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Molecular Modelling Analysis of the Metabolism of Naphthalene

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Abstract: Naphthalene is a bicyclic aromatic hydrocarbon widely used as an intermediate in chemical and plastics industry and in the manufacture of insecticides and fungicides. It is metabolized by microsomal enzymes to naphthols and dihydrodiols via the formation of an epoxide which has a very short half-life. Reactive metabolites of naphthalene can deplete glutathione and in the absence of sufficient glutathione, get covalently bound to tissue macromolecules. In this study, molecular modelling analyses based on molecular mechanics, semi-empirical and DFT calculations have been carried out to provide information on the relative stability of naphthalene and its metabolites, deemed to be useful in the understanding of naphthalene induced toxicity. The analyses show that although naphthalene has a low thermodynamic stability, the larger LUMO-HOMO energy difference makes it less labile kinetically and hence less toxic than its more labile metabolites. Although naphthalene-1,2-epoxide has the lowest thermodynamic stability, the larger LUMO-HOMO energy difference makes the epoxide also labile. Much lower LUMO-HOMO energy differences make the naphthoquinones more reactive and hence more toxic.

Key words: Naphthalene, glutathione, toxicity, molecular modelling

Introduction

Naphthalene is an aromatic hydrocarbon consisting of two fused benzene rings. It is used mainly as an intermediate in the production of phthalic anhydride required for the manufacture of plasticizers, leather tanning agents and the insecticide carbaryl and is a constituent of creosote (Schreiner, 2003). It is also used as a moth repellent, an air freshener, a deodorizer for nappy pails and toilets. It is present in cigarette smoke as a pyrolysis product. In the past, naphthalene was used as an antiseptic and dusting powder in the treatment of skin diseases (IRIS, 1998). Naphthalene can be absorbed through skin, mouth and respiratory route. A number of health hazards are associated with excessive exposure of naphthalene, including haemolytic anaemia accompanied by jaundice, headache, confusion, nausea and vomiting, cataracts and toxicity to respiratory tract. Children and infants exposed to naphthalene vapour or coming to its dermal contact from clothing or bedding stored in mothballs may also develop neurological symptoms characterized by lethargy and decreased crying, possibly due to decreased oxygen-carrying capacity of blood. However, cessation of exposure usually allows recovery from symptoms and toxic effects.

Because of high degree of lipophilicity and lack of functioual groups, naphthalene needs to undergo oxidative metabolism as the first step in its clearance. However, as naphthalene undergoes oxidative metabolism, more reactive and more toxic compounds are formed. Thus, the toxicity of naphthalene is considered to be due to its metabolic activation. The first step in the mammalian metabolism of naphthalene is oxidation catalysed by cytochrome P450 monooxygenases, to highly reactive electrophilic arene epoxide intermediate, naphthalene-1,2-epoxide. It is believed to be the main reactive intermediate responsible for the toxicity of naphthalene. It has a very short half-life of 3.6 min (Bounarati *et al*., 1989) and spontaneously rearranges to form naphthols

(mainly 1-naphthol), leading to the formation of naphthalene diols and naphthoquinones. The covalent binding of reactive metabolites to critical cellular macromolecules is believed to be an important step in the development of toxicity of a number of hepatic, renal and pulmonary toxicants (Cohen and Khairallah, 1997; Hinston *et al*., 1994). The epoxide can be enzymatically conjugated with glutathione S-transferase to form a variety of glutathione conjugates such as N-acetylcysteine conjugate. Figure 1 summarizes the proposed pathways for naphthalene metabolism in mammals. In humans, the major stable metabolite is 1,2-dihydro-1,2-naphthalenediol whereas in mice, it is the cytotoxic 1-naphthol (Bolton *et al*., 2000).

Naphthalene toxicity has been studied most extensively in mice where the principal target cell population is the noncilliated epithelial cells (Clara cells) that line the intrapulmonary airways of the lung (Plopper *et al*., 1997). Although human studies assessing the carcinogenic potential of naphthalene are not available, it is believed that naphthalene can contribute to human cancer risk (Preuss and Angerer, 2004). As stated earlier, the covalent binding of reactive metabolites to critical cellular macromolecules is a critical step in the development of toxicity due to a number hepatic, renal

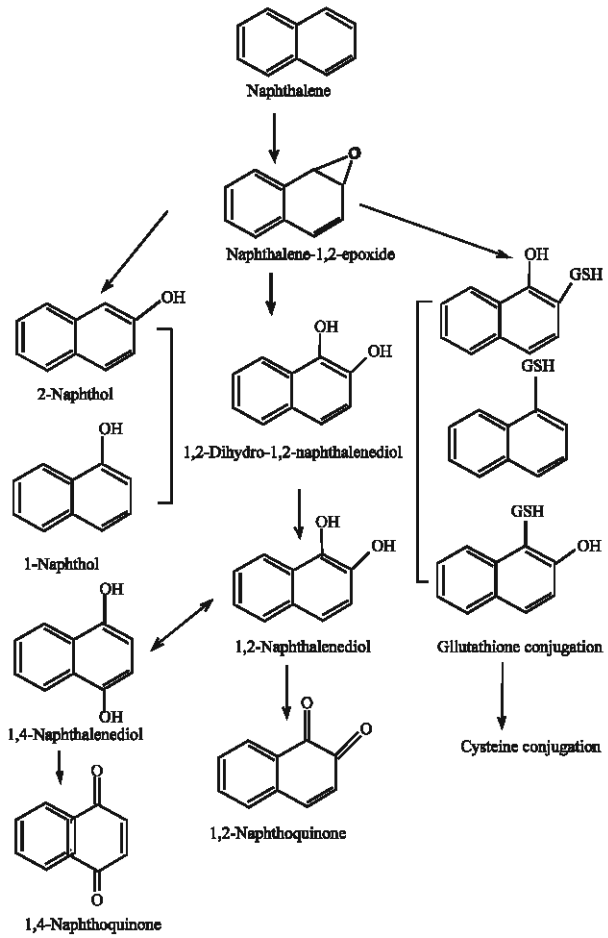


Fig. 1: Schematic representation of metabolism of naphthalene (Based on Agency for Toxic Substances and Disease Registry. Update toxicological profile for naphthalene. Final update. Atlanta, GA: U.S. Department of Health and Human Services, US Public Health Service (1995).) GSH: Glutathione-SH

and pulmonary toxicants (Cohen and Khairallah 1997; Hinston *et al*., 1994; Preuss and Angerer, 2004). However, what remains to be determined for many of these toxicants, are which macromecules they target and how the interaction with these targets causes cellular injury (Phimister *et al*., 2005). Loss of glutathione (GSH) is believed to be first step in a chain of events that lead to cytotoxicity for a number of metabolically activated toxicants (Warren *et al*., 1982). It is generally assumed that when the capacity of the cell to detoxify electrophilic metabolites is overwhelmed (due to exhaustion of GSH supplies), the level of adducts with proteins will increase, causing injury (Kyle and Faber, 1991). Epoxides have been shown to be cytotoxic and genotoxic to human mononuclear leucytes (Wilson and Kelly *et al*., 1995) because of their ability to undergo redox cycling and toxic oxygen species (Flescher and Snyder, 1995). Human lymphocytes are also sensitive to quinones (Buffinton *et al*., 1989) because of their ability to undergo redox cycling and form toxic oxygen species.

In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 (HyperChem, 2002) and Spartan '02 (Spartan, 2002) to investigate the relative stability of naphthalene and its metabolites in order to obtain knowledge on the role of metabolic activation in the toxicity of naphthalene.

Materials and Methods

The geometries of for naphthalene, naphthalene-1,2-epoxide, 1-naphthol, 2-naphthol, 1,4-naphthalenediol, 1,2-dihydro-1,2-naphthalenediol, 1,2-naphthalenediol, 1,2-naphthaquinone and naphthalene-2-glutathione conjugate have been optimised based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling programs Spartan, 2002 and HyperChem 7.0. Molecular mechanics calculations were carried out using MMFF 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. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimised structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO and LUMO. 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 than reaching global minima. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. 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 so 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, dipole moment and solvation energy, energies for HOMO and LUMO.

For the optimised structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment and solvation energy, HOMO and LUMO. The study was carried out in the School of Biomedical Sciences, The University of Sydney during the period July 2005 to February 2006.

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 naphthalene, naphthalene-1,2-epoxide, 1-naphthol, 2-naphthol, 1,4-naphthalenediol, 1,2-dihydro-1,2-naphthalenediol, 1,2-naphthalenediol, 1,2-naphthaquinone and 1,2-naphthalenediol-2-glutathione conjugate.

Table 1: Calculated thermodynamic and other parameters for aspirin and its metabolites

Molecule	Calculation type	Total energy (kcal mol^{-1}/ atomic unit*)	Heat of formation (kcal mol^{-1})	Enthalpy (kcal mol^{-1} K^{-1})	Entropy (cal mol^{-1} K^{-1})	Solvation energy (kcal mol^{-1} K^{-1})
Naphthalene	PM3	38.16	40.68	96.38	80.21	-2.52
	DFT	-385.90		97.02	79.20	-2.26
naphthalene-1,2-epoxide	PM3	-78.82	86.12	98.63	86.11	-7.30
	DFT	-460.98		99.43	86.19	-6.96
1-naphthol	PM3	-10.86	-4.12	100.91	87.52	-6.73
	DFT	-461.12		100.25	88.67	-6.56
2-naphthol	PM3	-11.58	-4.24	100.64	88.34	-7.34
	DFT	-461.12		100.21	87.76	-7.46
1,4-naphthalenediol	PM3	-57.56	-47.03	105.09	93.32	-10.54
	DFT	-536.34		103.63	93.78	-10.99
1,2-Dihydro-1,2-naphthalenediol	PM3	-59.18	-51.48	118.25	95.17	-8.70
	DFT	-537.50		118.89	94.58	-7.55
1,2-Naphthalenediol	PM3	-54.78	-45.95	104.67	94.93	-8.84
	DFT	-536.34		103.65	94.12	-8.57
1,2-Naphthoquinone	PM3	-27.89	-21.05	88.58	88.13	-6.84
	DFT	-535.11		88.81	92.31	-5.59
1,4-Naphthoquinone	PM3	-22.83	-22.83	89.29	92.14	-5.84
	DFT	-535.12		88.97	90.30	-4.88
1,2-naphthalenediol-2-glutathione conjugate	PM3	-245.80	-209.10	145.45	112.45	-36.71
	DFT	-1789.83		144.68	111.88	-34.45

Table 1: Continued

Molecule	Calculation type	Free energy (kcal mol^{-1})	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
Naphthalene	PM3	72.46	0.00	-8.84	-0.41	8.43
	DFT	73.41	0.00	-5.79	-0.96	4.83
naphthalene-1,2-epoxide	PM3	72.95	2.04	-9.26	-0.43	8.83
	DFT	73.73	2.52	-6.15	-1.20	4.95
1-naphthol	PM3	74.82	1.37	-8.59	-0.47	8.12
	DFT	73.82	1.41	-5.47	-0.94	4.53
2-naphthol	PM3	74.30	1.37	-8.65	-0.44	8.21
	DFT	74.05	1.59	-5.52	-0.92	4.60
1,4-naphthalenediol	PM3	77.27	0.54	-8.49	-0.43	8.11
	DFT	75.67	0.74	-5.12	-0.81	4.31
1,2-Dihydro-1,2-naphthalenediol	PM3	89.87	2.21	-9.33	-0.47	8.86
	DFT	90.69	2.46	-6.15	-1.16	4.99
1,2-Naphthalenediol	PM3	76.37	1.76	-8.37	-0.39	7.98
	DFT	75.59	2.51	-5.15	-0.75	4.40
1,2-Naphthoquinone	PM3	62.30	4.97	-9.77	-1.50	8.27
	DFT	61.29	6.07	-6.63	-3.19	3.44
1,4-Naphthoquinone	PM3	61.82	1.21	-10.29	-1.53	8.86
	DFT	62.04	1.37	-7.17	-3.17	4.00
1,2-naphthalenediol-2-glutathione conjugate	PM3	111.94	6.09	-8.87	-0.92	7.95
	DFT	111.34	6.67	-5.90	-1.37	4.53

* In atomic units from DFT calculations

Figures 2-10 give the optimised structures of naphthalene, naphthalene-1,2-epoxide, 1-naphthol, 2-naphthol, 1,4-naphthalenediol, 1,2-dihydro-1,2-naphthalenediol, 1,2-naphthalenediol, 1,2-naphthaquinone and 1,2-naphthalenediol-2-glutathione conjugate as per PM3 calculations using the program HyperChem 7.0. The structures also give 2D contours of total electrostatic potential. The solvation energies of naphthalene, naphthalene-1,2-epoxide, 1-naphthol, 2-naphthol, 1,4-naphthalenediol, 1,2-dihydro-1,2-naphthalenediol, 1,2-naphthalenediol, 1,2-naphthaquinone and 1,2-naphthalenediol-2-glutathione conjugate from PM3 calculations in kcal mol^{-1} are respectively -2.52, -7.30, -6.73, -7.34, -10.54, -8.70, -8.84, -6.84, -5.84 and -36.71, respectively. The corresponding values from DFT calculations in kcal mol^{-1} are, respectively -2.26, -6.96, -6.56, -7.46, -10.99, -7.55,

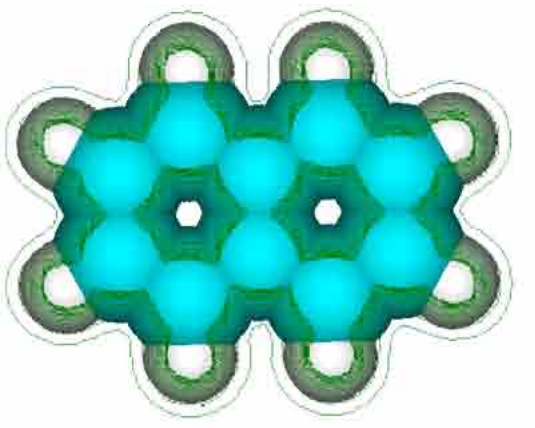


Fig. 2: Structure of naphthalene giving 2D contours of total electrostatic potential

-8.57 and -5.59, -4.88 and -34.45. (The metabolite that has the highest value for the solvation energy is 1,2-naphthalenediol-2-glutathione conjugate). The values indicate that the metabolites of naphthalene are more soluble in water than the parent compound and hence can be more easily excreted.

The other point to note is that all the metabolites of naphthalene listed in Table 1 have LUMO-HOMO energy differences similar to that of naphthalene (between 4.40 to 4.99 kcal mol^{-1} from DFT calculations), except the two naphthoquinones which have much lower values (1,2-naphthoquinone has the lowest value of 3.44 kcal mol^{-1} and 1,4-naphthoquinone has the next lower value of 4.00 kcal mol^{-1}). The values indicate that the ease of electronic excitation is much greater for naphthoquinones than the parent compound and other listed metabolites. This means that among the metabolites of naphthalene, naphthoquinones are kinetically most labile. The heats of formation of naphthoquinones are however lower those of naphthalene, naphthalene-1,2-epoxide , 1-naphthol and 2-naphthol, indicating that the naphthoquinones may be more stable thermodynamically than the parent compound and the metabolites naphthalene-1,2-epoxide , 1-naphthol and 2-naphthol. However, greater kinetic lability still means that naphthoquinones would react readily especially because biochemical reactions may be coupled such that the overall change in Gibb's free energy (ΔG) defined by the equation: $\Delta G = \Delta H - T\Delta S$ is negative.

When we compare the LUMO-HOMO energy differences and heats of formation of 1,2-naphthoquinone and 1,4-naphthoquinone, it is found that 1,2-naphthoquinone has smaller LUMO-HOMO energy difference (3.44 kcal mol^{-1} for 1,2-nahthoquinone and 4.00 kcal mol^{-1} for 1,4-naphthoquinone) and lower negative heat of formation (-21.05 kcal mol^{-1} for the former and-22.83 kcal mol^{-1} for the latter), indicating that 1,2-naphthoquinone is kinetically more labile and slightly less stable thermodynamically than 1,4-naphthoquinone.

The toxic metabolite naphthalene-1,2-epoxide has the largest positive of formation (86.12 kcal mol^{-1}), indicating that thermodynamically it may be the least stable metabolite. However, the larger LUMO-HOMO energy difference makes it kinetically less labile as compared to other metabolites and the parent compound.

For 1-naphthol which is cytotoxic in mice, the heat of formation is relatively high and the LUMO-HOMO energy difference is relatively low, indicating its low thermodynamic stability and high kinetic lability.

For the stable metabolite 1,2-dihydro-1,2-naphthalenediol, the HOMO-LUMO energy difference is high and the heat of formation is much more negative, indicating that the metabolite has lower kinetically lability and higher thermodynamic stability. The parent compound naphthalene also has relatively high heat of formation, indicating its low thermodynamic stability. However, the large HOMO-LUMO energy difference makes it less kinetically labile and hence less toxic as such.

The contours of total electrostatic potential show the concentration of negative charges around the oxygens in all the metabolites. In Fig. 3, it can be seen that there is concentration of negative charge on the oxygen of naphthalene-1,2-epoxide. Similarly, there are concentration of negative charges around oxygens of 2-naphthol (Fig. 4) and 1-naphthol (Fig. 5), 1,4-naphthalenediol (Fig. 6), 1,2-naphthoquinone (Fig. 7), 1,2-dihydro-1,2-naphthalenediol (Fig. 8), 1,2-dihydro-1,2-naphthalenediol (Fig. 9), 1,2-naphthoquinone (Fig. 10) and Naphthalene-2-glutathione conjugate (Fig. 11). The concentration of negative charges at positions close to oxygen centres indicates that the positions may be subject to electrophilic attack.

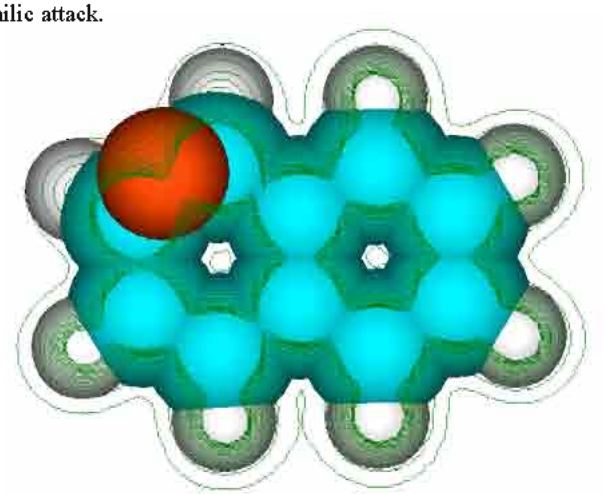


Fig. 3: Structure of naphthalene-1,2-epoxide giving 2D contours of total electrostatic potential where the black sphere denotes oxygen

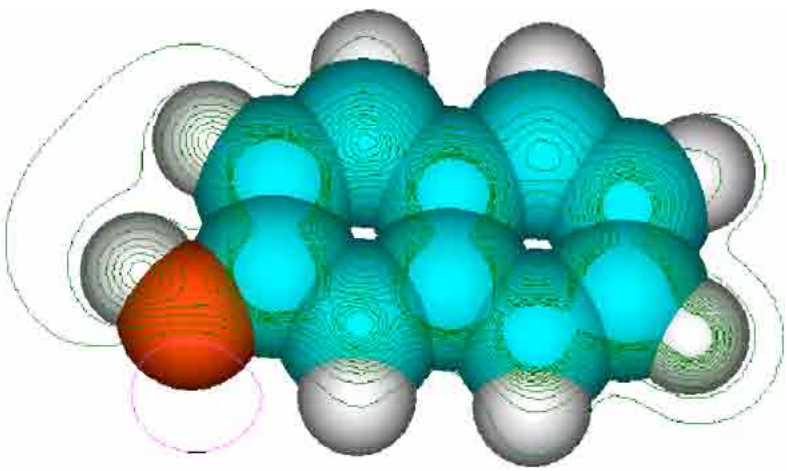


Fig. 4: Structure of 2-naphthol giving 2D contours of total electrostatic potential where the black sphere denotes oxygen

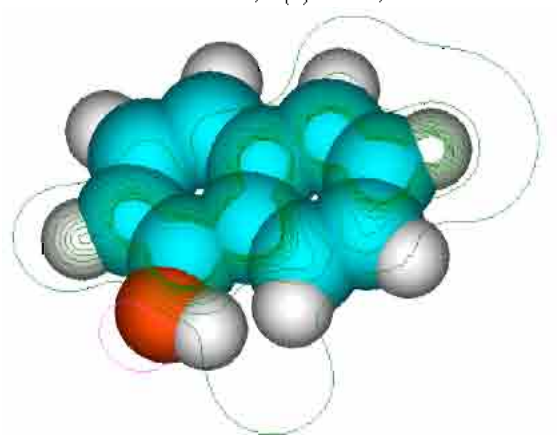


Fig. 5: Structure of 1-naphthol giving 2D contours of total electrostatic potential where the black sphere denotes oxygen

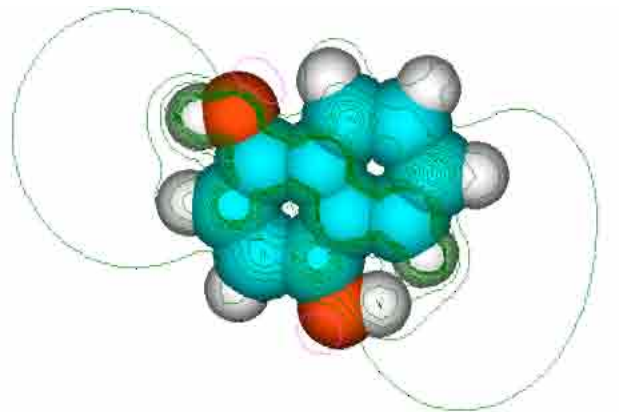


Fig. 6: Structure of 1,4-naphthalenediol giving 2D contours of total electrostatic potential where the black sphere denotes oxygen

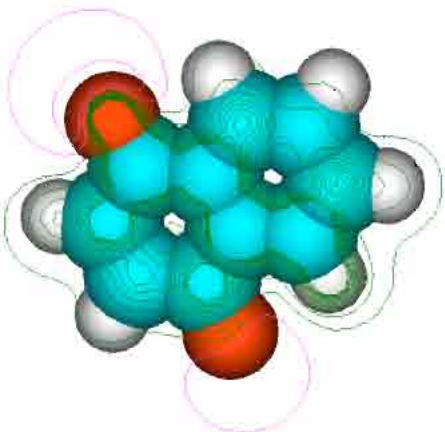


Fig. 7: Structure of 1,2-naphthoquinone giving 2D contours of total electrostatic potential where the black sphere denotes oxygen

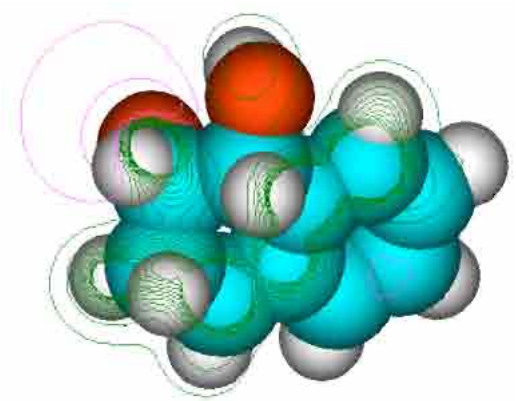


Fig. 8: Structure of 1,2-Dihydro-1,2-naphthalenediol showing 2D contours of total electrostatic potential where the black sphere denotes oxygen

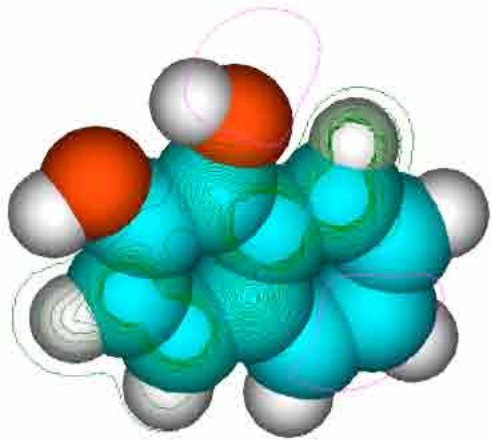


Fig. 9: Structure of 1,2-Dihydro-1,2-naphthalenediol showing 2D contours of total electrostatic potential where the black sphere denotes oxygen

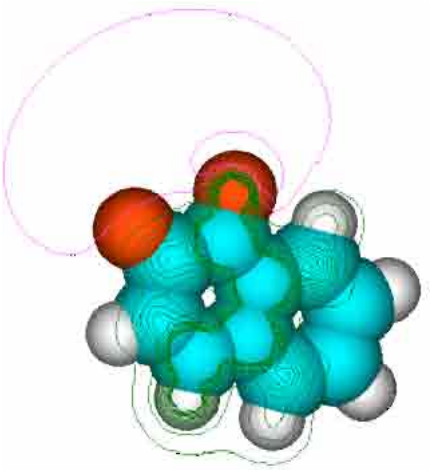


Fig. 10: Structure of 1,2-Naphthoquinone showing 2D contours of total electrostatic potential where the black sphere denotes oxygen

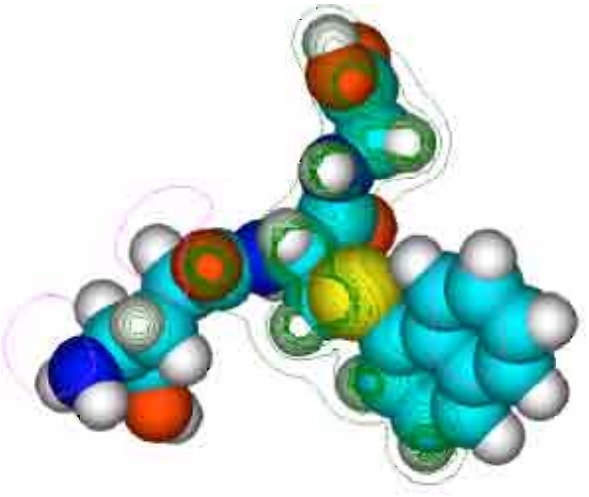
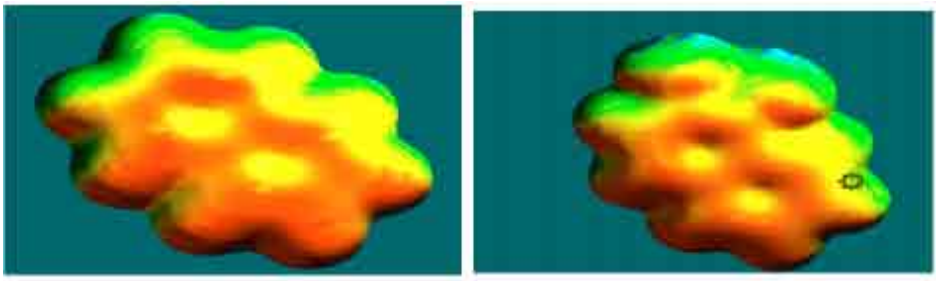
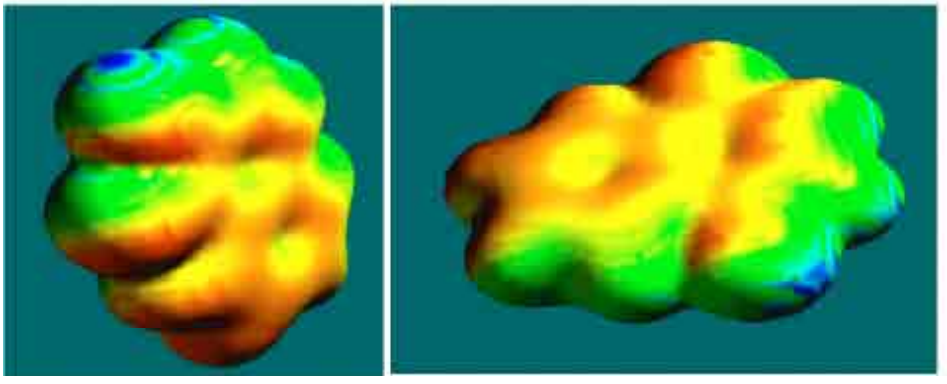


Fig. 11: Structure of naphthalene-1-glutathione conjugate showing 2D contours of total electrostatic potential where the black sphere denotes oxygen



Naphthalene 1,2-Napthalenediol



1,2-Naphthoquinone 1,4-Naphthoquinone

Fig. 12: Surface charges on naphthalene, 1,2-naphthalene-diol, 1,2-naphthoquinone and 1,4-naphthoquinone

Figure 12 gives the distribution of surface charges on naphthalene and three of its metabolites 1,2-naphthalenediol, 1,2-naphthoquinone and 1,4-naphthoquinone whereas red and yellow mean negative, green means neutral and blue means positive. It can be seen that although naphthalene is insoluble in water, the shown surface of the molecule is negatively charged. Actually this negative charge is associated with delocalised π-electrons above the fused biphenyl rings. In the case of the three metabolites, although the overall charge on the surface appears to be negative, there are positively charged patches on their surfaces. The presence of negative in the case of naphthalene and its metabolites indicates that the compounds may be subject to electrophilic attack.

Conclusion

Molecular analyses show that although naphthalene has a low thermodynamic stability, the larger HOMO-LUMO energy difference makes it less kinetically labile and hence less toxic than its more labile metabolites. Although naphthalene-1,2-epoxide has the lowest thermodynamic stability, the larger LUMO-HOMO energy difference makes the epoxide also labile. Much lower LUMO-HOMO energy differences make the naphthoquinones more reactive and hence more toxic.

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