

AHS

AN OVERVIEW—PART I

Cancer: Chemical factors in the environment

DAILY WE ARE EXPOSED to hundreds of chemical agents in an infinite number of combinations, by way of the air, the drinking water, the food, by skin contact, occasionally by way of medication, and in industrial working environments. A number of these agents have the ability to induce malignant tumors (are carcinogens).

The aim of this article is to provide a general background for nonspecialist readers of this journal in the light of which they may meaningfully correlate and to some extent critically evaluate the significance of scattered information obtained on cancer-inducing chemical agents. The article describes the methods of testing chemicals for carcinogenic activity, the guidelines for evaluating the significance of the test results, the major classes of chemical carcinogens and their mechanism of biological action, and the types of interaction that occur when several carcinogens act in combination.

Scope of the problem

Close to 200,000 new organic compounds are synthesized throughout the world yearly, and it is conservatively estimated that well over 1000 of these will eventually be put to some use, and, thus, into the environment. Hence it is necessary to test chemicals for carcinogenic activity before they come in contact with human populations. Yet, the "Carcinogenesis Technical Report" No. 1, released in May, 1976 by the National Cancer Institute,

stated that approximately only 350 chemical compounds were then on long-term tests in laboratory animals. The performance of these bioassays requires an average of 3 yr. The National Cancer Institute and its contractual-testing laboratory network represents by far the largest organization of this type in the world. At present the total world capability of testing chemicals for carcinogenic activity is at most twice that of the National Cancer Institute's organization. Hence, it is probable that a minimum of 300 new and untested or inadequately tested organic chemicals find their way yearly into consumer items or industrial use, world-wide. Some of the environmental pollutants are stable chemically and are biodegraded very slowly. Moreover, this already pessimistic picture does not take into account the melancholy fact that a considerable number of chemicals have been put to use in highly industrialized countries before their present stringent criteria of licensing for consumer use were established.

Testing procedures

Bioassays

Since humans are mammals, chemical agents are usually assayed in mammalian species. Small rodents (rats, mice, and hamsters) are generally used. This is because of the statistical necessity of using large groups, and because short-life-span species (average 2-3 yr) are preferred for reasons that will be discussed below. While a great variety of ways of administration have been tried, substances are most commonly assayed by:

1. Feeding; mixing the substance into the diet, dissolving it in the drinking water, or administering it directly into the stomach by special tube.

2. Skin "painting," where small volumes of a solution of the substance in a solvent are deposited on the shaved surface of the skin. The term painting originated from the fact that in the early years of such assays (around 1940) the solution of the substance tested was actually applied to the skin with a small brush.

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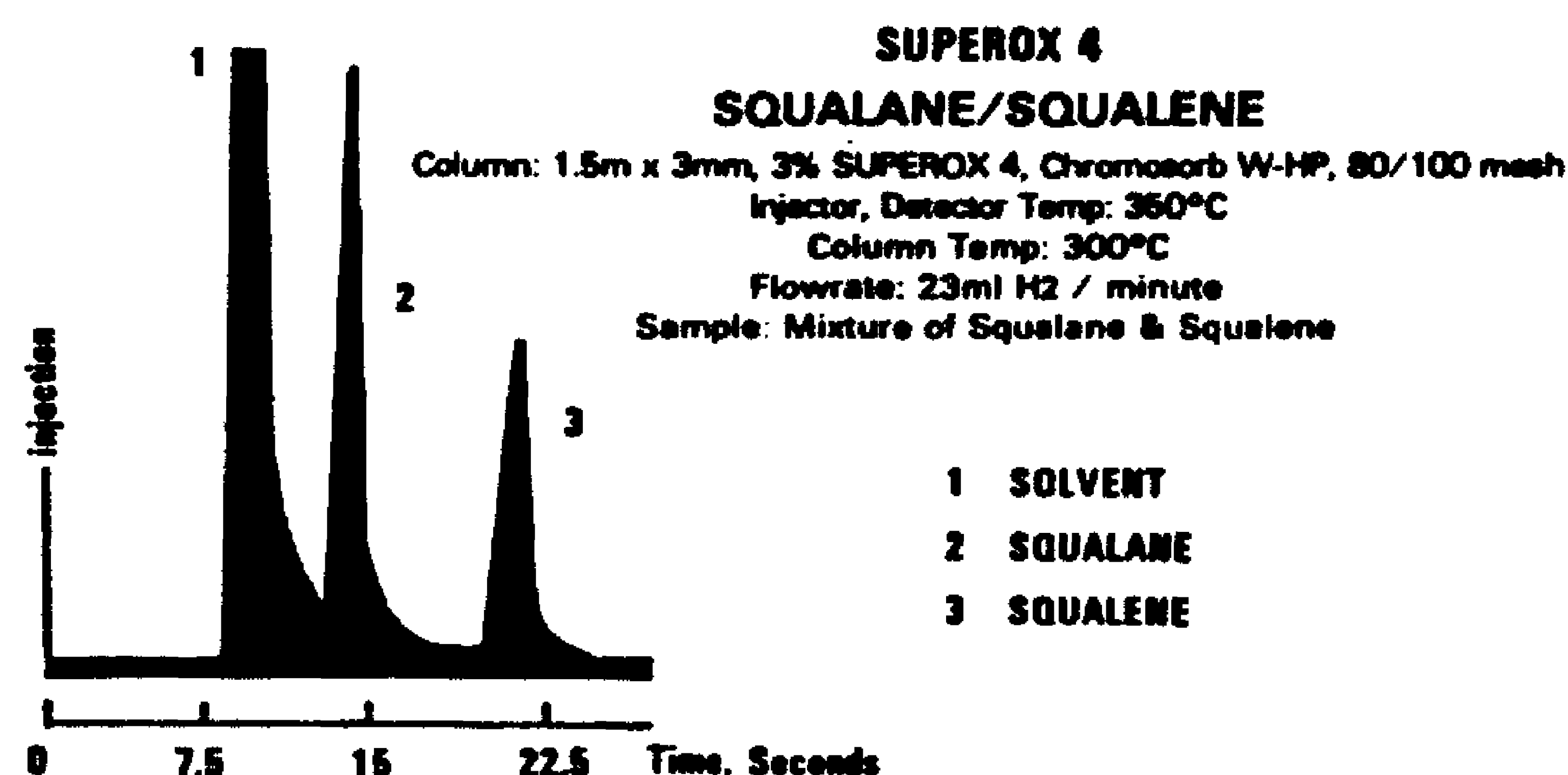
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CANCER continued

3. Subcutaneous injection of the substance or its solution once or at repeated intervals.

4. Inhalation of fine suspensions (aerosols) of the substance or introduction of its solution into the trachea by means of a special tube (intratracheal intubation).

When a bioassay is carried out for reasons of public health considerations of human safety (rather than as an experiment to explore a scientific problem, such as biochemical studies or structure-activity relationships), the route of administration of the chemical mimics human exposure whenever possible.

At the end of the testing period the surviving animals are killed, completely autopsied, and every abnormality is diagnosed by histopathological examination of tissue samples. The tissue samples are "fixed" (to forestall deterioration), stained with an appropriate dye or dyes to render them observable and provide contrast to microscopically visible tissue structure, and then are submitted to histopathology. This examination will reveal any change (resulting from the administration of the test compound) in the characteristic normal microscopic pattern of the particular tissue at hand, in the size and shape of the cells, the appearance and arrangement of intracellular components, etc. The totality

of this information enables the pathologist to diagnose whether or not the tissue has undergone a neoplastic change, to determine the cell origin (i.e., histological type) of any tumor, and to estimate the degree of its malignancy.

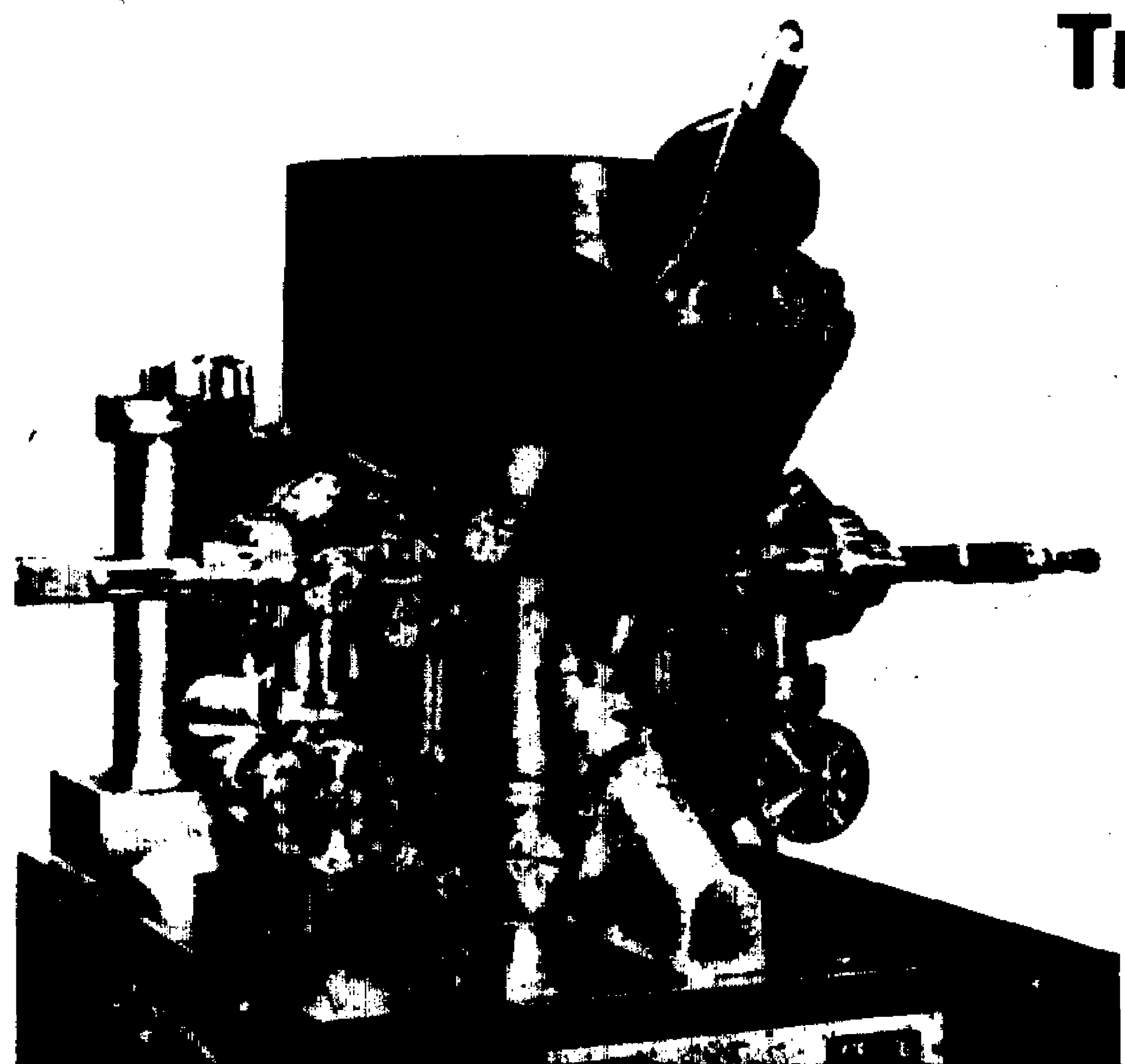
Two important parameters are obtained in a bioassay experiment. These are a) the tumor incidence, representing the percentage of animals bearing at least one tumor in the animal group considered (for example, 40 tumor-bearing animals in a test group of 100 would represent a tumor incidence of 40%); and b) the latent period, which is most commonly considered to be the number of days from the beginning of administration of the compound until the first tumor in the group is observed.

To characterize the carcinogenic potency of a chemical agent by one number, several carcinogenicity indexes have been proposed and used. These indexes are calculated from the tumor incidence and latent period, using various formulas. One of the most widely used is the Iball index:

$$\text{Iball index} = \frac{\text{tumor incidence (\%)}}{\text{latent period (days)}} \times 100$$

This index is based on the logic that the higher the tumor incidence, and the shorter the latent period (that is, the smaller the number of days) necessary

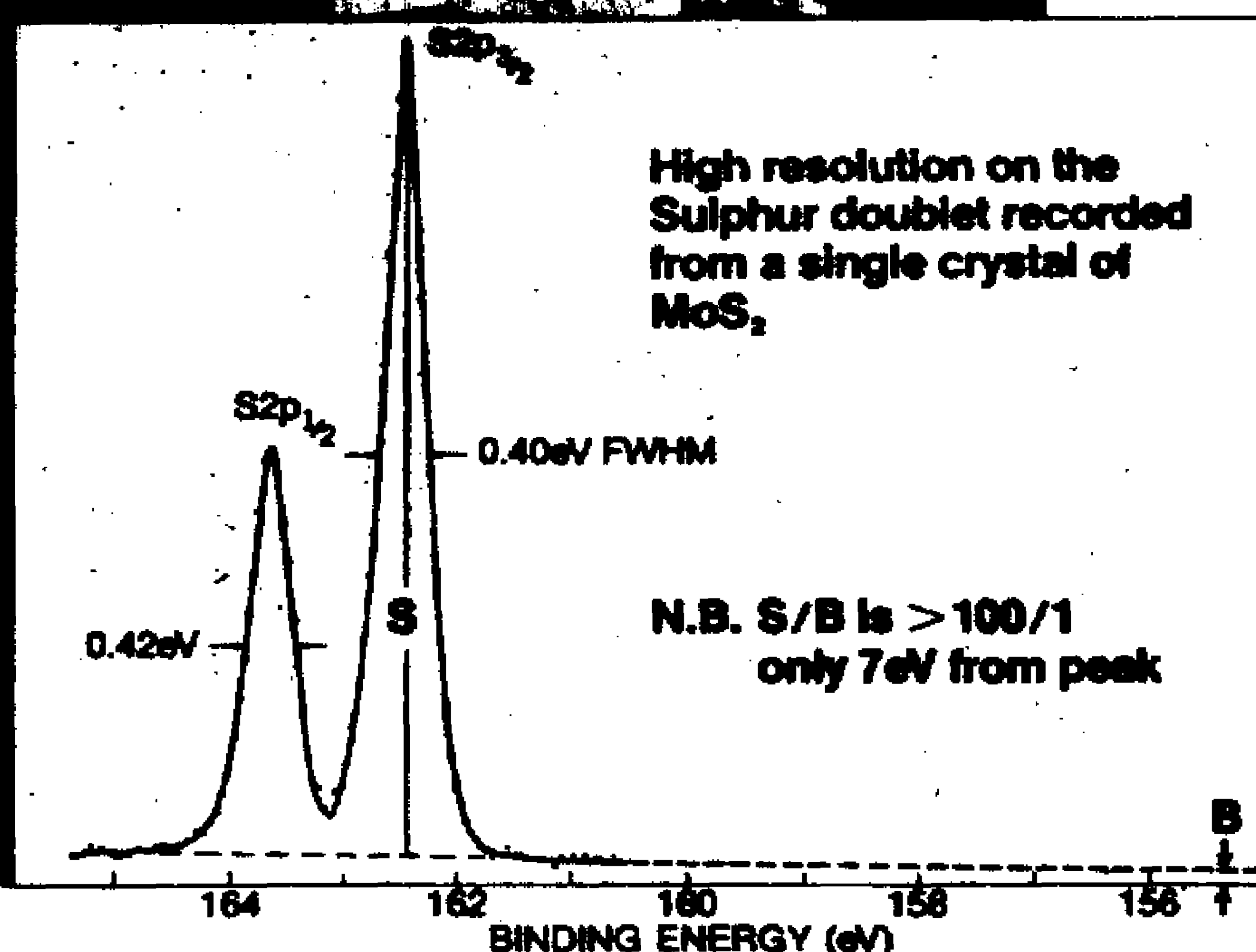
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for inducing the first tumor, the more potent the compound that has been tested.

The final results of bioassays are considered in the light of the following five principles.

1. A positive result, i.e., a chemical agent is found to be carcinogenic, carries a much greater weight than a negative result. This is because it can never be excluded that a compound, found inactive under certain experimental conditions, may prove to be carcinogenic when tested in another species by different routes and ways of administration, and under different dietary conditions.

2. It is a general consensus among investigators of chemical carcinogenesis that for any testing experiment to be meaningful at all, whether carried out for reasons of public health safety considerations or as a purely scientific study, it must be carried out for a minimum of 1 yr, unless a statistically significant tumor incidence would happen to become manifest earlier.

3. No chemical compound may be stated safely to be devoid of carcinogenic activity toward man unless it has been found inactive when tested in several mammalian species and by several routes of administration for a length of time corresponding to one half (or even better, the whole) life span of each species. This statement is based on these frequent

findings: A carcinogen that is inactive in one species may be highly active in another; the susceptibility of a species to a given carcinogen also depends on the genetic strain, sex, and dietary conditions; the detectability of carcinogenic activity often depends on the route of administration. Moreover, it is well established that the incidence of cancer in humans increases with the age group, and that the tumor, if its origin can be traced to a single or repeated exposures to a carcinogenic agent, will often appear only decades after exposure. Hence, the preference for using short-lived test species and to test for the entire life span can be understood.

4. Some considerations of experimental cancer research may not be carried over to public health considerations of human safety. A chemical agent that would induce a 1% net tumor incidence over that of a control group, even though statistically significant, would not be regarded as really meaningfully carcinogenic from the standpoint of laboratory experimentation. It is evident, however, that a chemical agent would be regarded as a major health hazard if it were known with certainty to produce 1,000 cancer patients per 100,000 population.

5. The latter considerations lead us to examine the statistical limitation of any bioassay procedure. To illustrate the point, consider that a compound is

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A



B



Figure 2 Two stages in the development of a skin tumor induced in the mouse using benzo[a]pyrene.

rings, a considerable variety of structures exists. Benzo[a]pyrene (also known as 3,4-benzopyrene), was the first hydrocarbon identified to be a strong carcinogen. It was isolated from coal tar, and the lead that pointed to this source was the unusually high skin cancer incidence in workers in the industries using coal tar. 3-Methylcholanthrene is another strong carcinogen; it has been obtained by synthesis and it is not present in tar. Benz[a]anthracene (also known as 1,2-benzanthracene) and dibenz[a,h]anthracene (also known as 1,2,5,6-dibenzanthracene) do occur in tar; however, they are weaker carcinogens than benzo[a]pyrene and arise during tar formation in lesser amounts. The four compounds shown in Figure 1 are only representative examples; a great variety of hydrocarbon carcinogens are known.

Benzo[a]pyrene is the most widely and ubiquitously distributed hydrocarbon carcinogen in the environment. It is also the main hydrocarbon carcinogen in cigarette smoke. Figure 2 exemplifies the result of application of this compound on the mouse skin. The mouse shown in Figure 2a received, twice weekly, applications of 0.3 mg of benzo[a]pyrene for 7 weeks. The tumor seen is a papilloma, a benign tumor, which most often is re-

sorbed and disappears if carcinogen application is discontinued at this point. However, if the application is continued for 11 weeks, the tumor turns malignant (Figure 2b) and will continue to grow and ultimately will kill the animal. Once the tumor has turned malignant the process is irreversible. (Histopathological examination has shown that the tumor in Figure 2b was a keratinizing epithelioma).

While hydrocarbons have a predominantly local effect at the site of application, such as the mouse skin tumor shown, oral administration of hydrocarbons produces tumors in distant tissue targets, mainly malignant mammary and lung tumors, and leukemia.

The reason why hydrocarbon carcinogens, and especially benzo[a]pyrene, are so ubiquitous is that they readily arise from almost any kind of organic material by pyrolysis. Thus, benzo[a]pyrene has been identified in food materials and in consumer and industrial products, the processing of which involves high temperatures. These include for example lubricating machine oils, medicinal paraffin oil used as a laxative, cigarette smoke, the exhaust fumes of internal combustion engines, charcoal-broiled steak, roasted coffee and chicory, smoked sausage, smoked fish, charred dough, caramelized sugar, and French fries, to name but a few. The pollution of lakes and rivers with polycyclic hydrocarbons originates mainly from the untreated effluents of industrial plants and from the lubricating oils and exhaust fumes of ships. Some of these pollutants eventually find their way into the water table and, hence, into the drinking water.

Figure 3 summarizes the chemical mechanism of the formation of benzo[a]pyrene from organic

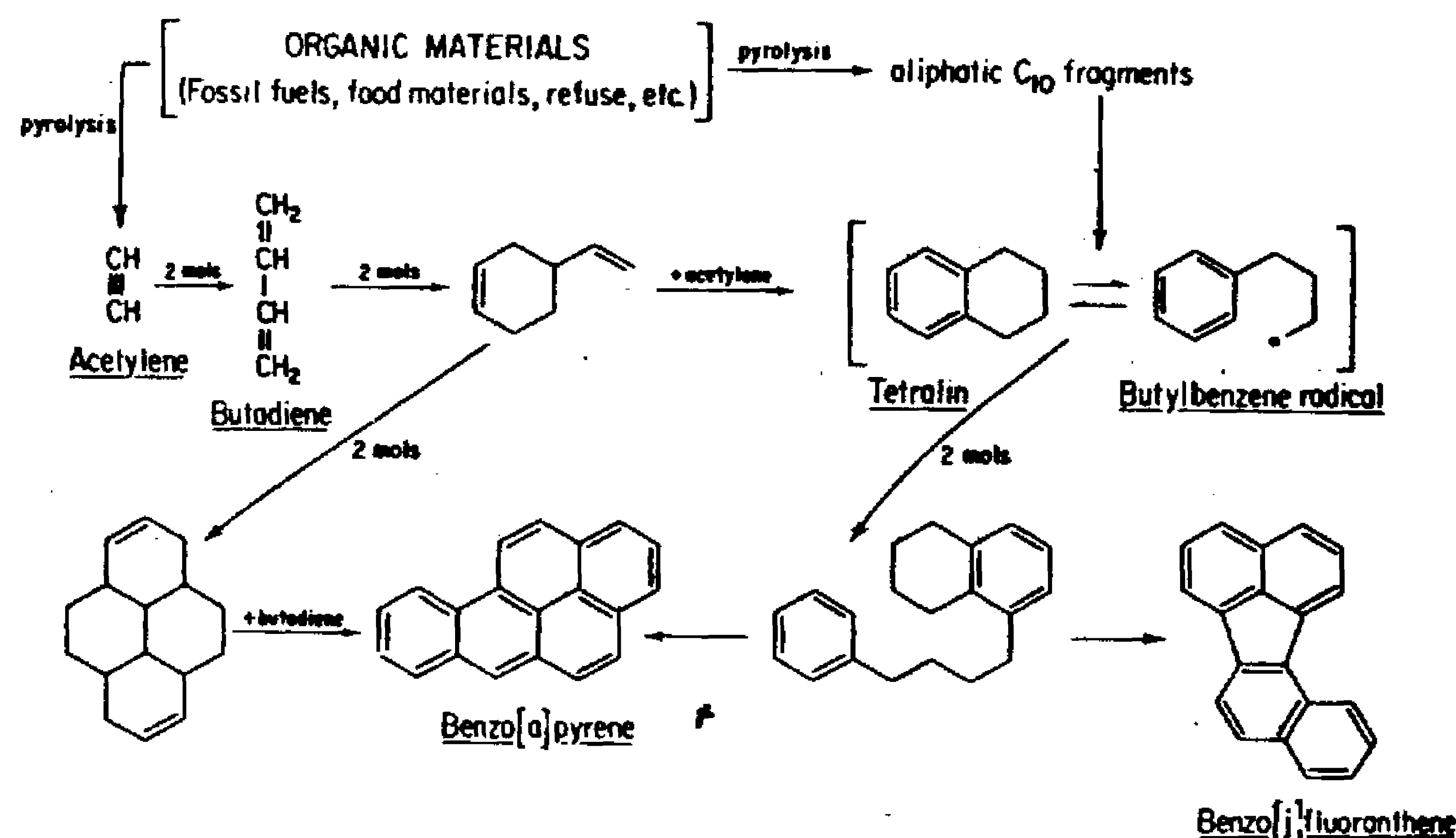


Figure 3 Molecular mechanism of the formation of benzo[a]pyrene and benzo[j]fluoranthene from lower hydrocarbons during pyrolysis.

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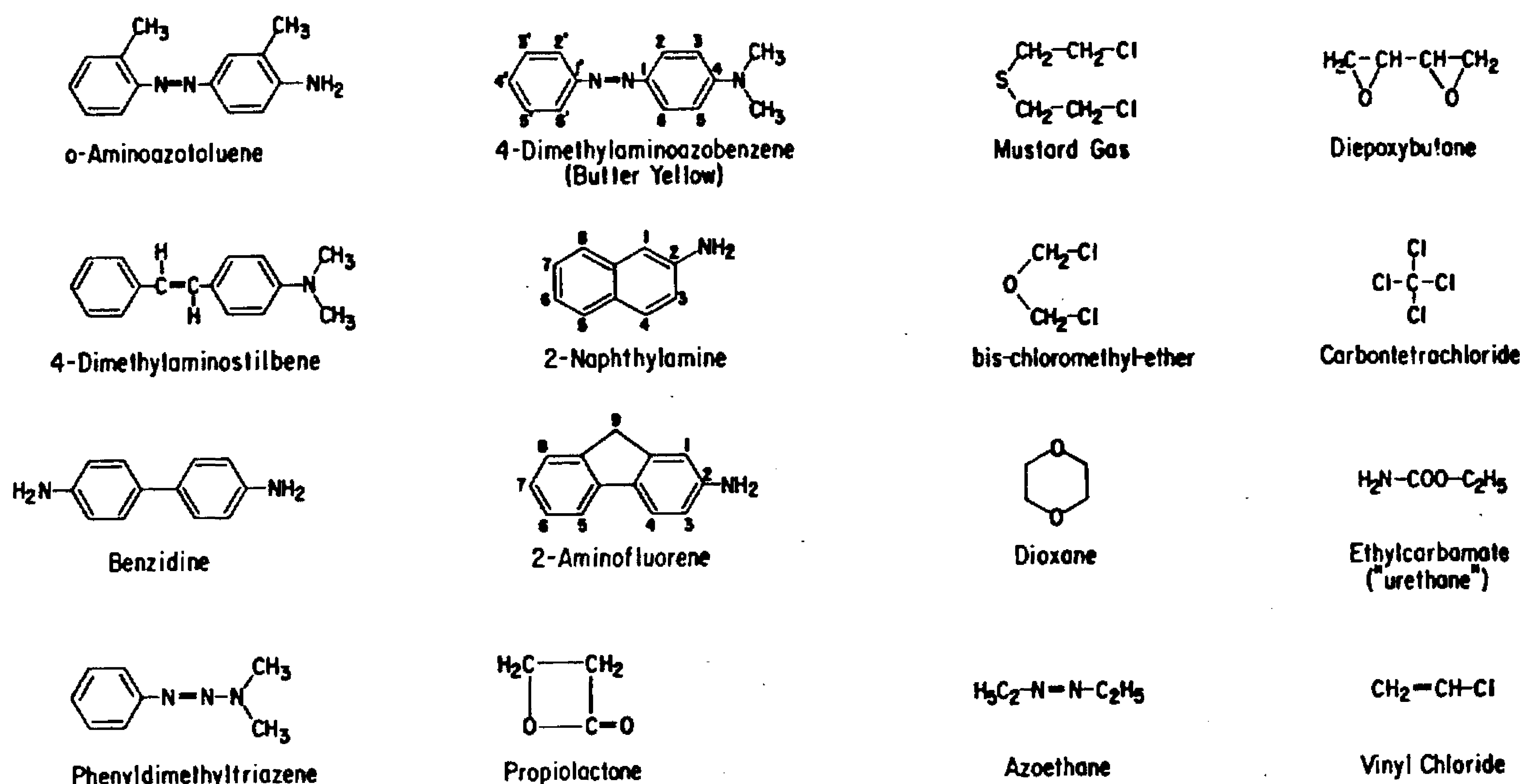


Figure 4 Varieties of chemical carcinogens.

materials during pyrolysis. A common pyrolytic decomposition product of all organic compounds is acetylene. However, molecules of acetylene in turn undergo gradual recombination at high temperatures to yield larger and larger structures, ultimately yielding polycyclic hydrocarbons. These compounds are relatively stable at high temperatures and, hence, accumulate as pyrolysis progresses. One of these hydrocarbons is benzo[a]pyrene.

As stated earlier, chemical carcinogens represent a considerable variety of structures. Figure 4 shows several structural types other than polycyclic hydrocarbons. The histories of these illustrate further the insidious hazard that chemical carcinogens represent, as well as their ubiquitous presence.

Aromatic amines

These have the general structure H_2N -aryl or (alkyl)₂N-aryl; attachment of an amino or dimethylamino grouping to an otherwise inactive, low-molecular-weight aryl moiety often confers powerful carcinogenic activity. 2-Naphthylamine and benzidine were extensively used before 1960 as intermediates in the manufacture of certain textile dyes. They are powerful bladder carcinogens to man and animals, and account for the fact that dye factory workers have a bladder cancer risk 200–300 times higher than the population at large. Manufacture of these compounds is now tightly restricted.

2-Aminofluorene (as *N*-acetyl) was proposed for use as an insecticide in the early 1940's. It is indeed an excellent insecticide. However, virtually by accident it was discovered, fortunately before it was released for use, that it is a very powerful carcinogen toward most abdominal organs, the ear duct, and the mammary gland in rodents.

4-Dimethylaminoazobenzene, as its alternate name “Butter Yellow” indicates, was used to color butter and cooking oils up to about 1935, when it was discovered to be a powerful carcinogen toward

the liver and to some extent toward the skin in rodents. It is still in use as an industrial solvent dye. A great variety of azo dyes has been found to be carcinogenic. Many of the “certified food colors” are azo dyes, the relative safety of which was established before licensing.

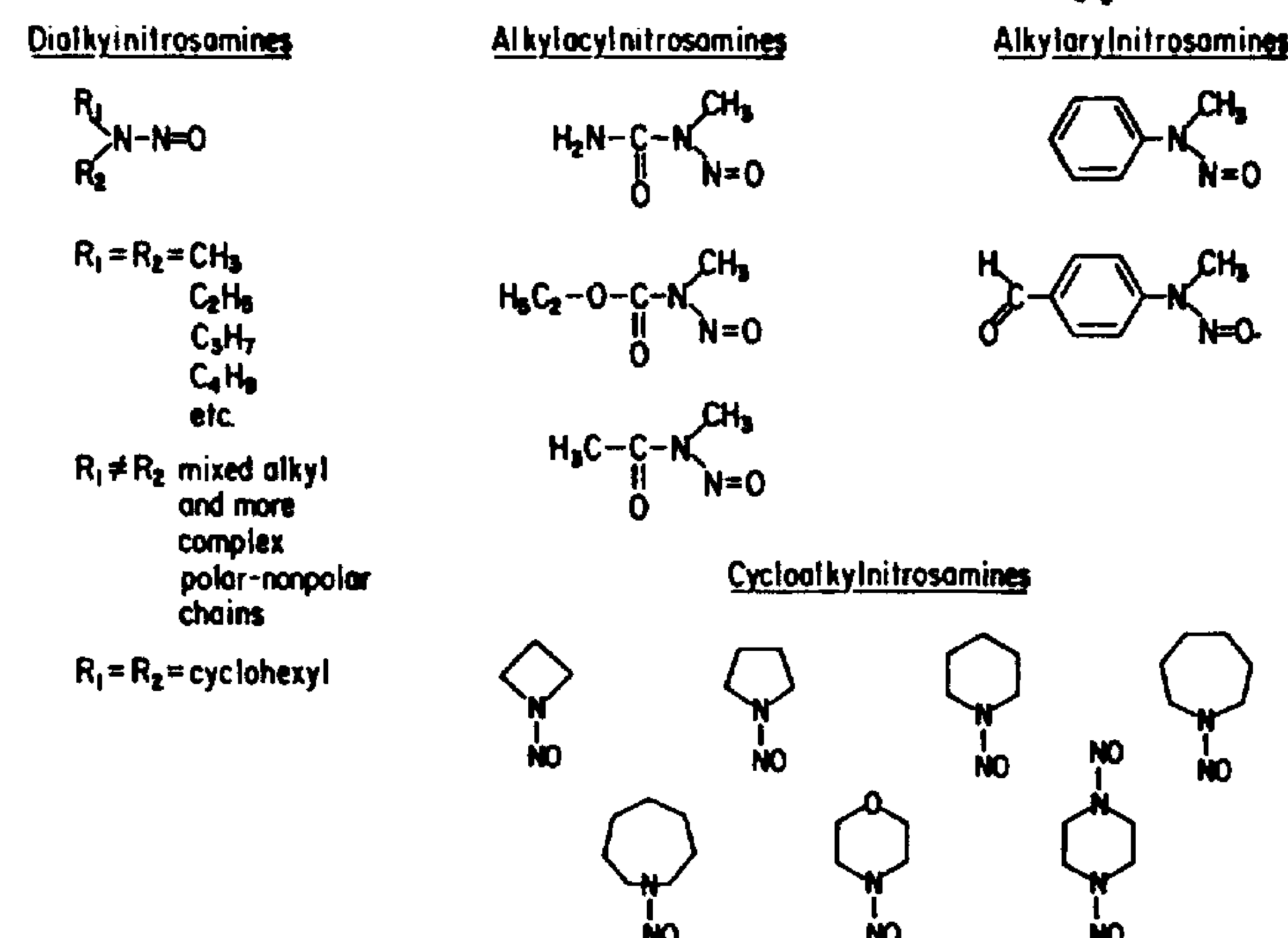
Aliphatic carcinogens

These are by themselves an extremely structurally heterogeneous group; their only common denominator is that they are chemically reactive as such or are transformed to chemically reactive agents (proximate carcinogens) by metabolism (see Part II of this article), and they interact with nucleic acids and proteins in the target tissue to bring about the change to malignancy. For example, β,β -dichlorodiethyl sulfide or sulfur mustard, as the compound is commonly known, was introduced in World War I by the German military high command as a potent vesicant warfare agent at the Belgian front, at Ypern, against the French troops. A number of the infantrymen who survived the gassing developed malignant lung tumors decades later. Subsequently, the compound was shown to be carcinogenic also in rodents. Carcinogenicity is maintained if the sulfur atom is replaced by an $-NH-$ group (nitrogen mustard). Various compounds in which a nitrogen mustard group is linked to another moiety were found to be carcinogenic.

Dioxane is a common solvent used in tank-car quantities in the chemical industry. It is extensively employed in laboratories in scintillation counting and has many other uses. It is a weak to moderately active liver carcinogen, active also to some extent toward the kidney and lung and toward the epithelium of the nasal passage in rodents.

Carbon tetrachloride, a medium-active liver carcinogen in mice and hamsters, was extensively used until not so long ago in the dry cleaning industry and was widely used in the chemical industry as an

Figure 5 Principal types of carcinogenic nitrosamines.



extractive solvent. Its close chemical relative, chloroform, is also carcinogenic. Chloroform was used several decades ago as a surgical hypnotic agent and until very recently as a component of cough syrups and ear drops.

Urethane (also known as ethyl carbamate) was used medicinally as a hypnotic agent; it is still used in veterinary medicine. This compound produces pulmonary tumors in mice, rats, and chickens; it also produces liver tumors in occasional animals.

Vinyl chloride is the starting material in the manufacture of the plastic polyvinyl chloride. It was also used until recently as the propellant gas, or component thereof, in spray cans. Recent industrial statistics demonstrated that vinyl chloride is a liver carcinogen to humans, and its carcinogenicity toward this organ was confirmed also in laboratory animals.

The insecticides aldrin, endrin, dieldrin, mirex, and DDT, the herbicide atrazine, and the polychlorinated biphenyls (PCB's) used as plasticizer additives (not shown in the figure), also belong in this structurally miscellaneous group. ~~These compounds are weak to moderately active in inducing liver tumors, lymphatic tumors, and leukemia in occasional animals.~~ The PCB's are among the most persistent organic chemicals in the environment.

Nitrosamines

In terms of total impact as environmental hazard, however, the chemical family of carcinogens most important next to the hydrocarbons comprises the

compounds known collectively as nitrosamines. Figure 5 illustrates the principal classes of carcinogenic nitrosamines. The common denominator of these compounds is that they all bear a nitroso group linked to a secondary nitrogen atom. Bioassay results with about 200 nitrosamines so far suggest that probably as much as 90% of all hypothetically possible nitrosamines will prove to be carcinogenic. The most common and widely distributed nitrosamine is dimethylnitrosamine. The primary tissue target of the carcinogenic action of dimethylnitrosamine in rodents is the liver; the secondary targets are the kidney and lung. Figure 6 illustrates the carcinogenic action of dimethylnitrosamine toward the liver in the rat. Figure 6a shows the exposed abdominal organs of a normal rat. Figure 6b shows the abdominal organs of a rat that received 0.4 mg of dimethylnitrosamine daily, 5 times weekly, for 28 weeks. Note the large hepatic tumor masses, increasing the overall size of the liver close to fivefold. These tumors are malignant (overwhelmingly hepatocellular carcinomas). Dimethylnitrosamine was shown to be carcinogenic in all the 16 species in which it was tested so far.

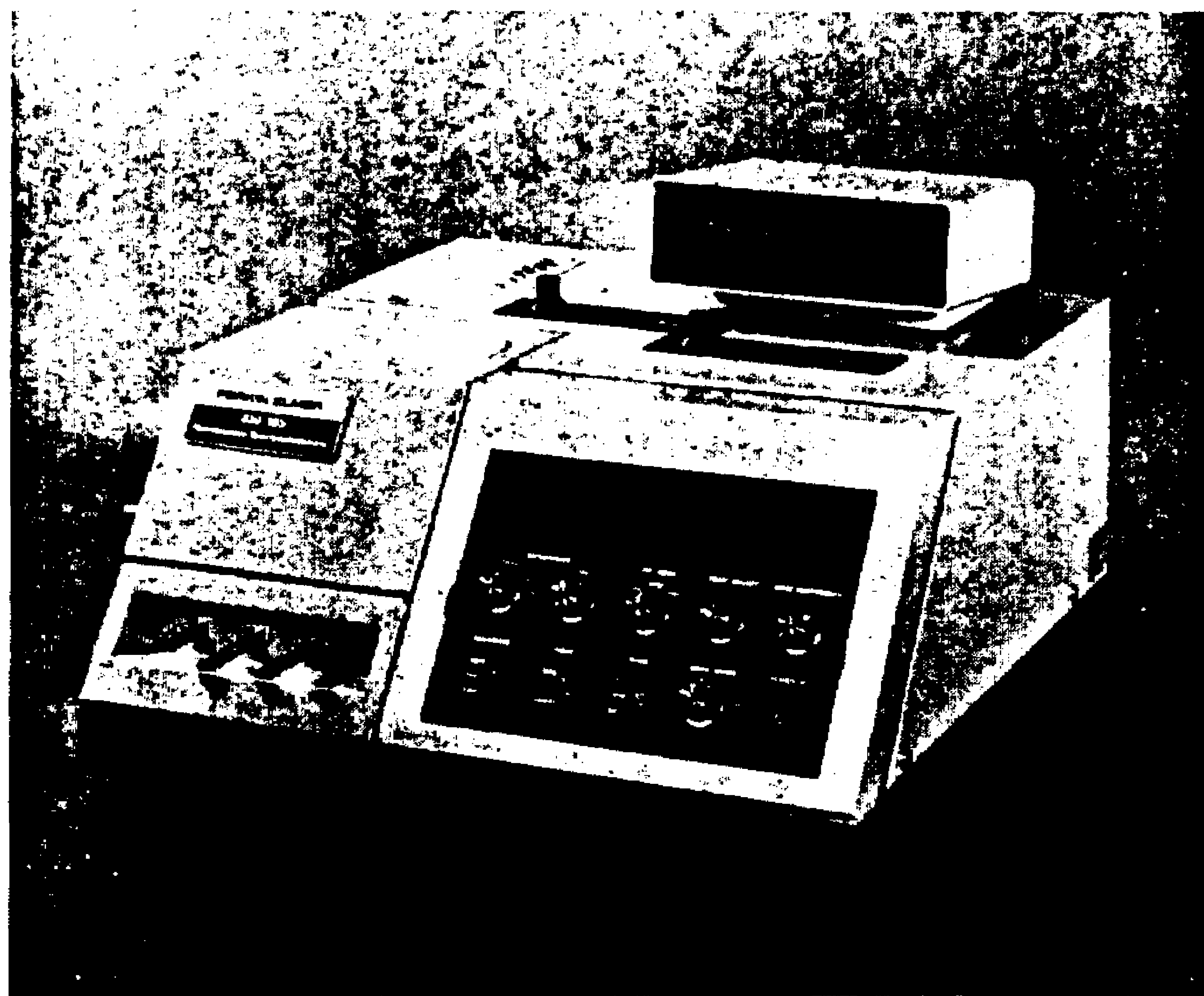
The nitrosamines are potent, multitarget carcinogens. Depending on the type of nitrosamine, the tissue targets attacked include the lung, liver, kidney, bladder, pancreas, stomach, and different regions of the nervous system; some induce leukemia.

Nitrosamines are formed with extreme ease from any secondary amine and nitrous acid; for example,



Figure 6 a) Abdominal organs of a normal rat and b) abdominal organs of a rat that was administered dimethylnitrosamine. In the carcinogen-treated animal, large liver tumor masses are seen.

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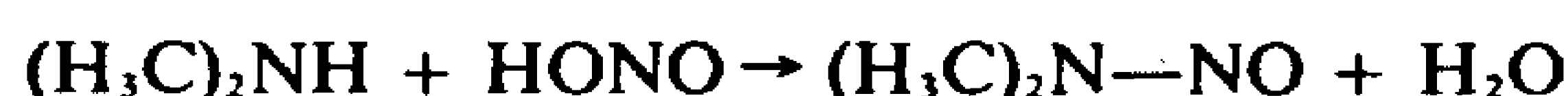
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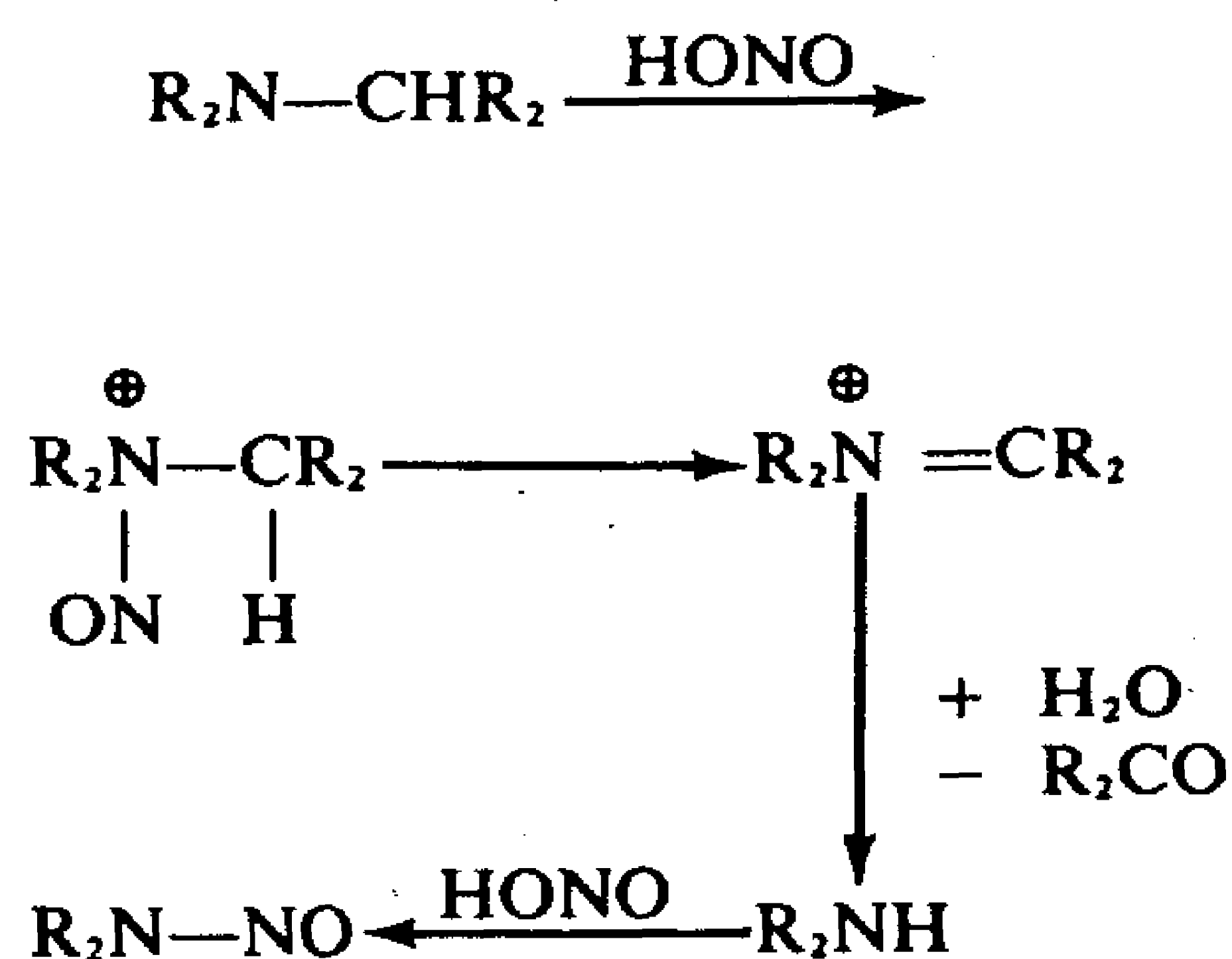
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the simple reaction



depicts the formation of dimethylnitrosamine. However, tertiary amines can also undergo nitrosation following the general reaction:



In the presence of nitrate-reducing bacteria, nitrates may serve as the source of nitrous acid. The nitrosation reaction is catalyzed by formaldehyde and thiocyanate ion, and is inhibited by ascorbic acid. There is evidence for photochemically induced nitrosation of amines by nitrogen oxides and this may be the

origin of dimethylnitrosamine detected in the atmosphere.

Consistent with the ease of formation, nitrosamines, and in particular dimethylnitrosamine, have been identified in a variety of consumer products, e.g., processed fish, certain cheeses, cured ham, and fried bacon. Moreover, most processed meat products contain sodium nitrite as an additive; processed meats and all fish also contain various secondary amines, sometimes at elevated levels. Nitrosamines that are already preformed are not usually found in commercially sold processed meat products. However, upon ingestion, owing to the low pH in the mammalian stomach, nitrous acid will be liberated from the sodium nitrite and will in turn rapidly react with the secondary amines also ingested with the meat, to yield nitrosamines. It has been amply documented that in rodents administration of a secondary amine *together with* sodium nitrite is just as carcinogenic as the preformed nitrosamine.

Part II of this article will discuss the naturally occurring carcinogens, the possible role of food formulation and preparation practices, the role of metabolism, the inorganic carcinogens, the cross-reactions between carcinogenic effects, and the external and internal factors influencing carcinogenesis. Part II will also provide a bibliography for further reading.

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