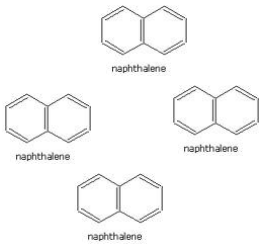
Carcinogenesis by Aromatic amines & N-Nitrosamines



1

Aromatic amines

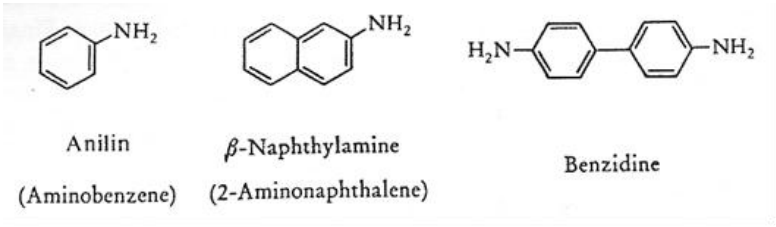
- Aromatic amine derivatives are employed in the manufactures of dyes polymers explosives and pesticides.
- Have Nitrogen atoms (–NH) attached to their ring carbon.

2

Aromatic Amines (cont.)

In 1895, Frankfurt surgeon, Rehn reported the increasing appearance of bladder cancer among dye factory workers. Rehn concluded that aniline might be responsible for these cancers.

Later, it was found that aniline itself did not cause these tumors. Whereas, several other aromatic amines, primarily β-naphthylamine (or 2-amino-naphthalene), but also benzidine and diphenylamine were observed to be carcinogenic.



3

Examples of Aromatic amines (cont.):

- **Benzidine:**
- Used as an intermediate in manufacture of dyes and as a hardener, as laboratory agents, for quantities' determination of Nicotine and spray reagent for sugar, sulfate and HCN.
- Exposure by inhalation and mainly induces Bladder cancer

4

Azo Dyes

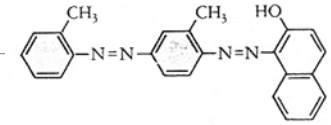
In 1906, Fischer-Wasels noticed epithelial proliferation in the ears of rabbits after multiple subcutaneous injections of a solution of Scarlet Red in oil.

After 30 years, Sazaki and Yoshida discovered the carcinogenic effect of o-Aminoazotoluene, a structural component of Scarlet Red. All the rats receiving a diet containing o-Aminoazotoluene developed hepatomas.

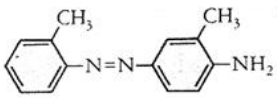
In 1936, Kinoshita identified N, N-dimethyl-4-aminoazobenzene as a stronger carcinogen than Aminoazotoluene.

Dimethyl-aminoazobenzene (DAB) is also known as 'butter yellow' because of the practice (now given up) of adding this dye to margarine and winter butter to add the color of summer freshness.

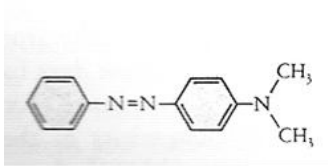
Azo Dyes



"Scarlet red"



O-Amino azotoluene



Dimethyl aminoazobenzene (DAB)
"Butter yellow"

Aminofluorenes

In 1940, 2-acetylaminofluorene (AAF) was patented as the principal ingredient of an insecticide.

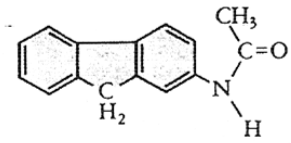
However, during the test of carcinogenic activity, this compound was found to be a highly effective carcinogen in 1941, hence was never used as an insecticide.

AAF induced tumors in rats, mice, dogs, cats, rabbits, and chickens, predominantly in bladder and liver but mammary, lung, and uterine tumors were also observed.

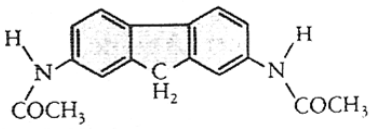
In most experiments, AAF had been added to the diet. It is locally inactive and does not produce tumors at the site of injection.

N, N′-2,7-fluorenyl(bis)acetamide produces several kinds of tumors in rats including mammary, skin, lung, stomach, intestine, uterine, liver, leukemias, and lymphomas.

Aminofluorenes



2-Acetylaminofluorene (AAF)



N-N′-2,7-Fluorenylbis-acetamide

Metabolic Activation of Aromatic Amines

β-Naphthylamine produces bladder tumors in experimental animals on feeding but is inactive on direct implantation (mixed with paraffin) into the bladder.

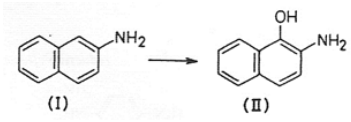
It is therefore assumed that there is a transformation into an active form somewhere in the body.

Hydroxylation of the parent compound has been linked with metabolic activation and increased carcinogenicity.

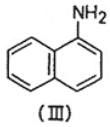
Ortho-ring Hydroxylation

Oxidation of aromatic rings results in phenols.

For example, β-Naphthylamine is converted to a new substance 1-hydroxy-2-aminonaphthalene (an aminophenol). The former compound produces no tumors whereas the latter is carcinogenic.



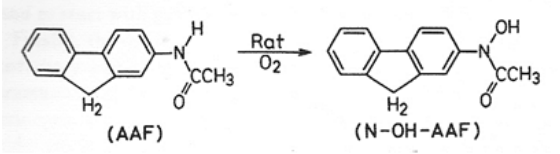
- α-Naphthylamine is non-carcinogenic because the 1-position is already occupied which cannot be oxidized and therefore cannot be activated.



- Although the ortho-hydroxylation hypothesis applies to some compounds it is not generally applicable because some o-hydroxy-amines are not carcinogenic

N-Hydroxylation

In 1960, Cramer and Millers reported a new substance isolated from the urine of rats who fed on 2-acetylaminofluorene (AAF) for many weeks. This new metabolite was identified as N-hydroxy-2-acetylaminofluorene (N-OH-AAF).



(AAF) (N-OH-AAF)

- N-OH-AAF was found to be more carcinogenic than AAF.
- N-OH-AAF can induce tumors in guinea pigs, even though AAF is non-carcinogenic in these animals.

N-Hydroxylation (cont.)

The N-hydroxy derivatives derived from many different carcinogenic amines and amides are more carcinogenic than non-hydroxylated compounds. However, in some cases, N-hydroxylation was ineffective for activation.

Even though in most cases, N-hydroxylation represents a necessary step in the activation of carcinogenic aromatic amines, it might not be entirely sufficient.

N-Hydroxylation (cont.)

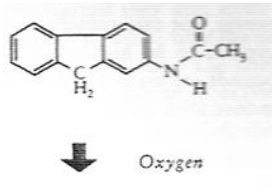
Millers coined the term “proximate carcinogens” for N-hydroxylated forms of carcinogenic amines. Proximate carcinogens are converted to “ultimate carcinogens” after further activation.

N-hydroxy esters appear to be active forms of many aromatic amines; these forms can directly react with cell constituents without the mediation of enzymes. Millers coined the term “ultimate carcinogen” for these active forms.

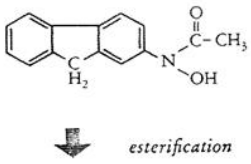
N-Hydroxylation (cont.)

The conversion of an aromatic amide (such as AAF) into its active form takes the following pathway: amide → N-hydroxy amide (proximate carcinogen) → N-sulfate ester (ultimate carcinogen).

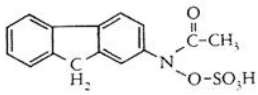
N-Hydroxylation (cont.)



2-Acetylaminofluorene (AAF)



N-Hydroxy-AAF (*Proximate Carcinogen*)



N-Hydroxy-Ester (Ultimate Carcinogen)

15

The biotransformation of Aromatic amines

❖**Metabolic activation:**

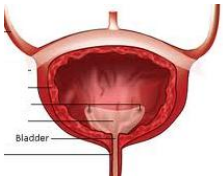
They are Acetylated by N-acetyltransferase in the presence of an acetyl-CoA.

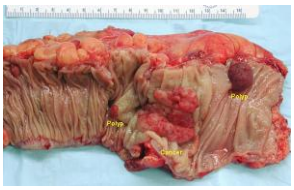
Acetylated reaction reversed (hydrolyzed) by microsomal enzymes.

16

Metabolic activation (cont.):

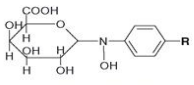
- Acetylation phenotype varies among individuals
  - Rapid Acetylation → **Colon cancer.**
  - Slow Acetylation → **Bladder cancer.**





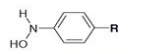
17

Final formation of aromatic amine-derived carcinogenic metabolites in the human bladder

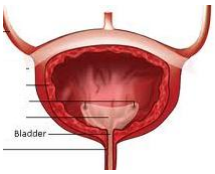


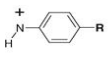
Aryl hydroxy-N-glucuronide

Acid pH



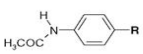
Aromatic hydroxylamine





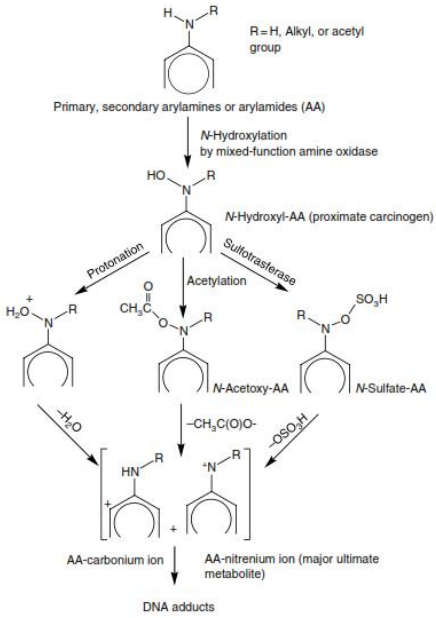
Nitrenium ion

Bladder acetylases



18

Metabolic activation pathways of aromatic amine and amide



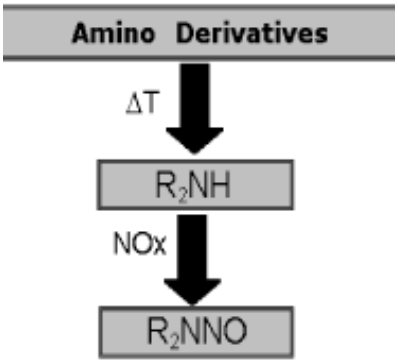
19

Nucleic acid alteration

- Activated aromatic amines derivatives form adduct with nucleic acids or their monomeric units in specific sites:
  - 2 amino, 6 Oxy purine.
  - 6 amino purine.
  - N_3 of pyrimidine.

20

Carcinogenesis by N-Nitrosamines



21

N-Nitrosamines

- Nitrosamines are simple compounds formed in the body.
- Formed in the body by the interaction of Nitrous acid + 2^{ed} or 3rd or 4th amines (e.g Trimethyl-amineoxide, in fish sea foods, meat, eggs, vegetables and spices).
- Mainly caused liver tumor.

22

N-Nitrosamines chemical structure

- $R_1R_2N-N=O$
- Many compound could produce nitrosamines like:
  - Drugs like Piperazine
  - Nitrogen-containing pesticides.
  - Tetracycline.

23

N-Nitrosamines chemical structure (cont.)

Example:

N-nitrosodimethylamine (NDMA).

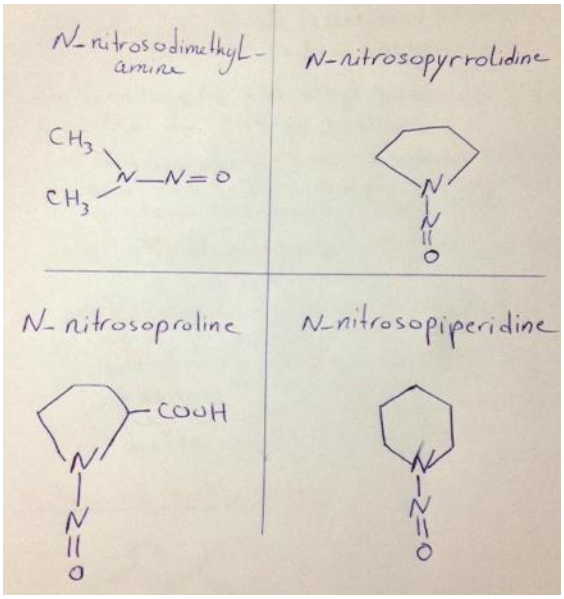
N-nitrosopyrrolidine.

N-nitrosoproline.

N-nitrosopiperidine.

24

N-Nitrosamines chemical structure (cont.)



25

N-Nitrosamines(cont.)

- Nitrates and Nitrites ⟹ used as food additives for preservation.
- Nitrates(inactive) ⟹ Nitrites (active).
- ***Microorganism***
- The rate of Nitrosation depend on:
  - [Reactant]
  - Basicity of amine.
  - pH and Temp.

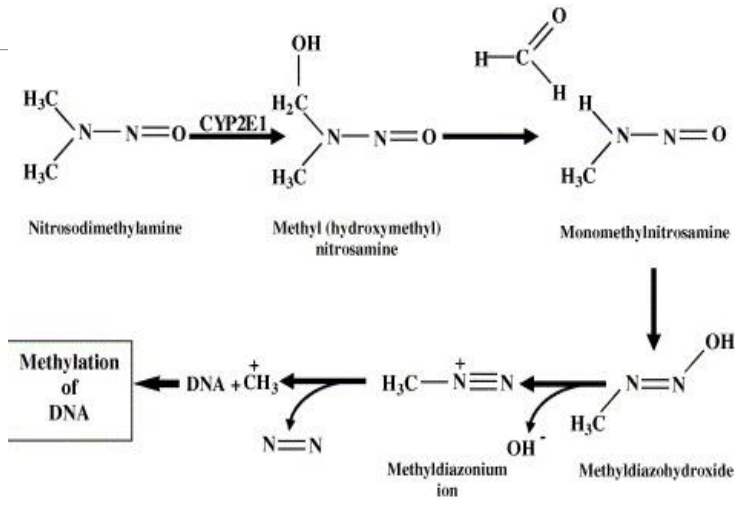
26

Factors affecting Nitrosation (cont.)

- Thiocyanate(HCN)+ Cl^- + Br^-
- In present of HCN → Optimum pH 1-2.
- In absent of HCN^- → Optimum pH 3-3.4.
- At alkali condition + HCHO → ↑ Nitrosation.
- Ascorbic acid + α-tocophenol + other antioxidants → Inhibit Nitrosation

27

Metabolism of Nitrosamines



28