FILE NAME: Exxon (EXX)

DATE: 1970 Oct

DOC#: EXX046

DOCUMENT DESCRIPTION: Journal Article - Innovations in Occupational Health: Toxicological Evaluation of Industrial Chemicals - Industrial Chemicals

Toxicological evaluation of industrial chemicals is increasingly sophisticated. Relatively simple tests once considered adequate are no longer enough today. The situation as well as the role of the occupational health worker in relation to it are discussed.

INNOVATIONS IN OCCUPATIONAL HEALTH: TOXICOLOGICAL EVALUATION OF INDUSTRIAL CHEMICALS

Robert E. Eckardt, M.D., Ph.D., F.A.P.H.A.

THERE used to be a saying that "If you develop a better mousetrap, the world will beat a path to your door." Today industry spends billions of dollars in research attempting to develop better products to get the world to beat a path to its market outlets. In the course of this research it has been estimated that 10,000 new chemicals are synthesized every year. Obviously, not each of these is a completely new chemical—some may represent a synthesis of a material previously identified in nature; others may represent modifications in the structural formulas of previously known compounds in an attempt at improvement. Not all of these new chemicals reach the market place.

Ehrlich synthesized 606 compounds before he found his first clinically useful antisyphilitic drug and had reached 909 before he was able to successfully improve 606. What happened to the remaining compounds is not recorded, but the likelihood is that they were completely abandoned. Industrial experience today is not so different from that of Ehrlich. Our company, for instance, synthesized 406 compounds before they found an effective antifungistat for agricultural use, now known as Captan.

There was a time when an individual or a company could develop a new product and put it on the market without any consideration for its toxic properties. However, many years ago certain responsible industries began to look at the toxic properties of their new products, just as they looked at other properties such as melting point, boiling point, viscosity, and flash point. In part this began as a recognition of product liability claims, but also in some companies, at least, as a recognition of a responsibility that the company had to face up to. Today, particularly as this concerns direct or indirect food additives, potentially hazardous household products, and pesticides, statutory requirements for toxicity hazard evaluation have been spelled out by federal regulations. In our company responsibility for review of new product toxicity considerations was delegated to our Medical Research Division about 20 years ago—long before this was required by government regulations. DuPont with its Haskell Laboratories, Dow Chemical with its Division of Biological Research, and Union Carbide with its Carnegie-Mellon Fellowship are all examples of corporations which recognized this responsibility before there were regulatory requirements. The increasing number and expanding size of private toxicological testing laboratories indicate that other smaller companies, who might not be able to justify their

own in-house toxicological testing programs, are increasingly aware of these problems and are seeking assistance with them.

Chemical Environment

Several committees of the U. S. Congress have held extensive hearings and issued reports, either directly or indirectly through groups of experts, on the importance of our chemical environment in health and other matters. Occupational health, therefore, must increasingly concern itself with potential product toxicity problems and with the impact of the total environment, as well as the occupational environment, on the health of the people. Increasingly, occupational health personnel are having to devote more and more time to actual or potential health problems occurring beyond the confines of the plant boundaries. Although perhaps not an innovation, this is one of the more significant changes in occupational health practice.

More and more occupational health personnel are being called upon to consult with and advise management on toxicological problems. Concurrently many innovations are being introduced into toxicological testing programs, so occupational health personnel are not only having to learn a new field but a field that is constantly expanding and changing.

When a chemist or chemical engineer announces the synthesis of a new chemical, the first step in toxicological evaluation is usually the determination of an oral LD_{50}. This test is usually conducted by administering graded doses of the new chemical to groups of five or more rats and observing the resultant mortality. The actual LD_{50} is determined by plotting deaths against dosage on log probability paper. Thus dosage is usually varied by a log factor, and the data plots out in a straight line from which the LD_{50} may be read. It is important to remember that the LD_{50} determined in this way is not a hard and fast figure but may have a considerable standard deviation. Further, different laboratories may come up with different LD_{50}'s because they use different methods, i.e., use of different strains of animals, fasted or fed animals, varying postdosage observation periods, and so on. A recent report[1] compared LD_{50} determinations on a series of compounds in a series of laboratories and emphasizes this variability. At best the LD_{50} is an inexact figure which provides the toxicologist with an indication of the degree of toxicity of the new chemical, i.e., is it highly toxic, moderately toxic, or essentially nontoxic. It is important to realize that adequate testing does not consist solely of counting dead animals. Observations of the behavior of the animals by trained observers at appropriate intervals after dosing may give important clues to toxicological mechanisms.

Sometimes the chemist will have synthesized so little of his compound that innovations may have to be introduced into the LD_{50} determination. Mice may have to be substituted for rats, two animals per group used instead of five or ten, and the intraperitoneal route substituted for the oral. It should always be remembered in such cases that a more adequate LD_{50} determination should be obtained when a sufficient amount of the new chemical becomes available.

Expanded Toxicity Testing

If the new chemical continues to look promising for some application by the company, larger quantities will be made available for expanded toxicity testing. Tests for dermal absorption and skin irritation are performed. Rabbits are generally the species of choice. The material is applied in graded doses to the clipped intact and abraded skin in such a way that the animals cannot lick it off and so complicate the experiment by oral intake. The chemical is also tested for eye irritation by instilling 0.1 cc or a solution of it into rabbit eyes. At

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laboratories on several differ
pounds.[2] The results of these
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consideration, depending upon its
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the preceding tests. Thus 90-day
feeding studies usually done in rats may
be made. Two-year or lifetime feeding
experiments usually using rats and dogs
may be indicated. More recently
primates are increasingly used as the
second species in this type of test. At
the conclusion of either of these tests,
sacrifice of the surviving animals and
careful study of gross and microscopic
pathology of as many as 24 tissues are
usually carried out. During the actual
testing, studies may be done periodically
of blood counts, urinalyses, and certain
enzyme systems in the animals. Thus
cholinesterase, SGOT, liver microsomal
enzyme systems, and other enzymes or
body constituents may be studied. Also,
if an analytical method exists for the
chemical, measurements in blood and
other body tissues may be carried out.

Recently, as a result of the study of
such enzyme systems, it was found that
lead interferes with delta-aminolevulinic
acid (dALA) dehydrogenase.[3] As a re
sult, delta-aminolevulinic acid accumu
lates in the blood from which it is re
moved by the urine and excreted. Al
though more work needs to be done on
this, measuring the dALA in the urine
may be a more sensitive test for lead
exposure than either blood or urinary
lead determinations. In addition, it is a
far simpler test to perform and not so
subject to contamination. Where lead
determinations are being made, lead is
usually being used and there is always
a great danger of contaminating the
blood or urine sample with lead.

If warranted, complex metabolic
studies may be carried out. In these,
all urine, feces, and expired air are
collected and analyzed for the chemical
or its metabolites. Frequently radio
isotope techniques are employed which
offer a degree of sensitivity that may
not be achieved with standard analytical
techniques. Metabolic studies have
shown that a metabolite of benzene,
namely phenol, is excreted in the urine;
therefore, it has been proposed[4] that
urinary phenol excretion be used as a
measure of benzene exposure. The de
velopment of the gas chromatograph
has made urinary phenol determinations
accurate and quite specific.

If use of the new chemical is apt to
involve frequent skin contact, the po
tential for cumulative toxicity by the
dermal route must be determined. This
can be done conveniently in rabbits by
repeated skin applications. This may or
may not be followed by a challenge dose
to test the possibility that the compound
may be a skin sensitizer. Often, how
ever, the guinea pig is used as the ani
mal of choice to test the skin sensitizing
properties of a new chemical.

Depending on anticipated use of the
chemical, carcinogenesis testing may be
indicated and undertaken. The procedure
has to be designed on the basis of the
suspected site of carcinogenesis. Thus

materials suspected of containing polynuclear aromatic hydrocarbons are usually tested by painting the shaved skin of groups of mice. This may be done two to three times weekly; inbred strains are frequently used. Amino-azo and nitrosamine compounds which may lead to liver cancer formation are more appropriately tested by feeding studies in rats and mice. Some amino compounds may lead to bladder cancer. Such tests usually use the dog since rats and mice may not metabolize the compound to a carcinogen. Recently, pulmonary cancers have been produced by suspending a PNA on hematite which is then injected intratracheally as a suspension into the Golden Syrian hamster.[5] Thus it can be seen that there is no "Standard Carcinogenic Test." Testing for carcinogenesis will have to be done in an intelligent fashion based upon one's index of suspicion of carcinogenic action.

Other tests that may be indicated in special situations are teratogenic or reproduction tests. The demand for teratogenic tests arose as a result of the thalidomide episode. The rabbit is usually used in this test and the compound administered within a specified period during pregnancy. Obviously the pregnant females are sacrificed near term and the fetuses examined carefully for teratogenic effects. In the reproduction studies, rats may be carried through three generations, measuring the number of pregnancies, the number of live offspring, the ability of the mother to nurse and wean the litter, and the number of deaths among the litters—all this while the compound is being administered. A variation of this test may be to measure the egg production and fertility of the eggs in chickens to which the material is being administered.

Cosmetic Testing

When the chemical is intended for use on human skin, as for instance in a cosmetic, testing may actually even be carried to the human being. Thus material may be patch-tested in hu beings and, by subsequently applyin challenge, patch-tested for its ability cause skin sensitization. By using pa of human beings known to have a s tization to a particular compound, possibility of cross-sensitization can tested; finally, actual use tests may conducted with human beings.

A few years ago our company veloped a compound which would m women's lips stay moist-appearing, w added to lipsticks. We tested this c pound in a double blind experiment a group of women who were given ei the lipstick with the new chemical i or a control lipstick without it. The perimenter himself did not know w was which. The women were told to the lipstick for two weeks, then to t it in for the other one. They were asked to compare the two lipsticks to include any irritation they exp enced. Not only did this give us a cha to evaluate consumer reaction to and ceptance of the new compound in the stick, but also to see if the new c pound would cause any lip irritat This use test was quite valuable s we had received some reports that compound might cause a blistering the lips. Detergent manufacturers to usually ask a panel of women (for a f to put their hands—for several hour into a 5 per cent solution of a new tergent before they put it on the mar Also other panels will actually use it a report any skin irritation they obser

Subacute (90-day) or chronic (t year or lifetime) inhalation toxicity t may also be undertaken. These tests complicated and difficult to perform an must be accompanied by engineering and analytical support to assure accura determination and control of exposur atmospheres. Usually multiple specie are used and may include rats, rabbit guinea pigs, dogs, or monkeys. Recently the donkey has been recommended as an experimental animal for

certain types of inhalation studies.[6]

If water pollution is a concern, shrimp and certain fish species may be used. Here concentrations may have to be quite low because these species may exhibit exquisite sensitivity to certain compounds. Also it may be necessary to test the effect of the compound on ducks and other wildlife, not only from the standpoint of the ability of the duck to swim but also for toxic effects. A recently discovered toxin, aflatoxin, is now known for toxicity through its ability to induce liver damage in ducks.[7] This toxin is present in moldy ground peanuts. It is produced by the mold, Aspergillus flavus, which can grow on peanuts and other similar materials after harvesting.

Russian toxicologists have been devoting a lot of study to the effect of chemicals on certain higher nervous functions, e.g., effects on certain conditioned reflexes, the chronaxie of the optic nerve, and so on. The significance of their observations is not fully understood at this time but, increasingly, more of such experiments are being performed in this country in an attempt to understand the implications of the Russian findings.

I have not exhausted the list of possible tests used to evaluate the toxicity of industrial chemicals. Others would include effects on tissue cultures, effects on paramecia, and even effects on domestic commercial animals such as cows, horses, sheep, pigs, and others. It seems that the more we study toxicology the more innovations are introduced. It is possible that 10 or 20 years from now someone could discuss the same topic and barely acknowledge the approaches we are using today.

Summary and Conclusions

The toxicological evaluation of industrial chemicals is becoming an increasingly sophisticated exercise. Whereas once it may have been considered sufficient to have oral and skin LD_{50}'s and inhalation LC_{50}'s, today the range and variety of tests are constantly expanding as innovations are introduced to meet growing needs for safety evaluation. The occupational health worker must understand the utility of these as well as the limitations and shortcomings since, increasingly, he is asked to advise and counsel management in the total environmental health field.

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This paper was presented before a Joint Session of the Industrial Medical Association and the Occupational Health Section of the American Public Health Association at the Ninety-Seventh Annual Meeting in Philadelphia, Pa., November 11, 1969.