BULL. PHIL. BIOCHEM. SOC. I(1): 4-9 (1978)

MUTAGENICITY POTENTIAL OF SOME DRUGS

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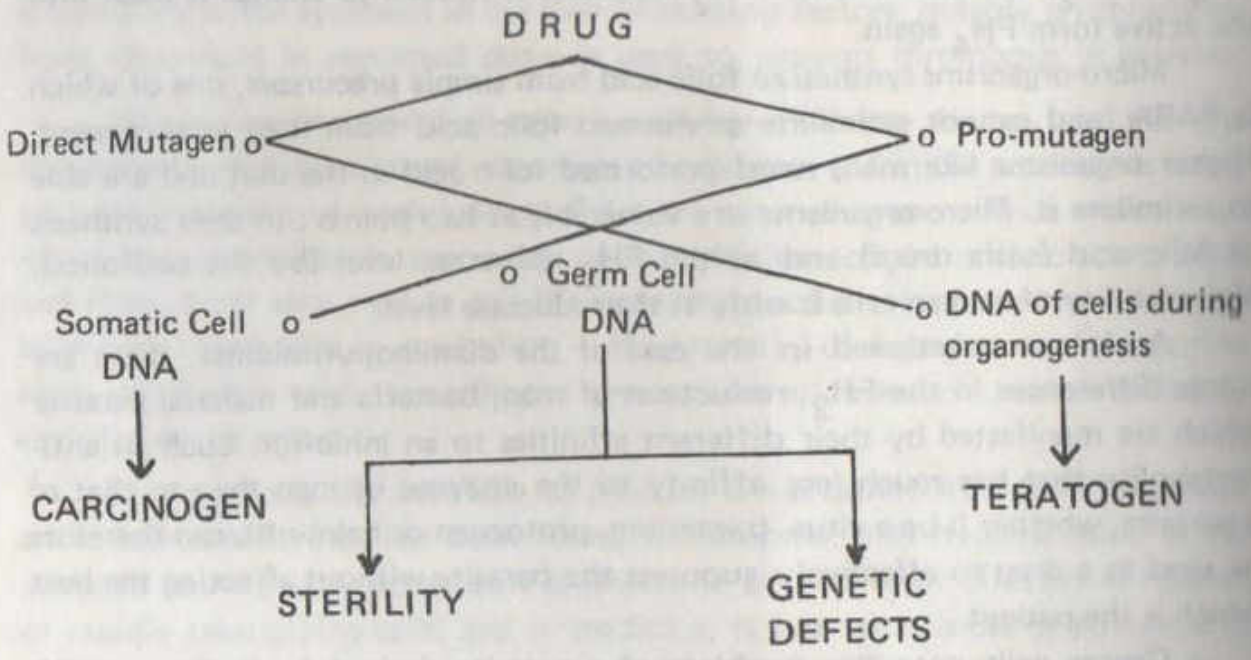
A number of man-made chemicals in widespread use have been shown to be mutagenic in subhuman experimental systems. This poses the possibility that such chemicals may constitute a potential genetic hazard for man both for the contemporary generation and for the future generation.

There has been expanded efforts in evaluating chemicals to which humans are exposed. Several of these chemicals which otherwise serve a useful function have either not been tested in this regard or only fragmentary or conflicting data are available for them. Thus, there is an urgent need to investigate possible mutagenic properties of chemicals introduced regularly to the human body such as drugs, especially those used for long periods of time and those that are employed for mass therapeutic practices.

When a drug interacts directly with the DNA of the cell or when it is metabolized to something that interacts with the DNA of the cell, it may induce mispairs and misrepairs. In some cases only sub-toxic levels are required to induce subtle changes leading to mispairs and misrepairs. When the DNA of the somatic cells are affected, cancer may develop after a latent period of 20 years or so. Effects on the DNA of cells during oraganogenesis may lead to physical defects.



It is therefore possible that a mutagenic drug can be a carcinogen or a teratogen or can induce sterility and genetic disorders.

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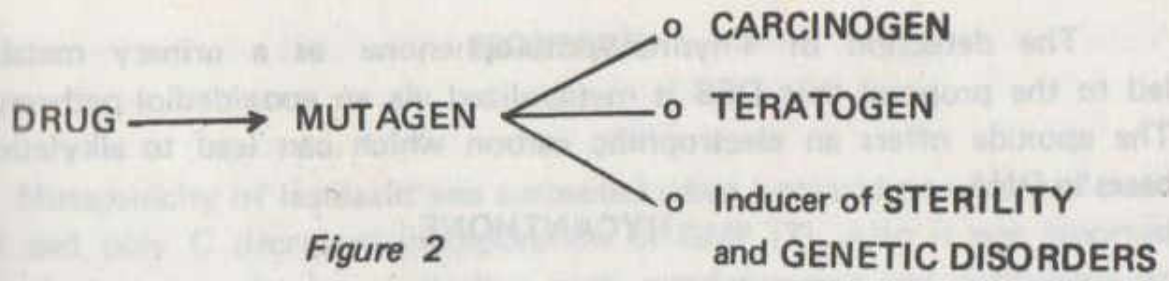


Figure 2

DIETHYLSTILBESTEROL

Diethylstilbesterol, a mutagenic drug, used to be a drug of choice for menopausal symptoms, as an antiabortive agent and also for the fattening of cattle. It is a non-steroidal estrogenic substance with the following structure:

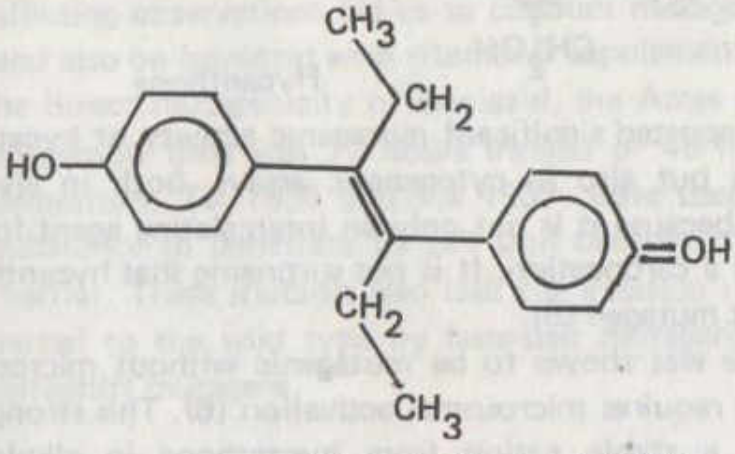


Figure 3 Diethylstilbesterol (DES)

The use of DES was stimulated in 1940's by many reports that its administration could prevent spontaneous abortion in high-risk pregnancy.

In 1971, Herbst, et al. reported (1) that young females with clear-cell adenocarcinoma of the vagina were linked to mothers who used DES during pregnancy. Seven out of 8 mothers whose daughters developed cancer had been treated with 4-hydroxypropiophenone DES for high-risk pregnancy. Over 90% of individuals who developed this cancer have been 14 years or older, the median ranging from 18 to 19 years (2)

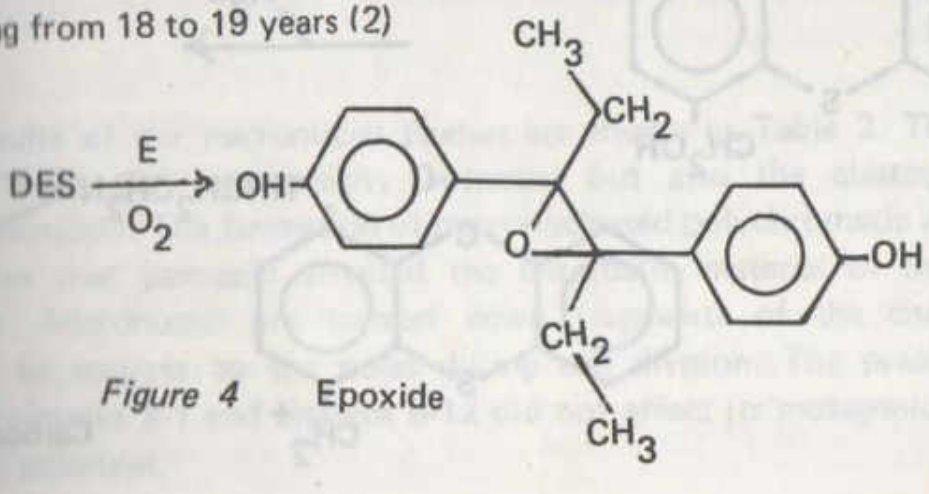
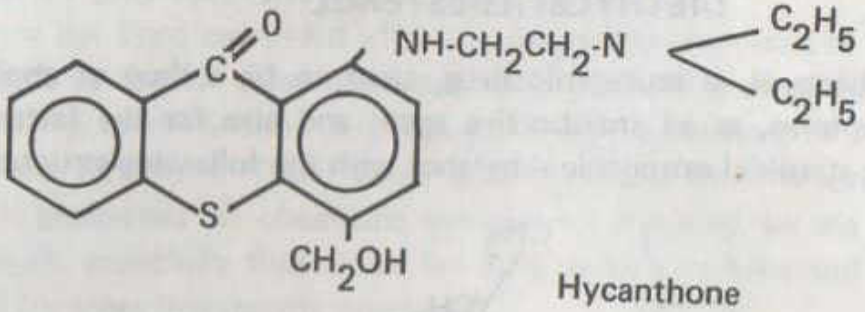


Figure 4 Epoxide

The detection of 4-hydroxypropiophenone as a urinary metabolite led to the proposal that DES is metabolized via an epoxidediol pathway (3). The epoxide offers an electrophilic carbon which can lead to alkylation of bases in DNA.

HYCANTHONE

Hycanthone is one of those drugs used widely in endemic areas all over the world where *Schistosomiasis mansoni* is a big problem. Its structure is shown:

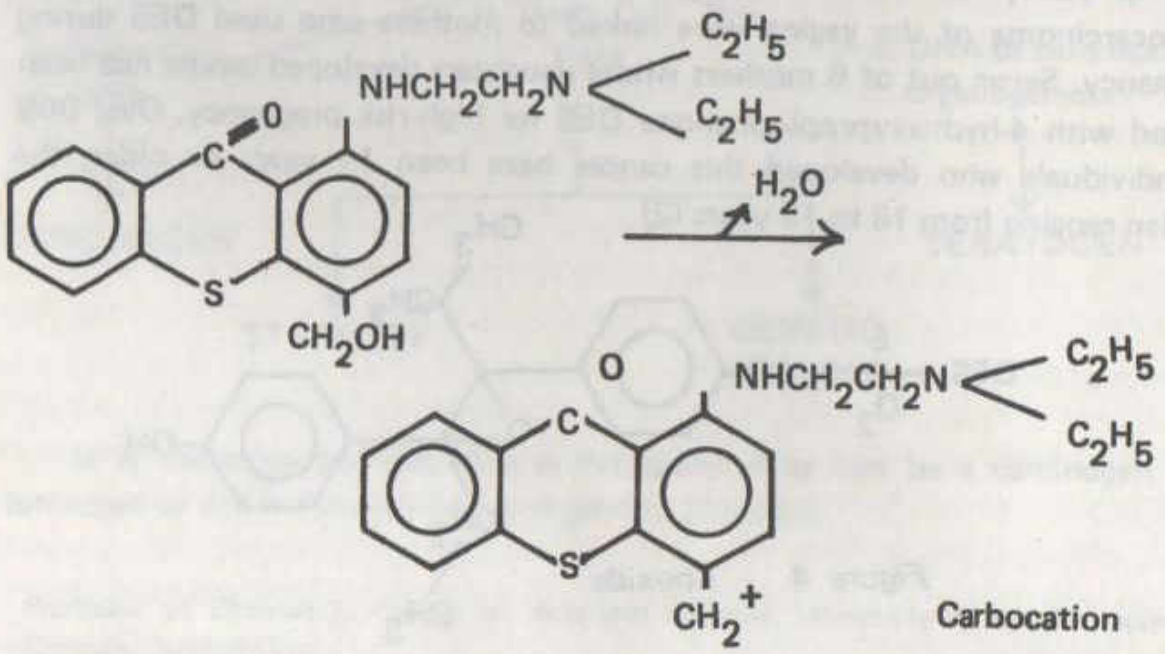


Hycanthone

Rey, et al. (4) reported significant mutagenic activity of hycanthone not only in bacterial tests but also in cytogenetic assays, both in vivo and in vitro. This is expected because it is not only an intercalating agent for DNA but also one that can give a carbocation. It is not surprising that hycanthone was shown to be a frameshift mutagen (5).

Hycanthone was shown to be mutagenic without microsomal activation while lucanthone requires microsomal activation (6). This strongly indicates the participation of a stable cation from hycanthone in alkylation reactions. Lucanthone cannot form readily a carbocation because it has a methyl group instead of a hydroxymethyl group that is in hycanthone.

Not only has hycanthone been shown to be carcinogenic in vitro and in vivo but it has also been shown to be a teratogen (6). This finding has led to stepped-up efforts in developing schistosomacides that are not mutagenic.



Carbocation

ISONIAZID

Mutagenicity of isoniazid was suspected when isoniazid treatment of both DNA and poly C decreased incorporation of GMP (7). Also it was reported that substituted hydrazines including both antidepressant and antitubercular drugs also produce hydrogen peroxide and inactivated transforming DNA (8). Interaction of isoniazid with DNA was suggested by Rosenkranz in their studies involving pol A mutants of *E. coli* (9). However, Rohrborn, et al. (10) found out that isoniazid was negative in the dominant lethal test for mutagenicity.

These conflicting observations led us to conduct mutagenicity studies on isoniazid alone and also on isoniazid with vitamin B supplementation.

To test the direct mutagenicity of isoniazid, the Ames method (11) was used but the incubation time was 72 hours instead of 48 hours. Mutants of *Salmonella typhimurium,* TA 1535 and TA 1537 were used. These mutants allow the test substance to penetrate its cell wall because of an altered lipopolysaccharide barrier. These mutants also lack the excision repair mechanism. TA 1535 is reverted to the wild type by base-pair mutagens while TA 1537 is reverted by frameshift mutagens.

Mutagenic and clastogenic effects were studied using the micronucleus technique of W. Schmid (12). This method can reveal the effect of the mutagen on the chromatin material. A mutagen that affects the chromatin material can induce the formation of micronuclei in erythrocytes of bone marrow cells.

Our studies show that after 72 hours of incubation, isoniazid induced base-pair mutations in *Salmonella typhimurium.* The data are given in Table 1. Isoniazid did not induce frameshift mutations. The direct mutagenicity of isoniazid (base-pair) can be a consequence of its reactivity with cytosine of DNA. This reactivity has been elucidated by Hayatsu, et al. in studies with nucleic acids (13). The addition of vitamin B-6, B-1 and B-12 did not affect its mutagenicity potential.

The results of our micronuciei studies are shown in Table 2. The data suggest not only the mutagenicity potential but also the clastogenicity potential of isoniazid. The formation of micronucleated polychromatic erythrocytes indicates that isoniazid affected the chromatin material of the bone marrow cells. Micronuclei are formed when fragments of the chromatin material fail to migrate to the poles during cell division. The presence of vitamin B-6, vitamin B-1 and vitamin B-12 did not affect its mutagenicity and clastogenicity potential.

Table 1. Induction of Base-Pair Mutations by Isoniazid-Containing Preparations

	No. of Revertant Colonies Per Plate	
	TA 1535	TA 1537
Control	5	9
Isoniazid	38	10
Isoniazid + vitamin B-6	34	11
Isoniazid + B-6, B-1, B-12	33	8

Isoniazid tablets used contained 100 mg of isonicotinyl hydrazine

Isoniazid-vit. B-6 preparation contained 100 mg of isonicotinyl hydrazine with 100 mg of vitamin B-6 (pyridoxine HC1) per tablet.

Isoniazid-B-6, B-1, B-12 preparation contained 100 mg of isonicotinyl hydrazine with 10 mg each of B-6, B-1, B-12 per tablet.

TA 1535 and TA 1537 are mutants of *Salmonella typhimurium*. These were gifts from Dr. B.N. Ames, University of California, Berkeley.

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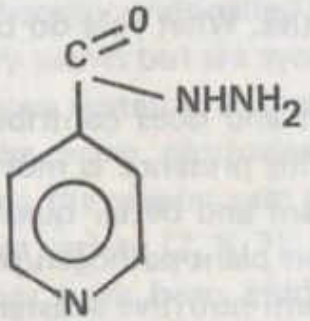
Table 2. Mutagenicity and Clastogenicity Potential of Isoniazid-containing Drug Preparations

	No. of micronucleated polychromatic erythrocyte per thousand
Control	3.14 ± 0.21
Isoniazid	19.64 ± 0.32
Isoniazid + B-6	17.82 ± 0.76
Isoniazid + B-6, B-1, B-12	18.73 ± 0.58

Ten mice were used for each trial

Our studies, therefore, indicate potential mutagenic activity of isoniazid. This should open avenues for studying carcinogenic potential of this drug to humans. There has been isolated, unrecorded reports of patients cured of tuberculosis but died of lung cancer.



Isoniazid

LITERATURE CITED

1. Herbst, A. L., et al. *N. England J. of Med. 284:* 878 (1971).
2. Herbst, A. L. *J. Toxicol. and Environ. Health. Suppl. 1:* 13, (1976).
3. Metzler, M. *J. Toxicol. and Environ. Health. Suppl. 1:* 21, (1976).
4. Rey, V. A. et al. *J. Toxicol. and Environ. Health. 1:* 21, (1975).
5. Hartman, P. E. and Hubert, P. B. *J. Toxicol. and Environ. Health. 1:* 243 (1975).
6. Sieber, S. M. and Adams, R. H. *J. Toxicol. and Environ. Health. 1:* 309 (1975).
7. Klamerth, O. L. *Mutation Res. 35:* 53 (1976).
8. Freese, E., et al. *Mutation Res. 5:* 343 (1968).
9. Rosenkranz, H. L. and Carr, H. S. *Lancet. 1:* 1354 (1971).
10. Rohrborn, G., et al. *Mutation Res. 16:* 189 (1972).
11. Ames, B. N. *Chemical Mutagens. 1:* 267 (1971).
12. Schmid, W. *Agents and Actions. 3:* 77 (1973).
13. Hayatsu, et al. *Biochem. Biophys. Acta. 123:* 445 (1967).