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# Toxicology of *N*-Nitroso Compounds

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Toxicology of *N*-Nitroso Compounds. SHANK, R. C. (1975). *Toxicol. Appl. Pharmacol.* **31**, 361–368. The acute and chronic toxicity of *N*-nitrosamines and *N*-nitrosamides are reviewed in general terms. Emphasis is made of the relationship between metabolism and toxicity.

Interest in the toxicology of *N*-nitroso compounds was greatly stimulated by the recognition of industrial hazards presented by *N*-nitroso-*N*-methylurethane (Watrous, 1947; Wrigley, 1948) and dimethylnitrosamine (Hamilton and Hardy, 1949; Barnes and Magee, 1954). Dimethylnitrosamine drew special interest. In an American automobile factory, where dimethylnitrosamine was used as a solvent, two men were accidentally poisoned. One man recovered after signs of liver damage; the other died in a clinical accident and a necropsy revealed a cirrhotic liver with regenerating nodules. Two of three men in a British industrial research laboratory, working with the solvent over a period of 10 mo showed signs of liver injury. One died of bronchopneumonia and a necropsy found liver cirrhosis. The other technician developed a hard liver with an irregular surface but recovered after exposure to the solvent was terminated. In characterizing the toxicity of dimethylnitrosamine, Magee and Barnes (1956) were able to demonstrate that the compound was a potent hepatocarcinogen. The toxicology of the *N*-nitroso compounds has recently been reviewed by Magee and Barnes (1967), Druckrey *et al.* (1967), and Magee (1971).

## ACUTE TOXICITY

For the purpose of discussing the toxicity of the *N*-nitroso compounds, it is convenient to divide them into two groups, the *N*-nitrosamines and the *N*-nitrosamides (Fig. 1). In general, the nitrosamines require metabolic transformation to active intermediates by an enzyme system similar to, if not the same as, the microsomal drug-hydroxylating system. The nitrosamides are not stable at physiological pH and decompose, probably to active intermediates analogous to those derived from nitrosamines.

The potency of the *N*-nitroso compounds in causing acute tissue injury and death varies considerably (Table 1). Acute toxicities, expressed as single dose oral LD50 values in adult rats, range from 18 mg/kg. for *N*-nitrosomethylbenzylamine to more than 7.5 g/kg for *N*-nitrosoethyl-2-hydroxyethylamine (Druckrey *et al.*, 1963a).

A great deal of work remains to be done in studying the relationship between structure and acute toxicity. It does seem, however, that acute toxicity decreases with chain length of dialkylnitrosamines. Cyclic nitrosamines such as *N*-nitrosohexamethyleneimine and

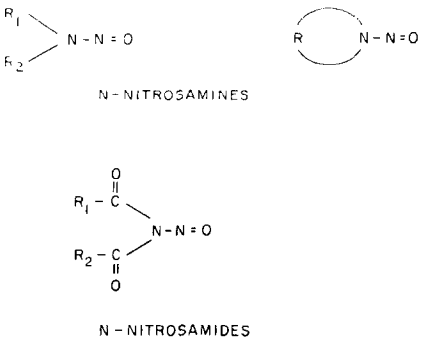


FIG. 1. General structures for *N*-nitroso compounds.

*N*-nitrosomorpholine are also acutely toxic. Nitrosamides, such as *N*-methyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourethane, have moderate LD50 values.

The histopathology of dimethyl- and diethylnitrosamine acute poisoning has been well studied; unfortunately, this is not true for most other *N*-nitroso compounds. Dimethyl and diethylnitrosamine are hepatotoxins causing centrilobular necrosis with hemorrhages in 24–48 hr; death occurs in 3–4 days or the animals survive and recover completely in approximately 3 wk. Detailed studies of the acute toxicity of the *N*-nitroso compounds as a class have not been common because the striking carcinogenicity of many of these compounds has commanded such intense interest.

TABLE 1

ACUTE TOXICITY OF SOME *N*-NITROSO COMPOUNDS

| Compound | LD50[a] | Reference |
|---|---|---|
| Dimethylnitrosamine | 27–41 | Heath and Magee, 1962 |
| Diethylnitrosamine | 216 | Heath and Magee, 1962 |
| Di-*n*-propylnitrosamine | >400[b] | Pour *et al.*, 1973 |
| Di-*n*-butylnitrosamine | 1200 | Druckrey *et al.*, 1964b |
| Di-*n*-amylnitrosamine | 1750 | Druckrey *et al.*, 1961a |
| Methyl-*n*-butylnitrosamine | 130 | Heath and Magee, 1962 |
| Methyl-*t*-butylnitrosamine | 700 | Heath and Magee, 1962 |
| Ethyl-*n*-butylnitrosamine | 380 | Druckrey *et al.*, 1963a |
| Ethyl-*t*-butylnitrosamine | 1600 | Druckrey *et al.*, 1963a |
| Ethyl-2-hydroxyethylnitrosamine | >7500 | Druckrey *et al.*, 1963a |
| Di-2-hydroxyethylnitrosamine | >5000 | Schmähl, 1963 |
| Methylphenylnitrosamine | 200 | Heath and Magee, 1962 |
| Methylbenzylnitrosamine | 18 | Druckrey *et al.*, 1963a |
| Nitrosomorpholine | 282 | Druckrey *et al.*, 1961a |
| Methylnitrosourea | 180 | Druckrey *et al.*, 1961a |
| Methylnitrosourethane | 240 | Druckrey *et al.*, 1962a |
| Nitrosohexamethyleneimine | 336 | Goodall *et al.*, 1968 |
| Nitrosoheptamethyleneimine | 283 | Lijinsky *et al.*, 1969 |
| Nitrosooctamethyleneimine | 566 | Lijinsky *et al.*, 1969 |

[a] LD50 units: mg/kg, single oral dose, adult male rats.
[b] Male and female Syrian golden hamsters.

## CARCINOGENICITY

Table 2 lists some of the *N*-nitroso compounds and some sites where they induce tumors. It is immediately obvious that tumors can be produced in a great many tissues. These compounds, in several instances, are highly potent carcinogens; single administrations to infant animals can result in high tumor incidences when the animals reach adulthood (Magee and Barnes, 1959; Druckrey *et al.*, 1964c). A single administration of *N*-nitrosoethylurea to pregnant rats results in malignant tumors of the vagina, uterus, or ovaries (Druckrey and Ivankovic, 1969).

TABLE 2

TUMOR SITES OF SOME *N*-NITROSO COMPOUNDS

| Site | Compound | Reference |
|---|---|---|
| Skin | Methylnitrosourea | Graffi and Hoffmann, 1966 |
| Nose | Diethylnitrosamine | Herrold, 1964 |
| Nasal sinus | Dimethylnitrosamine | Druckrey *et al.*, 1964a |
| Tongue | Nitrosohexamethyleneimine | Goodall *et al.*, 1968 |
| Esophagus | Nitrosoheptamethyleneimine | Lijinsky *et al.*, 1969 |
| Stomach | Ethylbutylnitrosamine | Schmähl *et al.*, 1963 |
| Duodenum | Methylnitrosourea | Druckrey *et al.*, 1963b |
| Colon | Cycasin | Laqueur, 1965 |
| Lung | Diethylnitrosamine | Dontenwill and Mohr, 1961 |
| Bronchi | Diethylnitrosamine | Dontenwill and Mohr, 1961 |
| Liver | Dimethylnitrosamine | Magee and Barnes, 1956 |
| Pancreas | Nitrosomethylurethane | Druckrey *et al.*, 1968 |
| Kidney | Dimethylnitrosamine | Magee and Barnes, 1959 |
| Urinary bladder | Dibutylnitrosamine | Druckrey *et al.*, 1962b |
| Brain | Methylnitrosourea | Druckrey *et al.*, 1965 |
| Spinal cord | Nitrosotrimethylurea | Ivankovic *et al.*, 1965 |
| Thymus | Nitrosobutylurea | Yokoro *et al.*, 1970 |
| Lymph nodes | Ethylnitrosourea | Vesselinovitch *et al.*, 1971 |
| Blood vessels | Nitrosomorpholine | Bannasch and Mueller, 1964; Newberne and Shank, 1973 |

Both nitrosamides and nitrosamines appear to induce neoplasms transplacentally. *N*-Nitrosoethylurea given to pregnant rats on the 15th day of gestation produced brain and spinal cord tumors in the offspring (Ivankovic and Druckrey, 1968); exposure during days 10 through 21 of gestation have resulted in renal tumors in the offspring several months after treatment (Wrba *et al.*, 1967). Diethylnitrosamine given subcutaneously to pregnant rats on days 9–15 of gestation induced tracheal papillomas in almost half the offspring within 25 wk of the first administration (Mohr *et al.*, 1966).

Some of the nitrosamines are capable of rapid induction of tumors. Lijinsky and co-workers (1969) obtained esophageal papillomas in only 7 wk of treatment with 200 mg *N*-nitrosoheptamethyleneimine.

There is good correlation between metabolism of nitrosamines and tumor induction (Montesano and Magee, 1971). Dimethylnitrosamine is readily metabolized by rat liver, less so by rat kidney and lung; liver tumors are the most frequent neoplasms

obtained with dimethylnitrosamine administration; renal tumors are much less frequent, and lung tumors are seen only under special experimental conditions (Magee and Barnes, 1967). Diethylnitrosamine is readily metabolized by hamster lung and much less so by hamster liver, and its administration to hamsters results in more lung tumors than liver tumors. The nitrosamides, presumably because of their instability in neutral and alkaline solutions, produce tumors at the site of administration or where decomposition is favored. Enzyme-catalyzed breakdown of nitrosamides may also be a factor but this has not yet been established.

## MUTAGENICITY AND TERATOGENICITY

The nitrosamides, but not the nitrosamines, are mutagenic in in vitro bacterial systems; the nitrosamines appear to require metabolic activation by mammalian enzyme systems before they can exert a mutagenic effect as demonstrated with dimethylnitrosamine in the host-mediated microbial assay of Gabridge and Legator (1969) and liver microsomal-activated microbial system of Ames and co-workers (1973) proved by Czygan and co-workers (1973). Even mammalian systems may fail to detect mutagenic activity. Dimethylnitrosamine does not cause mutations in Bateman's (1966) dominant lethal test where males are exposed to the test compound and then mated with untreated females (Epstein and Shafner, 1968). The inactivity of dimethylnitrosamine is probably due to the inability of the germ cells in the male to metabolize the compound.

*N*-Nitroso compounds can also be potent teratogens. *N*-Nitrosomethylurea given to rats on the 13th or 14th day of gestation results in fetal deaths and resorption and deformities in those that reach term (von Kreybig, 1965). When *N*-nitrosoethylurea is given to rats before the 12th day of pregnancy, the compound is not carcinogenic, but it is a powerful teratogen (Napalkov and Alexandrov, 1968).

## METABOLISM

Over a period of several years of research on the metabolism of nitrosamines, the following pathway has been proposed (Magee and Vandekar, 1958; Rose, 1958; Druckrey *et al.*, 1961a; Magee and Hultin, 1962). Dialkylnitrosamines undergo an enzymatic hydroxylation at the $\alpha$ carbon in one of the aliphatic chains. Further oxidation may produce the acylalkylnitrosamide. Hydrolysis produces an aldehyde (or carboxylic acid in the case of the nitrosamide) and the monoalkylnitrosamine. Until recently it was thought that the monoalkylnitrosamine was rearranged to the corresponding diazohydroxide, which then becomes the diazoalkane and then the carbonium ion, which alkylates nucleic acids and other macromolecules. Lijinsky and co-workers (1968), however, have shown that in the case of dimethylnitrosamine, alkylation is a transmethylation with no exchange of hydrogen atoms involved in deriving the methonium ion. This, then, argues against the formation of diazomethane as an intermediate in dimethylnitrosamine metabolism. Ethylation of nucleic acids has been demonstrated with administration of diethylnitrosamine (Magee and Lee, 1964) which supports $\alpha$ oxidation of one ethyl group and transethylation of the second.

Recently, Kruger (1972) has provided evidence that $\beta$-oxidation of dialkylnitrosamines may also occur. The first step would require enzymatic dehydrogenation between the $\alpha$ and $\beta$ carbons of one of the alkyl chains. Adding water to the double bond produces the $\beta$-hydroxylated nitrosamine, as the nitroso group would have an inductive effect which would be analogous to the effect the activated carbonyl group has in $\beta$-oxidation of fatty acids.

Oxidation of the hydroxylated nitrosamine would continue, yielding acetyl-CoA and methylalkylnitrosamine; the nitrosamine might then undergo $\alpha$-oxidation producing either the methonium ion or the carbonium ion corresponding to the second alkyl group. Studies on the metabolism of [$^{14}$C]di-*n*-propylnitrosamine labeled in the 1 or 2 position and [1 – $^{14}$C]di-*n*-butylnitrosamine support this pathway. Kruger used alkylation of guanine in liver RNA as evidence for the formation of carbonium ions. [1 – $^{14}$C]Dipropylnitrosamine metabolism yielded both [7-methyl-$^{14}$C]guanine and [7-propyl-$^{14}$C] guanine in rat liver RNA. When the 2 position of the propyl chain was labeled, only the 7-propylguanine carried label. Similarly, administration of [1-$^{14}$C] dibutylnitrosamine resulted in both [7-methyl-$^{14}$C]guanine and [7-butyl-$^{14}$C]guanine.

## MECHANISM OF ACTION

The role of nucleic acid methylation in acute toxicity and carcinogenesis has been the object of a great deal of research. Alkylation of the 7 position of guanine appears to be closely associated with acute toxic tissue injury, but the mechanism is not yet clear. Methylation of messenger RNA resulting from dimethylnitrosamine poisoning effectively inhibits translation in protein synthesis (Villa-Trevino, 1967; Shank, 1968) by inactivating guanine in the genetic code (Wilhelm and Ludlum, 1966).

Ludlum (1970) has shown, however, that polyribonucleotides containing 7-methylguanylic acid serve as normal templates for RNA polymerase. Incorporation of 3-methylcytosine in polycytidylic acid, however, not only lowered template efficiency in producing polyguanylic acid, but also produced incorrect polymers of guanylic and uridylic acids. This suggests that alkylation of cytosine at the 3 position may be more closely related to carcinogenesis than alkylation of the 7 position of guanine.

There are a number of instances in which there is poor correlation between the formation of 7-methylguanine and a compound's ability to induce tumors in specific tissues. Methylnitrosourea is a potent brain carcinogen and its administration produces approximately the same level of 7-methylguanine in brain DNA as does methylmethanesulfonate, which is only a weak brain carcinogen (Swann and Magee, 1969; Kleihues and Magee, 1973). Methylnitrosourea treatment produced O$^6$-methylguanine which was not detected in brain DNA from methylmethanesulfonate-treated rats. Loveless (1969) has suggested that this minor base may be more important in mutagenesis and carcinogenesis than 7-methylguanine.

## CONCLUSIONS

At this time, it seems that no one specific alkylated base can be considered critical in carcinogenesis. Just what role alkylation of nucleic acids and other macromolecules plays in cancer is not yet clear, but the great variety of structures of carcinogenic

*N*-nitroso compounds and the great variety of tissues which respond with tumors should serve as powerful tools in elucidating the mechanisms of chemical carcinogenesis. For these same reasons, we must also assess the extent to which these compounds occur in our environment, for there is no toxicological evidence to suggest that man, unlike other mammals, is resistant to the effects of these carcinogens.

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