	16.38
	DFT	1.61	2.10	-7.92	1.70	9.62

* in atomic units from DFT calculations

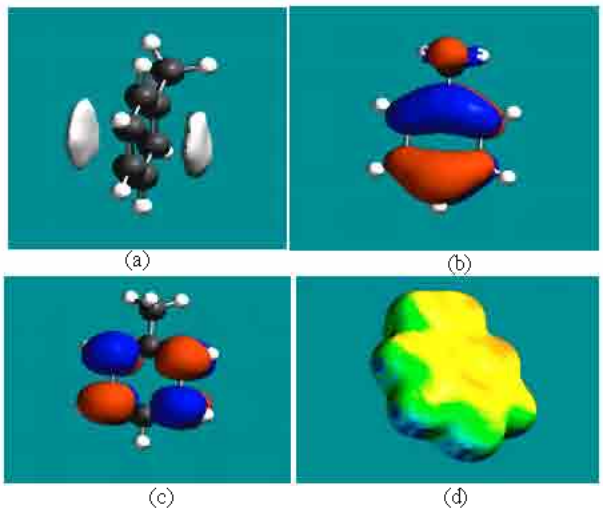


Fig 2 Structure of toluene giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

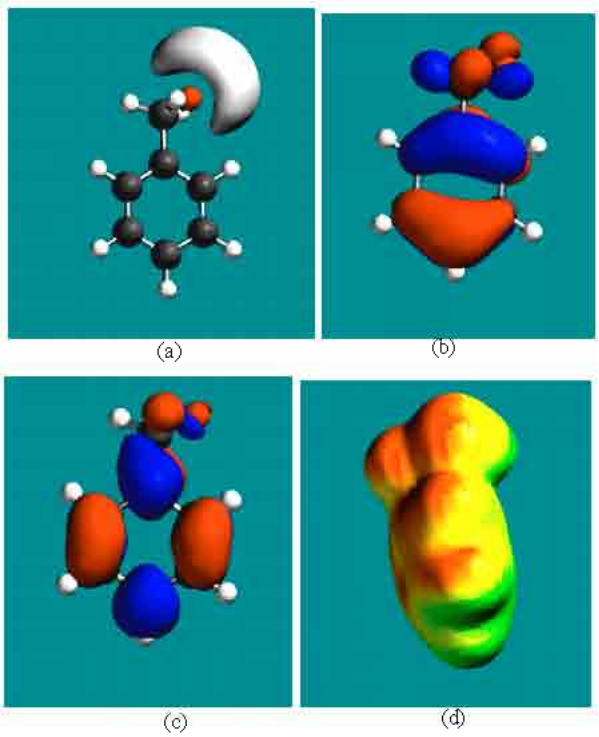


Fig 3 Structure of benzyl alcohol giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

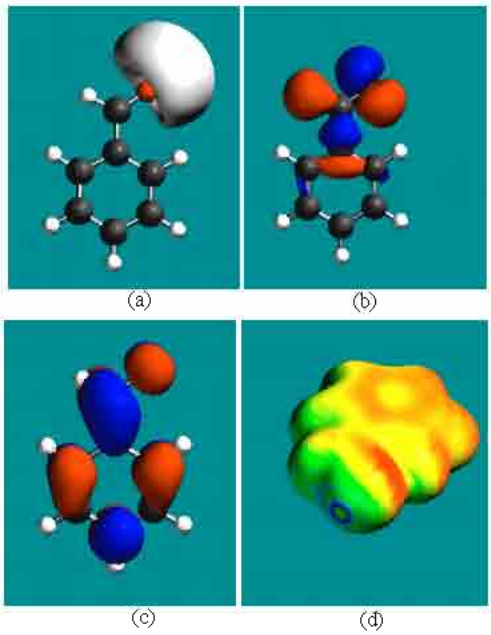


Fig 4 Structure of benzaldehyde giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

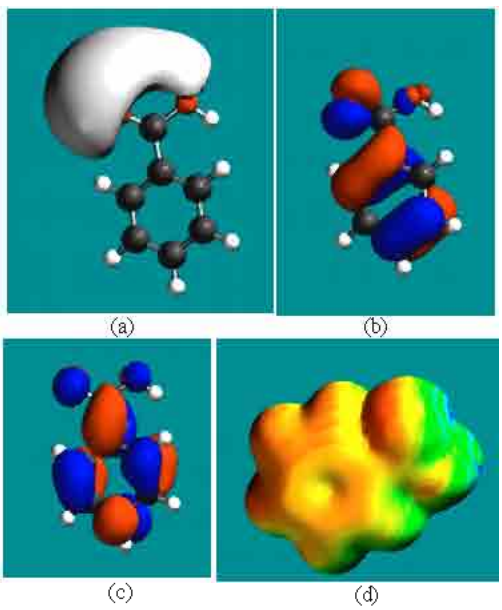


Fig 5 Structure of benzoic acid giving in (a) the electrostat ic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

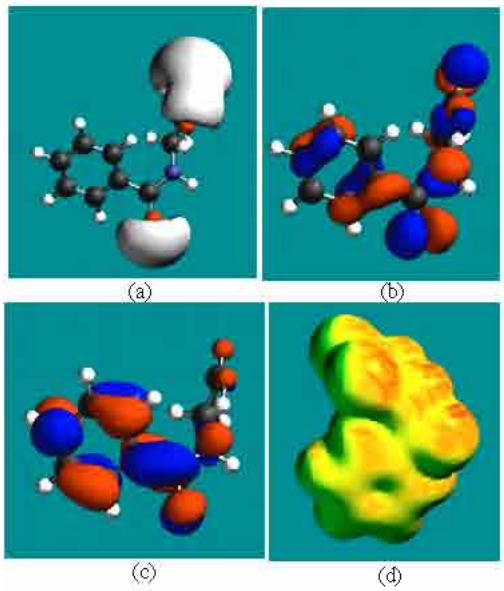


Fig 6 Structure of hippuric acid giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

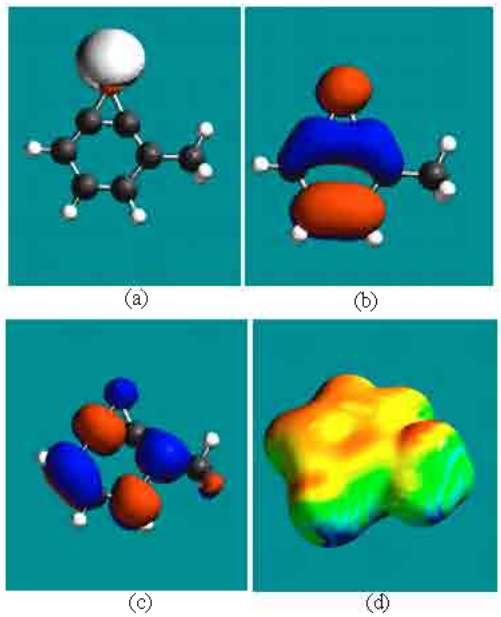


Fig 7 Structure of toluene-2,3-epoxide giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

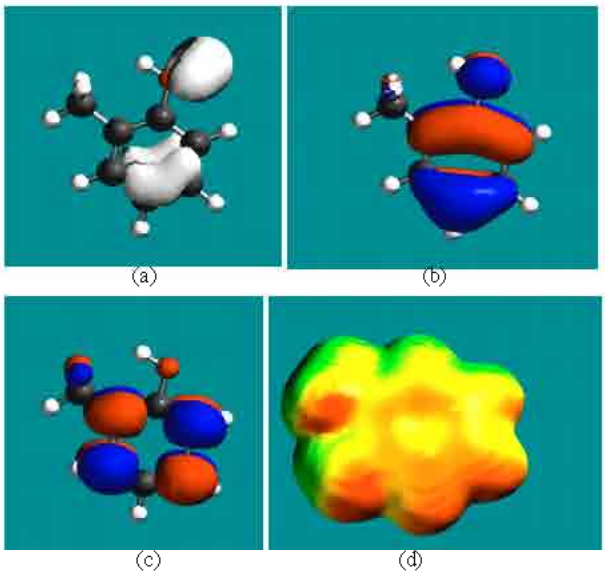


Fig 8 Structure of ortho-cresol giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

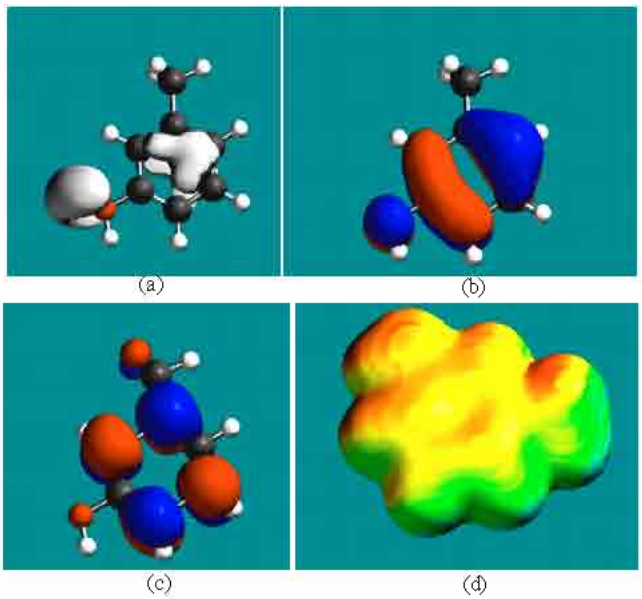


Fig 9 Structure of meta-cresol giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

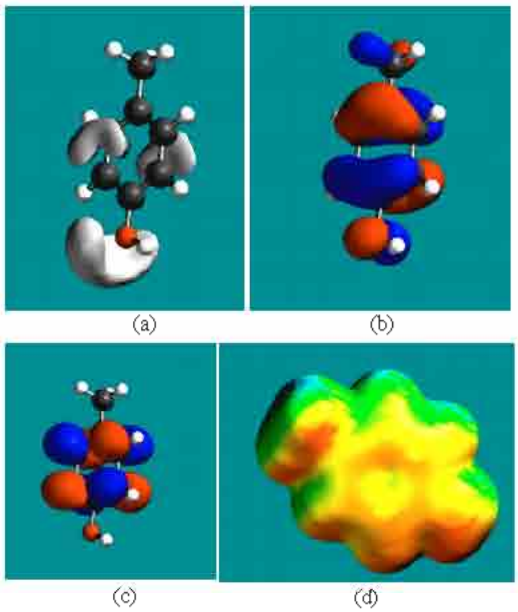


Fig 10 Structure of para-cresol giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

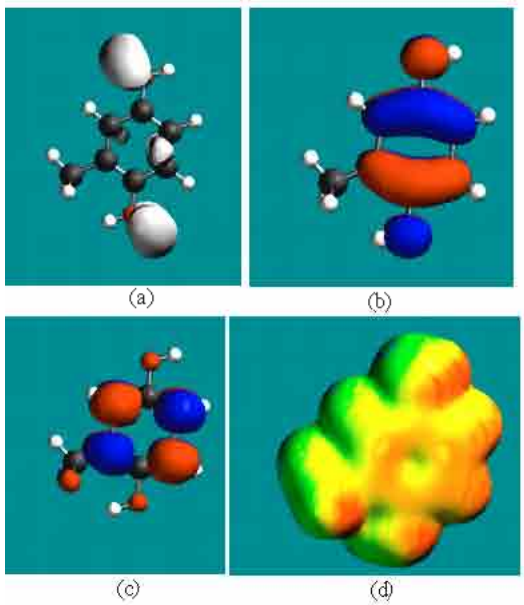
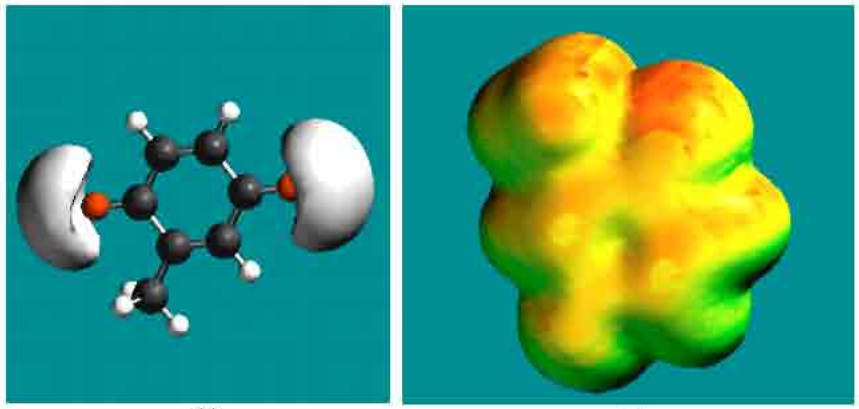


Fig 11 Structure of MHQ giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)



(a) (b)

Fig. 12: Structure of MBQ giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

After MBQ, benzaldehyde and MHQ have greater kinetic lability than other metabolites such as benzoic acid and ortho-cresol.

The reaction in which the major metabolite benzyl alcohol is formed from toluene can be written as:

$$C_6H_5CH_3 + 1/2O_2 \rightarrow C_6H_5CH_2OH \quad (1)$$

The Gibb's free energy change (ΔG) for the above reaction can be calculated according to the equation:

$$\Delta G = [\Delta G(\text{Benzyl alcohol})] - [\Delta G(\text{toluene}) + 1/2 \times \Delta G(O_2)].$$

Thus, based on DFT calculations,

$$\Delta G = 63.56 - [61.09 + 0.5 \times -8.75]$$
$$= 6.72 \text{ kcal mol}^{-1}$$

The positive value for ΔG indicates that the conversion of toluene to benzyl alcohol cannot be spontaneous. It should however be noted that reactions in biological systems are coupled such that the change in Gibb's free energy for the overall process is negative.

The reaction in which tolunene-2,3-epoxide is formed from toluene can be written as:

$$\text{Toluene} + 1/2O_2 \rightarrow \text{Toluene-2,3-epoxide} \quad (2)$$

The Gibb's free energy change (ΔG) for the above reaction can be calculated according to the equation:

$$\Delta G = [\Delta G(\text{toluene-2,3-epoxide})] - (\Delta G(\text{toluene}) + \tfrac{1}{2} \times \Delta G(O_2)]$$
$$= -9.99$$

The negative value for ΔG indicates that the conversion of toluene to toluene-2,3-epoxide would be spontaneous even though the heat of formation of toluene-2,3-epoxide (88.40 kcal mol^{-1}) is much greater than that for toluene (21.47 kcal mol^{-1}).

The conversion of benzaldehyde to benzoic acid can be written as:

$$C_6H_5CHO + 1/2O_2 \rightarrow C_6H_5COOH \quad (3)$$

Based on reaction (3) ΔG for the conversion of benzaldehyde to benzoic acid can be calculated to equal to -4.92 kcal mol^{-1}, indicating the conversion of benzaldehyde to benzoic acid would also be spontaneous. Table 2 gives the calculated free energy changes for the various steps in the metabolism of toluene.

The electrostatic potential is found to be more negative in the case of: toluene: above and below the phenyl ring, benzyl alcohol: around the hydroxyl oxygen atom, benzaldehyde: around carbonyl oxygen atom, benzoic acid and hippuric acid: around oxygen atoms of the carbonyl group and hydroxyl groups, toluene-2,3-epoxide: Around the epoxide oxygen atom, ortho-, meta- and para-cresols: around the oxygen atom of the hydroxyl group and above and below the phenyl ring, MHQ: around the two hydroxyl oxygen atoms and MBQ: around the two carbonyl oxygen atoms. The negative electrostatic potential indicates that the positions may be subject to electrophilic attack.

The HOMOs with high electron density are found in the case of: toluene-above or below all the non-hydrogen atoms, benzyl alcohol: above or below or close to all the non-hydrogen atoms, benzaldehyde-around the carbonyl group and first, second and sixth carbon atoms of the phenyl ring, benzoic acid-above or below or around all non-hydrogen atoms, hippuric acid-around or above or below nearly all non-hydrogen atoms, toluene-2,3-epoxide-above or below all non-hydrogen atoms except methyl carbon atom, ortho-, meta- and para-cresols-above or below essentially all non-hydrogen atoms, MHQ-above or below all non-hydrogen atoms except methyl carbon atom and MBQ-above or below or around all non-hydrogen atoms.

The LUMOs are found in the case of: toluene-below two ortho-and two meta-carbon atoms, benzyl alcohol, benzaldehyde, hippuric acid and toluene-2,3-epoxide-above or below or around essentially all non-hydrogen atoms, ortho-, meta- and para-cresols and MHQ- above or below all non-hydrogen atoms except hydroxyl oxygen atoms and MBQ-above or below or around all non-hydrogen atoms except methyl carbon atom. The LUMOs appear to be more localised (localised to single atoms) whereas the HOMOs with high electron appear to span two or more atoms except in the case of the most toxic metabolite MBQ where the situation is reversed (more about this will be considered later in the section). It may be noted that the greater delocalisation of HOMOs with high electron density correspond to the delocalisation of π-electrons. In the case of MBQ where the HOMOs with high electron density are found to be more localised, the π-electrons are also more localised.

As noted earlier, the most toxic metabolite MBQ has the smallest LUMO-HOMO energy difference. It was also noted that in the case of the metabolite, LUMOs are more delocalised than HOMOs with high electron density. The localisation of HOMOs with high electron density in the case of MBQ is more clearly shown in Fig. 13. It appears that the compound may be subject to both electrophilic and nucleophilic attack. The latter may explain why the compound can cause oxidative damage to DNA (more exactly to purine bases in DNA).

Table 2: Free energy changes associated with various steps in the metabolism of toluene

Metabolism step	ΔG (kcal mol^{-1})	
$C_6H_5CH_3 + 1/2O_2 \rightarrow C_6H_5CH_2OH$	6.72	Not spontaneous
Toluene + $1/2O_2 \rightarrow$ Toluene-2,3-epoxide	-9.99	Spontaneous
$C_6H_5CHO + 1/2O_2 \rightarrow C_6H_5COOH$	-19.07	Spontaneous

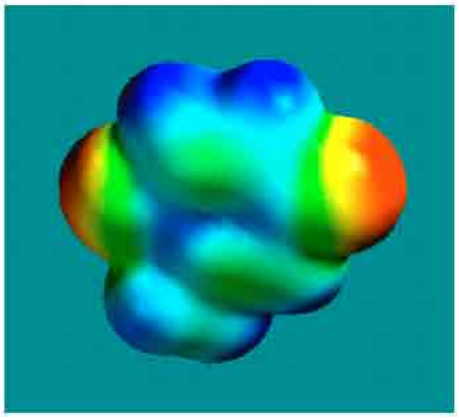


Fig. 13: Distribution of HOMOs with high electron density in MBQ whereas red indicates high electron density and blue indicates low electron density (that may correspond to LUMOs)

Conclusions

Molecular modelling analyses based on semi-empirical and DFT calculations give support to the idea that toxicity of toluene is associated with its metabolic activation. The analyses show that the metabolite MBQ has the lowest LUMO-HOMO energy difference and hence would be most reactive kinetically. MBQ is expected to undergo both electrophilic and nucleophilic attack, the latter providing explanation as to why it can cause oxidative damage to DNA.

Abbreviations

HQ:	Hydroquinone
BQ:	1,4-benzoquinone
LUMO:	Lowest energy unoccupied molecular orbital
HOMO:	Highest energy occupied molecular orbital
DFT:	Density functional theory

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