EX 1712
LEGACY 1

(17)

Biological and Ambient Monitoring of Benzene in the Workplace

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Surveillance of workers potentially exposed to benzene is focused on detecting evidence of bone marrow toxicity. A particularly thorough hematological evaluation should be conducted upon entrance to the work force at risk. This should be followed by semiannual surveillance using the routine complete blood count as the major monitoring technique. Attention should also be placed on increases in the mean corpuscular volume and decreases in the lymphocyte count as possible early indicators of benzene toxicity. Other hematological findings that may indicate early benzene toxicity depend upon alterations in the characteristics of blood cells, eg, a prolongation of the red cell glycerol hemolysis time, need to be more fully evaluated. No single finding or constellation of findings is pathognomonic of benzene hematotoxicity, and all findings must be carefully interpreted in terms of the individual or group at risk.

Biological monitoring of workers as a tool to detect early evidence of benzene toxicity has been in use for close to half a century. During this time, the workplace standard for exposure to benzene has been made more stringent as evidence has accumulated of its hematotoxic potency. It is anticipated that the new workplace standard being developed by the Occupational Safety and Health Administration (OSHA) will decrease allowable workplace levels, perhaps by an *order* of magnitude, from the current 10 ppm (time-weighted average). The difficulty in establishing a workplace standard is not only in determining what the allowable exposure level should be, but what screening and routine medical surveillance should be conducted for workers who are exposed or who have the potential to be exposed. A related question, presenting perhaps even more difficulty, is what should be stated in the regulations concerning the definition of abnormal biological monitoring data and the necessary response to such a finding.

In the case of benzene, a major problem in using laboratory tests for screening and surveillance is that there is not a specific end point definitive of benzene hematoxicity. It first is necessary to determine the tests to perform, to decide how often they should be conducted, and to determine the baseline normal values. Very often, these determinations are complicated by the fact that we are using tests in a public health setting that were really designed for use in a clinical setting. A related problem, present in many existing biological monitoring programs, is confusion between the different strategies needed to fulfill two separate aims: the clinically oriented approach of detecting persons who have been significantly affected, and the more public health-oriented approach of detecting subtle changes in the work group as a whole.

Most deaths from workplace exposure to benzene are a result of acute effects on the CNS. The neurotoxic effects of benzene are very much like those of all other alkylbenzenes. Structure activity relationships, molecular weight, boiling point, and other physicochemical characteristics can be invoked to explain the slight differences in neurotoxic potencies of these compounds. However, of all the alkylbenzenes, only benzene damages bone marrow and produces a hematological effect.[1-3]

The hematological disorders associated with benzene are described in the Table. Although opinion differs about the ability of benzene to cause some of these disorders, there is complete agreement that benzene causes aplastic anemia. This has been known since the nineteenth century. Benzene also causes acute myelogenous leukemia (AML) and its variants, including erythroleukemia and a variety of other leukemias, all of which fit under the rubric of acute myelogenous leukemia.[1] The evidence for these effects is primarily from epidemiological and case studies. Myelogenous leukemia

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0096-1736/86/1010-1061$00.00/0

Table
Association of Hematological Disorders With Benzene Exposure

Causality proven
Aplastic anemia (pancytopenia)
Acute myelogenous leukemia (including variants such as erythroleukemia)
Causality likely
Chronic myelogenous leukemia
Chronic lymphocytic leukemia
Paroxysmal nocturnal hemoglobinuria
Hodgkin's disease
Myelofibrosis and myeloid metaplasia
Causality suggested
Acute lymphocytic leukemia
Non-Hodgkin's lymphoma

has also been reported in laboratory animals exposed to benzene, but the findings have not been conclusive.[4]

The inherent difference between aplastic anemia and AML as a consequence of exposure to benzene must be considered in the selection of appropriate surveillance. Aplastic anemia due to benzene has not been demonstrated in situations where people are routinely exposed to levels within the current OSHA standard. A high dose of benzene seems to be necessary to cause this disorder, and it is reasonably clear that there is a threshold for its induction in animals and in humans. Although the threshold response for aplastic anemia can be replicated in animal studies, there are lesser degrees of bone marrow damage (pancytopenia) that may extend to levels that are not clinically apparent. In view of the large bone marrow reserve, hematopoietic stem cells can be affected without a decrease in circulating blood count below the normal range.[1] Whether there is a threshold for clinically inapparent hematoxicity caused by benzene is unknown.

When considering the concept of threshold, one must clearly distinguish between the population threshold, as a reflection of the average human, and the individual threshold for sensitive persons. The threshold for sensitive workers is of particular concern to public health officials and will always be lower than the average population threshold. One can consider two distributions of the population of individual thresholds: a normal bell-shaped curve in which standard expressions of variance will describe the statistical relation between the average person and more susceptible persons; and a biphasic distribution in which there is a subset of particularly sensitive persons, perhaps related to genetically determined differences in metabolism. For benzene, there is no clear-cut evidence for the existence of a specific subset of sensitive persons, in contrast to the idiosyncratic reaction observed with chloramphenicol, where a few persons exquisitely sensitive to small doses of the drug develop a severe aplastic anemia. Although some data on benzene suggest a familial tendency, and perhaps an effect of sex and of body size on susceptibility, there is not good evidence of an exquisitely sensitive group.[1,5,6] This simplifies the approach to biological monitoring of benzene.

When the disease of concern is acute myelogenous leukemia, instead of aplastic anemia, we are talking about cancer. For regulatory purposes, in the United States we usually assume a linear no-threshold dose-response curve for cancer. In essence, once a chemical is shown to be a carcinogen, it is treated as "guilty until proven innocent" of having no threshold for this effect in humans. However, it has been argued that benzene could be "innocent," that the dose-response curves for leukemia and aplastic anemia are the same, because aplastic anemia, or at least clinically overt pancytopenia, is a necessary precursor of leukemia. However, there is no convincing evidence for this supposition. Therefore, the prudent public health approach in a case such as this, where we do not have convincing evidence either way, is to assume the worst case, that is, that there is no threshold, and therefore any one molecule of benzene has a finite risk of producing acute myelogenous leukemia.

There is evidence in animals of toxicity to bone marrow stem cells occurring from exposure at or below the current standard of 10 ppm in an eight-hour day.[7,8] Animals exposed to benzene at 10 ppm, for six hours each day, five days every week for 6 months have developed evidence of stem cell damage. Therefore, it is clear that stem cells are affected at the current OSHA standard, which supports decreasing the standard, no matter which dose-response curve is considered to be accurate.

Detection and measurement of benzene in blood are not reasonable indicators of benzene exposure because of the relatively rapid half-life of disappearance of benzene: 50% is exhaled unmetabolized.[9] Further, in animal studies, it has been shown that the rate of disappearance of benzene in the blood varies according to the number of times the animal has been exposed.[10] Assuming that this holds true for humans, blood levels of benzene cannot be used except to indicate recent exposure.

Another approach to biological monitoring, measuring metabolites in body fluids, presents another problem. Benzene itself is not hematotoxic, but we do not know which of its metabolites is responsible for hematotoxicity. The work of Irons and his colleagues has suggested a role for a polyhydroxylated derivative of phenol, a major metabolite of benzene.[11] We have suggested that an opened-ring product of benzene participates in hematotoxicity.[12] Urinary phenol has been used as an indicator of benzene exposure. In rabbits, approximately one quarter to one half of administered benzene is metabolized to phenol, and roughly the same proportion is metabolized to phenol in humans. Therefore, urinary phenol is a reasonably good indicator of recent benzene exposure. However, it is not itself hematotoxic. There are other sources of urinary phenol, including dietary. When exposures are to relatively low levels of benzene, the background level of phenol obscures the benzene exposure. Measurement of phenol may be useful to determine whether someone has been recently exposed to high levels of benzene, but it is not likely to be useful for screening at the workplace, particularly if the benzene standard is lowered. There is no evidence that any

other metabolite will be of value unless we can identify the metabolite that is responsible for hematotoxicity.

Before discussing the complete blood count, the biological monitoring approach usually used to determine exposure, it would be useful to look at other end points that have been reported in the literature to be early indicators of benzene hemotoxicity. One indicator is simply that the red cells get larger (macroerythrocytosis). This occurs fairly early in the course of benzene poisoning in humans and in laboratory animals.[13,14] There really is no satisfactory explanation why the red blood cells enlarge, although it is tempting to relate the change to macroerythrocytosis present in disorders such as folic acid deficiency or pernicious anemia, where there is interference in DNA metabolism. Macroerythrocytosis is readily detected as an increase in the red cell mean corpuscular value (MCV), routinely reported in automated blood cell counting readouts or obtainable by dividing the hematocrit by the red cell count. A major problem in using enlarged red blood cells as an early indicator of benzene exposure is that there are competing causes. For example, macroerythrocytosis is very useful as an early indicator of alcoholism.[15] Other causes of large red cells include liver disease, hypothyroidism, and B_{12} and folic acid deficiencies. Despite the difficulty in distinguishing the cause of macroerythrocytosis, it remains a useful confirmatory tool in combination with other indicators of benzene hematotoxicity.

Another early indicator of benzene poisoning is lymphocytopenia.[1,16] In the bone marrow, the primary stem cell that is affected in benzene toxicity is the stem cell responsible for platelets, granulocytic white cells, and red blood cells. The lymphocytes are also severely affected and, in studies of occupationally exposed groups where benzene toxicity is clearly evident, lymphocytepenia has been a relatively early and consistent finding.[16] In rodent.. lymphocytopenia is tho earliest finding, occurring before granulocytopenia.[17]

A major problem with using lymphocytopenia as a diagnostic aid is the large degree of inherent error in measuring total lymphocytes based on the usual white blood cell differential in which only 100 cells are counted. A lymphocyte percentage of between 20% and 35%, for example, is not important to the clinician. Although a count of below 10% may implicate a variety of diseases, the normal range and tho inherent variability in tbc count are so large that it is not possible to use this parameter in a program aimed at early detection of benzene effects.

What may be of value b those who plan and design tests for studying workers exposed to benzene is that automated counting devices for tho white cell differential count are coming bb use. If 1,000 white blood cells are counted, instead of 100, the inherent variability in the lymphocyte count may be sufficiently lowered so as to be useful in surveillance of a work force exposed to benzene. Using automated equipment, the incremental costs for counting more white blood cells than the 100 needed for clinical determinations may be justified.

Although there is no question that benzene produces cytogenetic abnormalities, results are negative in the Ames test and related in vitro tests for mutations.[18–20] The question is whether cytogenetic tests are valid as screening tests. In my judgment, there are so many problems with using cytogenetic abnormalities as the basis for screening that its use has not yet been justified in the work force. However, a number of new and elegant approaches to studying chromosomes have yet to be used in medical screening. These warrant exploration.

An alteration in serum immunoglobulin levels has been reported in Eastern European workers exposed to benzene which might reflect lymphocytopenia.[21] Altered porphyrin levels, and both decreased and increased leukocyte alkaline phosphatase activity have also been reported in workers.[1] My laboratory previously found marked prolongation of red cell glycerol hemolysis time in mice exposed to benzene.[22] This may be related to whatever is causing the large red cells. The validity of this test in humans exposed to benzene has not been evaluated because, fortunately, it is now hard to find a group of workers poisoned with benzene. If there were a large work force with benzene poisoning, the red cell glycerol hemolysis test could be tested for its value in predicting benzene toxicity.

Undoubtedly, the most important biological monitoring test of benzene hemotoxicity at present is the complete blood count (CBC), including a platelet count and a white cell differential as well as the usual CBC components: white blood cell count, red blood cell count, hemoglobin, hematocrit, and red cell indices. It must be emphasized that these tests have been developed for the clinician, not for public health screening.

Major problems with the CBC are the wide range of "normal" values and the lack of specificity of findings just above or below this range of normal. If we are to be serious about surveillance, we are going to have to pay attention to minor variations in blood-cell counts. Failure to understand these changes may lead us to remove people from the work force for inappropriate reasons, or ignore early indicators of benzene-induced effects.

In my opinion, surveillance in the work force exposed to benzene cannot be useful in *detecting* early cases of acute myelogenous leukemia. The natural history of acute myelogenous leukemia is such that even if blood tests are conducted every 3 months, it will not be detected early in its course, particularly because there is at least a week or two between the time the white count is taken and the physician talks to the worker. By that time, if the person really has acute myelogenous leukemia, the usual explosive development of clinical symptoms will mean that the worker will have come to medical attention in all but the rarest of cases. Accordingly, the aim of surveillance in the work force exposed to benzene should be to *prevent* AML, not detect it.

One aim of biological monitoring is to detect an early indication of a benzene effect that should have been prevented by the usual type of primary prevention—ambient monitoring. Ambient monitoring, although absolutely necessary, will present difficulties in identifying local hot spots: the worker who is splashing benzene on

skin, the person who has bad work practices, and the undiscovered leaks.

Another aim of biological monitoring is to protect the worker; it would be most helpful to have a specific laboratory marker, for example, a blood count below which we must start looking very closely at what is happening to the worker to be sure that he or she is not being adversely affected.

Choosing the numbers is not difficult, but the choice must be recognized as an arbitrary one. It is even less useful to look at just one count at a time. Here again, the importance of clinical judgment must be emphasized. If a worker has a white count of 4,500/μL, the appropriate response would be to look at the red count, the platelet count, the mean corpuscular volume (MCV), and the lymphocyte count at the same time. Benzene would be expected to produce a pattern of response: eg, the likelihood that a white count of 4,500 is due to an adverse effect of benzene rather than due to a normal statistical variation will be much greater if the platelet and red counts are at the lower rather than upper range of normal. Although that seems obvious, in some workplaces it is difficult to obtain those data because the computer has not been programmed to do so.

It is also necessary to be alert to the fact that there could be a worker whose white count is normally 10,000 μL, whose platelet count is normally 300,000 μL, and whose hematocrit value is normally 50% (ie, at the upper ranges of normal). These numbers could fall precipitously and still be within the normal range. In order to detect such a benzene effect, it is necessary to have a surveillance program to look for this type of change, one that follows each person over a period of time.

It is a challenge to those who write computer programs and design surveillance approaches to take into account as much as possible the variety of effects that occur. It is also a challenge to realize that finding an abnormal count must lead to an analysis of all the data. We must be aware that apparently abnormal data can actually hide normalcy, and data that are supposedly within the normal range can hide an adverse effect.

Let me conclude by posing what to me is the most difficult question that OSHA may be facing right now: what to do about the affected workers. Suppose that a worker has a white count that drops down to 3,500 μL following known exposure to an inappropriately large amount of benzene. If the person is removed from that work environment, rapid clinical recovery would be expected, based on what is reported in the literature. However, do you let the person return to a work environment with a potential for benzene exposure, even after correcting the cause of the untoward exposure? In the situation where it is not known whether benzene is responsible, but the laboratory data are suggestive, what should be done? It is prudent to temporarily remove such a person from the potential exposure. However, in the absence of conclusive evidence, do you let that person go back to the same workplace? What is the dividing line? There is no simple answer. What is necessary is the use of informed judgment on the part of occupational health staff in an open process that includes discussion with the worker.

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