Environmental Health Perspectives
Vol. 29, pp. 45–48, 1979

Introductory Remarks: Session on Genetic Factors Affecting Pollutant Toxicity

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Occupational and environmental toxicologists are faced with the responsibility of providing sound scientific data that can be used to ensure individuals that they can conduct their daily activities without undergoing any undue risk which might potentiate the development of disease. With the majority of chemicals in the environment, it has been generally assumed that there is some safe level of exposure that is of no threat to human health. To be able to predict the absolute safe level is the sought after goal; however, the possibility of achieving this level of precision is remote, and in fact no chemical is absolutely safe. Thus, it becomes vitally important to carefully define the population at risk, the mode of exposure, and the lowest concentration that will cause an adverse health effect.

Great strides have been made from the days when the toxicologists were extrapolating low-level health effects from studies performed at high concentrations (LD_{50}), with single pollutant exposures (O_3, NO_2, SO_2), inadequate analytical and monitoring techniques, and "unnatural" routes of exposure.

It is now recognized that in many instances the action of one toxic agent can be modified by exposure to other agents. The possibility of various environmental pollutants interacting to cause effects that may be additive, synergistic, or antagonistic is always present. The inability to perceive ill effects from a single agent is now no longer a guarantee that, when combined with another test substance, an adverse health effect on the individual might not be noted.

In addition to those studies that use complex mixtures of chemical agents, more and more research is being designed to evaluate the possibility of interaction with other environmental factors, such as diet, temperature, humidity, other stressors, and infectious agents. Such studies are attempting to simulate the entire milieu in which man lives. Although scientists at the present time are unaware of the precise way in which host variations, diet, genetic constitution, or prior disease state directly influence the degree and type of toxicity experienced by the various individuals, they are in agreement that these factors are capable of modifying the host response and often increasing their susceptibility and vulnerability to disease.

Thus, environmental toxicologists are now conducting studies that are specifically designed to predict the health consequences resulting from exposure to complex mixtures. Practically every study utilizes normal, healthy, adult animals to model the human population at risk. However, compared to laboratory animals, humans are extremely heterogeneous with respect to the multitude of factors that can influence the biological effects of toxic chemicals. Animal toxicity studies must now begin to take into account various individuals who may be at higher risk with respect to the toxicological activity of the pollutant in question.

When a biological system encounters a large enough dose of any chemical, its defensive capacity will, at some point overcome, and this failure is usually accompanied by some change in function and/or structure. Often at low concentrations of exposure the significance of these changes may be only an expression of compensation of the individual's biological system to the chemical encountered.

However, it is now recognized that such subtle measured changes cannot be assumed to occur to

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the same degree or have the same consequence in every individual exposed. There are some genetic and environmental factors which may moderate or aggravate the degree of the host response. Many of these genetic abnormalities such as various disorders of the red blood cell (sickle-cell trait, thalassemia, glucose-6-phosphate dehydrogenase deficiency), blood serum (α-1-antitrypsin, pseudocholinesterase), homeostatic regulatory factors (cystinunia, cystinosis, tyrosinemia) and immunological deficiencies (immunoglobulin A hypersensitivity) have been shown to predispose individuals to toxic effects of various chemicals. The recent published book by Calabrese (*1*) has effectively reviewed the biological basis of increased susceptibility to environmental and occupational chemicals and has identified a number of specific high risk groups. Thus, there may be certain segments of the population that will manifest a severe response to chemical agents that would cause only minor, hardly significant changes in the majority of the people exposed. This conference has been convened to examine the biological basis of this increased susceptibility and to identify these individuals who, because of various biological factors, may be predisposed to certain environmental and occupational chemicals.

This particular session looks at a number of different types of studies that have been conducted to identify some of the high-risk segments of the population, especially with regard to some genetically determined degree of variability.

At the present time in our laboratory we are using an animal model to look at the possible extrapulmonary effects of ozone and the role that genetic factors may have in the development of increased susceptibility to ozone. Since this work has just begun, I can only illustrate the basic approach and present some preliminary data.

It was formerly believed that the action of ozone upon the respiratory tract was accompanied by the destruction or neutralization of the ozone and was not absorbed into the body. Evidence is accumulating that ozone exposure can also produce nonpulmonary effects, such as lymphocyte chromosome aberrations, sphering of red blood cells, lipid peroxidation of red blood cells, and the desaturation of oxyhemoglobin. However, it may be argued that these effects could have been produced by the action of ozone on these cells during their passage through the pulmonary capillaries, rather than the result of ozone or its reactive by-product acting at some distant site. Still, there are other extrapulmonary effects of ozone reported, such as structural changes in parathyroid gland and in heart muscle fibers, as well as neonatal mortality, that give evidence that the toxic action of ozone is not limited to the respiratory tract.

Our investigation examines the interaction between ozone and pentobarbital sleeping time which indicates that alteration in response to a barbituate can be brought about by a gaseous environmental pollutant and that this sensitivity may be genetically controlled. The precise technique employed in these studies has been described elsewhere by Gardner et al. (*2*). Briefly, groups of female mice (Charles River, CD-1 strain), 6 weeks old and weighing 21 $\pm$ 4 g were randomly selected to be exposed to filtered air or to a mixture of air and ozone. All exposures were for 3 hr (9:30 am to 12:30 pm) per day for periods up to 17 days. Immediately after the exposure, groups of mice were removed from the exposure chamber and immediately administered 50 mg/kg of pentobarbital sodium (Nembutal, Sodium, Abbott Labs., North Chicago, Ill.) by an intramuscular injection. Since environmental temperature and the circadium rhythmic cycle are known to influence sleeping time duration, all tests were performed from 1:00 pm to 4:00 pm in an environmentally controlled room where the temperature and humidity were carefully controlled.

Following the injection, two parameters were measured: induction time (time interval between injection of pentobarbital and the loss of righting reflex) and sleeping time (the elapsed time between the loss of righting reflex and the first time the animal righted itself spontaneously). Each animal was used only once and then removed from the study in order to prevent the influence of barbiturate tolerance or hypersensitivity.

In none of the studies did ozone have any statistical effect on the induction time following the pentobarbital injection. However, there was a significant increase in sleeping time as a result of exposure to ozone at concentrations as low as 0.1 ppm. This data is illustrated in Figure 1, which shows that as the concentration of ozone is reduced, the number of daily exposures required to cause an increase in sleeping time is increased. For example, at 5.0 ppm, a single 3-hr exposure produced a statistically significant increase in sleeping time of 35 min as compared to control. However, at the lowest concentration studied (0.1 ppm), no increase in sleeping time was evident until the animals had been exposed for 15-16 times. It is of interest to note that after the effect (increased sleeping time) reached a maximum, there appeared to be no further effect on sleeping time.

Goldstein and co-workers (*3*) have investigated the effects of O_3 on 21 different strains of inbred mice and have observed a genetically determined difference in response to lethal levels of this oxidant

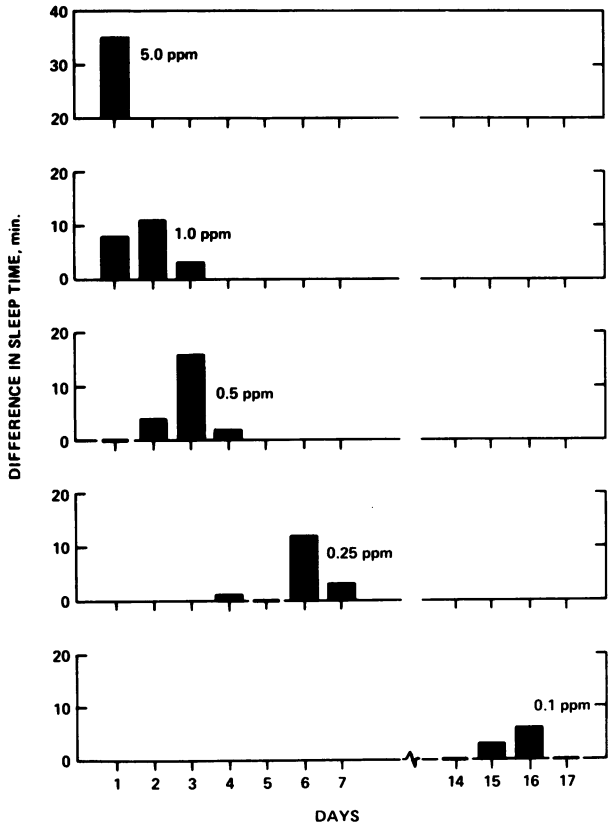

FIGURE 1. Effect of O_3 on sodium pentobarbital-induced sleeping time in CD-1 mice.

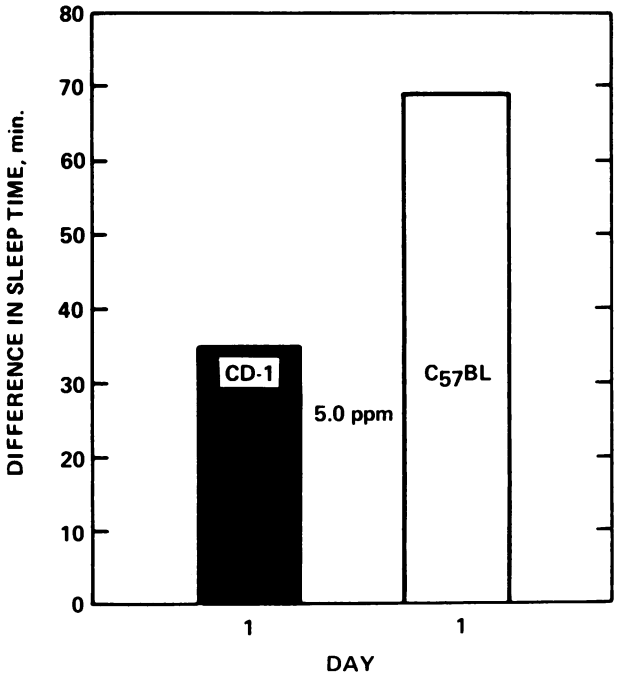

FIGURE 2. Effect of O_3 sodium pentobarbital-induced sleeping time in CD-1/C_{57}BL mice.

gas. The mice used in the above study (CD-1) were the most susceptible to the lethal effects of O_3.

Thus, in our study, the next step was to select a strain of mice that was more resistant to the action of O_3. The C57BL strain was chosen since it required a much higher mean ozone concentration to produce the same effect as that found for the CD-1 mice. Thus, since this strain was more resistant to oxidant effect, it was originally hypothesized that these animals would sleep less than the more sensitive CD-1 mice.

The length of sleeping time following a 3-hr exposure to 5.0 ppm O_3 is given in Figure 2. In this study, the C57BL strain was the more sensitive to the ozone exposure since they slept twice as long as the CD-1 animals when each was compared to their appropriate nonozone exposed controls.

In attempting to analyze this apparent paradox, it is important to understand that the duration of sleeping time induced by a barbiturate is primarily correlated to the biotransformation of the drug in the liver. The group of enzymes which would be expected to be involved are collectively known as the cytochrome P-450 system. Various studies have provided substantial evidence that the cytochrome P-450 system is genetically controlled (*4–6*). Pronounced strain differences in the oxidative metabolism of drugs such as hexobarbital and pentobarbital has been shown. Thus, the possibility exists that the two best strains used in these studies may be different in this respect. At the present time, our laboratory is currently in the process of comparison of the mixed function oxygenaces in these two strains.

If a difference in cytochrome activity is present, it could be expected to influence the intensity and duration of the drug action. Therefore, if O_3 or some reactive by-product has an interfering effect with the functioning and/or concentration of these hepatic enzymes, then one might hypothesize a difference in sensitivity of the C57BL strain to the action of O_3. It is important to note that this last study is preliminary in nature. Studies are under way which will provide dose-response data so that a better comparison between the two strains of mice can be made. In addition, we are collaborating with Dr. Daniel Menzel, Duke University, to investigate the mechanism that the oxidant effect has on pentobarbital induced sleeping time. Menzel et al. (*7, 8*) have demonstrated that certain partially oxidized species, such as fatty acid ozonides, could be responsible for systemic effects by peroxidation of hepatic membranes.

The possible implications of these effects of O_3 vary according to whether the alterations are sec-

ondary to lung damage or whether specific enzyme injury at distal sites occur. If specific enzyme damage does occur, the health consequences may be far-reaching. These membrane-bound enzymes are responsible for metabolizing a wide range of xenobiotics, such as polycyclic aromatic hydrocarbons, insecticides, aromatic amines, chemotherapeutic agents, drugs, steroids, strong mutagens and numerous other compounds. These enzyme systems may detoxify a reactive compound to an inactive product or may metabolically potentiate the detrimental effects of a parent compound by converting it to a reactive or toxic intermediate. It is thought that within the tissue there is a keen balance between enzymes that potentiate and those which detoxify. It has been shown that this relationship may be effectively altered by differences in genetics, age, nutritional and hormonal levels and now perhaps environmental chemicals at lower concentrations (0.1 ppm), such as ozone, may also upset this delicate balance.

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