EXPOSURE TESTS IN INDUSTRIAL TOXICOLOGY

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The purpose of exposure tests for industrial poisons or other hazards is to determine if sufficient quantities of the harmful agent are being (or have been) absorbed to cause intoxication. In interpreting such tests, the three major factors which determine whether or not intoxication occurs should be considered.

The first of these is the time factor. Over a short period the probability of intoxication may be proportional to the length of exposure. Over longer periods this is not true but as a general rule, the longer the exposure the greater the likelihood of injury.

The second factor is the susceptibility of the individual. This cannot be measured quantitatively and in many instances, cannot even be qualitatively estimated. This factor not only varies among different individuals but will, undoubtedly, change considerably with the same individual from time to time, reflecting stresses from infections, fatigue, and other causes.

The third factor, and the one which concerns us primarily, is the concentration of the toxic agent; not the concentration in the environment nor even, ordinarily, in the body as a whole but the concentration in that organ or tissue of the body which is most sensitive to attack by the toxic agent in question. For purposes of simplicity we will call this the critical organ.

In *Figure 1* a highly simplified version of the flow of a toxic substance through the body is depicted. It is seen that there are four primary media which we can analyse, indicated by the asterisks. These are the air, blood, urine, and breath. None of these analyses will give us directly the information we want most, namely, the concentration in the critical organ (C_c) which may be the brain or liver for example. Ordinarily it is impossible to measure this value directly (except, perhaps, at autopsy) and we can only infer that it rises or falls with increases or decreases in the concentrations which we can determine.

There are, of course, analyses which can be made—other than those mentioned. Environmental tests may include analysis of water or food, wipe tests of bench tops, etc. or the contamination of the skin can be determined.

Biological specimens sometimes analysed include lung tissue, fat and skin, as well as blood, while other excretion products, in addition to urine and breath, are sweat, faeces, hair, and nails.

For purposes of classification we can divide industrial poisons into four categories:

(1). Volatile vapours or gases relatively insoluble in water; examples—methyl chloroform, ether, and hexane. The solubilities of these substances in blood and urine are not high, and the analysis of breath is the most

Figure 1. A highly simplified arrangement showing the flow of a toxic substance through the body

promising test for exposure. Ordinarily the sample should be taken at the end of the workday or, at most, within a few hours of the exposure period. A special case in this group is carbon monoxide; because of its chemical affinity for Hæmoglobin, it is present in the blood in substantial amounts, as well as in the expired air.

(2). Water soluble vapours; examples include acetone, methanol, ethyl alcohol. These may be present in the blood and urine in relatively high concentrations; the amounts in the breath depend on their volatility.

(3). Metals and salts; e.g. lead, mercury, fluoride. Members of this group may present a complicated behaviour pattern since chemical combination with various body constituents, as well as physical solution in body fluids, is the rule rather than the exception. While reportedly significant amounts of some toxic elements, such as arsenic, mercury, selenium, and tellurium, are eliminated in the breath, analysis of expired air has not, to date, been used as an exposure test for such substances. Analysis of urine, and to a less extent, blood, has usually been employed to measure the absorption of these toxic elements. In certain cases analysis of hair and nails has been used to estimate arsenic absorption and relatively recently, other heavy metals such as lead¹.

(4). Metabolites of organic compounds; e.g. phenol, hippuric acid, trichloroacetic acid, bromide. This group differs from the others in that ordinarily we are not measuring the toxic agent itself but an oxidation or hydrolysis product thereof. The value of the test lies in the assumption that the amount of metabolite is proportional to the quantity of toxic substance which has been absorbed.

As can be seen from Figure 1 the concentration most closely associated with the concentration in the critical organ is that in the blood. Concentrations in urine and breath presumably are dependent to a large extent on blood levels. Other things being equal, therefore, analysis of blood would seem to provide more meaningful results than analysis of either urine or breath, to say nothing of workroom air.

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As a practical matter, however, it is much easier to obtain adequate and, especially, repeated samples of urine or breath than of blood. For this reason we have more data on the excretion of toxic substances than on the levels maintained in the blood or body tissues. In two cases where both types of data are available, blood analysis is considered the more reliable measure of body burden for both lead and bromide.

It is interesting to note, however, that for tetraethyl lead, urinalysis is believed to provide the better index of exposure². Moreover, when chelating agents (such as EDTA) are administered to workers with lead absorption there is little or no rise in blood lead, whereas urine lead is increased several-fold.

If we compare blood and urine levels of toxic substances, we frequently find great variations in the ratios of one concentration to the other among different individuals. There usually is a statistical correlation with the average concentration in the urine varying from five times that in the blood to less than one-fifth the blood level, if a few typical substances are examined (Table 1).

Table 1. Average ratios of urinary and blood concentrations

Substance	$C_{\text{Urine}}/C_{\text{Blood}}$	Reference
Mercury	5	3
Thiocyanate†	1.2	4
Lead		
(1) Exposed	0.34	5
(2) Controls	0.21	6
Nickel	0.25	7
Vanadium	0.17	8

† Based on serum analysis, corrected for red-cell content.

These ratios are not only highly variable among individuals but in many cases they seem to be concentration-dependent. Thus the average ratios for "normal" blood and urine values would frequently be quite different from those from intoxicated or heavily exposed workers. And depending on the range of concentrations found, as well as other factors, the average ratios obtained by different observers would be different.

Many of the discrepancies are probably due to analytical errors. Thus, if we compare the results of Goldwater *et al.*³ with the earlier report by Benning⁹, the former's ratio of urinary to blood mercury is only half as high with samples of low mercury content (less than 10 $\mu\text{g}/100\text{ g}$ blood). But if samples containing more mercury are compared, the average ratio of urinary to blood values are almost identical (3.7 and 3.8) in the two reports. This may be coincidence but it also may be related to the fact that the analytical results are more reliable in the samples of higher mercury content.

To the extent that blood and urine concentrations of heavy metals or other toxic substances do not correlate, we must consider one or both methods of estimating exposure as inaccurate. Care should be taken not to condemn a test as useless, however, on the basis of a very few comparisons unless adequate

data on length of exposure, time of sample collection, etc. as well as the reliability of the analysis, are at hand.

In the U.S.A. the term "intoxication" means, to the layman, under the influence of alcohol. A more vulgar term is "drunk". The law forbids driving an automobile on the public highway when drunk. In order to determine if a driver is in this condition, the police will analyse his breath; if it contains more than a certain amount of alcohol, he is considered drunk and away he goes to jail.

Industrial toxicologists take a more equivocal position regarding exposure tests, however. Most would hesitate to diagnose intoxication on the basis of such a test alone. In fact there is a school of thought that holds that urine test results are of no value in the diagnosis or prevention of intoxication, at least for many substances^{10, 11}. The logic of this reasoning seems to be that intoxication depends on the amount of poison retained, not the quantity absorbed. Thus, if two workers each absorb 100 mg of a poison and A excretes 50 mg and B eliminates 75 mg, A is the more likely to be affected since his retained dose is 50 mg, as opposed to 25 mg for B. An extreme example is where a lead worker is administered a chelating agent. The level of lead in his urine will greatly exceed that of a coworker who is not treated, but his chances of becoming intoxicated are probably less.

In general, however, this reasoning is applicable only if we know the amount absorbed; and where this is not known with any accuracy, as is usually the case in industrial exposures, it can lead to ridiculous conclusions. As a rule, the higher the concentration of a toxic agent or its metabolite in urine or breath, the greater the probability of intoxication. The same generalization applies to an even higher degree to concentrations in the blood.

Much of the preceding reasoning does not apply to an important group of industrial health hazards, namely, those whose harmful effects are primarily on the lungs, the upper respiratory passages, or even the eyes. Ordinarily the most meaningful concentration that can be readily measured in such cases is that in the air. While the degree of exposure to respiratory irritants may be reflected in blood or urine concentrations of the agent or its metabolite, their relationship to the hazard is not as direct as with systemic poisons. The nature of most such substances is such that little or none is found in the expired air.

Excretion exposure tests, such as those mentioned above, have little

Figure 2. Possible exposure tests for hazards with local effects

meaning unless the time of collection of sample, relative to exposure, is taken into consideration. The optimum time for the collection of urine samples varies greatly for different substances. Some, such as phenol (from benzene), are eliminated within a few hours, following exposure. Samples should preferably be taken at the close of the workday. Most substances that can be evaluated by breath analysis fall in this category.

Other substances are excreted quite rapidly but there is some carry-over from day to day; in such cases the Friday afternoon result will be higher than that obtained Monday afternoon. Trichloroacetic acid from trichloroethylene appears to fall in this category, as do fluoride, uranium, and some of the substituted phenols (dichlorophenol, *p*-nitrophenol).

Other substances can be found in the urine quite soon after exposure, but the amount increases for several days or weeks until equilibrium is reached. Mercury would seem to belong to this group.

Finally, there are a few elements that require a week or more of exposure before a detectable increase in excretion occurs and a correspondingly longer period before maximum levels are attained. Lead and cadmium behave in this fashion.

Examples of the varying behaviour of different metallic poisons, in so far as excretion following acute exposure is concerned, are shown in *Table 2*. In the case of the divinyl mercury there were no clinical symptoms; in the other cases the men were admitted to hospital with arsine poisoning and severe pneumonitis (from cadmium), respectively.

Table 2. Excretion of metals following acute exposure

<i>Hazard</i>	<i>Concentration in urine (mg/l.)</i>	
	<i>1-2 days after exposure</i>	<i>7 days after exposure</i>
Divinyl mercury	1.2	0.05
Arsine		
(1)	1.1	0.95
(2)	2.2	0.8
Cadmium fume	None detectable	

We have spoken about concentrations in blood, urine, and breath in the same terms as used in conventional chemistry, implying some such units as mg per litre, μ g per 100 g, etc. Actually there are several ways of expressing such concentrations, and the results can vary considerably, depending on which method is used.

Concentrations in blood are usually given as mg (or μ g) per 100 g (or 100 ml). Sometimes, however, the concentration in serum, rather than whole blood, is reported. It is obviously important to know which of the two methods is used, and frequently, difficult to compare results obtained by the two different procedures.

Concentrations in breath can be expressed as p.p.m. (or mg/l.) of alveolar air, or they can be related to the carbon dioxide content of the breath. Conceivably the rate of elimination in expired air in mg per minute or similar units could be used. It would seem to me that the level of physical

exertion of the subject at the time of and immediately before the sample is taken, might be of some importance.

Urinary excretion is most commonly expressed in terms of concentration (mg/l.). Rate of excretion (mg/24 h) and concentration adjusted for specific gravity are frequently employed. Occasionally the ratio of toxic agent or metabolite to creatinine is used. We have made studies using the osmolality instead of the specific gravity as an index of urine concentration.

Considerable variation may occur in the results, depending on which method of expressing urinary excretion is used. Variations in fluid intake will cause differences as great as six-fold between "unadjusted" and "specific gravity-adjusted" concentrations in a significant proportion of cases (*Table 3*).

A similar degree of variation will result if we compare rate of excretion (timed samples) with unadjusted concentration. Somewhat smaller fluctuations are obtained if the osmolality adjustment is employed (*Table 4*).

Table 3. Effect of specific gravity adjustment

<i>Sp. gr.</i>	<i>Concentration in Urine (mg/l.)</i> <i>Unadjusted</i> <i>Adjusted</i> <i>(to sp. gr. 1.024)</i>		<i>Osmolality</i>	<i>Unadjusted</i> <i>(mg/l.)</i>	<i>mg/osmol.</i>
1.006	0.10	0.40	0.300	0.10	0.33
1.036	0.10	0.07	1.200	0.10	0.08

Table 4. Effect of osmolality adjustment¹

The greatest discrepancies result if we compare concentration in urine (unadjusted) with the metabolite-creatinine ratio (*Table 5*). This is because when a worker perspires he loses water, salt, urea, and other solids in his sweat to a much greater degree than creatinine, which is therefore concentrated in the urine.

If we compare the other methods of adjustment with that for specific gravity, we still obtain considerable variation, as shown in *Tables 6, 7 and 8*.

Table 5. Effect of creatinine adjustment¹

<i>Creatinine concn</i> <i>(g/l.)</i>	<i>Unadjusted</i> <i>(mg/l.)</i>	<i>mg/g</i> <i>creatinine</i>	<i>Osmolality</i>	<i>Urine of 1.024 sp. gravity</i> <i>(mg/l.)</i> <i>(mg/osmol)</i>	
0.4	0.10	0.25	0.58	0.10	0.17
4.0	0.10	0.025	(unusual) 1.01	0.10	0.10

Table 6. Effect of osmolality adjustment²

Table 7. Effect of creatinine adjustment²

<i>Creatinine</i> <i>(g/l.)</i>	<i>Urine of 1.024 sp. gravity</i> <i>(mg/l.)</i> <i>(mg/g creatinine)</i>		<i>Volume</i> <i>(per 24/h)</i> <i>(ml)</i>	<i>Urine of 1.024 sp. gravity</i> <i>(mg/l.)</i> <i>(mg/24 h)</i>	
1.0	0.10	0.10	400	0.10	0.04
4.0	0.10	0.025	1700	0.10	0.17

Table 8. 24 hour excretion vs. sp. gravity adjustment

EXAMPLES OF EXPOSURE TEST APPLICATION

There are several situations in which analyses of urine, blood, breath, or other biological or excretory samples are particularly advantageous in comparison with air or other environmental tests.

The first is where an incident has occurred, such as a leak or spill, and workers have been thereby exposed. Usually it is undesirable, and frequently it is impossible, to duplicate the conditions which occurred. The toxic material will usually be present in the body, to some degree at least, for a few hours or longer. Hence analysis of blood, urine, or breath will frequently reveal the seriousness of the exposure.

I have already given some data on the excretion of arsenic following arsine poisoning. Two workers became sick, with hematuria the main symptom, after cleaning the inside of an aluminium tank with phosphoric acid. Exposure to arsine was suspected and verified by a finding of about 1 mg of arsenic per litre of urine. The tank had been used six months previously for transport of arsenite solution. There is nothing very remarkable about analysing urine for arsenic to detect a recent exposure. Methods for the microdetermination of arsenic in urine have been available for at least fifty years. Yet I was amazed, in reviewing the literature of arsine poisoning, to find that in only a handful of cases were urinary arsenic measurements made.

Another common situation is where the air is known to be contaminated so a mask is worn. A worker engaged in painting the inside of a water tank fell and was seriously injured. It was suspected that he was overcome by vapours of the paint-solvent, of which toluene was a major component, in spite of the fact that a protective mask was provided.

Tests for hippuric acid in the urine of a painter engaged in similar operations revealed that substantial quantities of toluene were inhaled in spite of the respiratory protection.

If absorption through the skin can occur, or ingestion of a toxic material is probable, measurements of concentrations in the air will not provide a true index of the hazard. A mercury boiler was dismantled over a period of several months. Air tests for mercury vapour reportedly indicated little or no exposure yet urine samples from a number of workers showed relatively high levels of this metal, indicating the need for additional precautions. It was believed that skin absorption might have been responsible in part, at least, for the high concentrations of mercury in the urine.

Many studies have been made in lead industries where air tests yielded low results but high urinary leads were found. In most cases it was found that certain dusty operations, not in process when tests were done, were frequently carried out. In others there was apparently significant ingestion of lead compounds.

In other cases it is virtually impossible to estimate the average exposure of a number of workers, due to the irregularity of their attendance at any one location. Such a condition was found in the churn rooms of rubber spreading plants. Measurements of phenol in urine established the degree of exposure to benzene (an impurity in the naphtha solvent) with an apparent high degree of reliability.

In our experience the concentrations of trichloroacetic acid (TCA) in the

urine of operators of trichloroethylene degreasers have usually been consistent with air findings. In a few cases the TCA values were unexpectedly high. Addiction to trichloroethylene or, at least, intentional inhalation of the vapour was suspected and in some instances verified.

A man was found drowned in the pit of a snow melting unit at a municipal airport. It was assumed that he accidentally fell into the pit during a snow storm. Analysis of his blood, however, revealed a high content of carbon monoxide. Further investigation revealed that the oil burner on the snow melting machine was improperly adjusted and gave off large amounts of carbon monoxide. It is probable that the workman was incapacitated by carbon monoxide asphyxiation before falling into the pit.

SUMMARY

Tests of blood, urine and breath are of great value in estimating the degree of exposure to industrial poisons. These are especially important when, if for any of several reasons, air tests fail to reveal the proper magnitude of the exposure. In addition, in many situations tests of biological or excretion product samples are more economical than adequate air tests.

There remains a great deal of uncertainty in the interpretation of the results of many such tests. Timing of the collection of samples relative to exposure is of major importance, and in many cases no uniform method of expressing the results of the analyses has been agreed upon.

In general, the analysis of urine has been the most widely practiced procedure due, in part, to the relative convenience of sample collection. In some cases, blood samples are found to give more reliable results. Frequently there appears to be quite a lack of correlation between the findings in blood and urine. Much additional work is necessary in order to determine the significance of these discrepancies.

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