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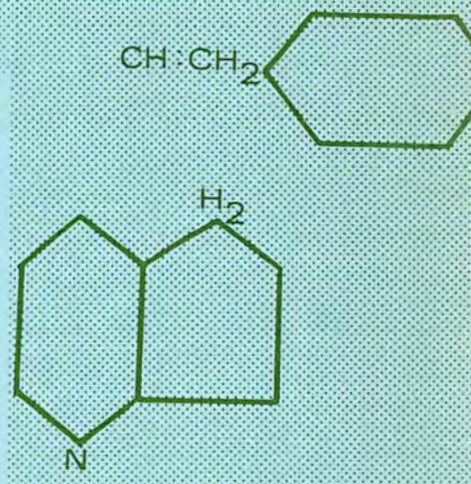
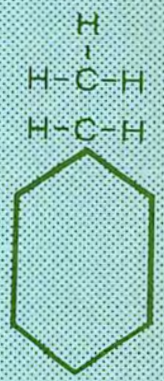
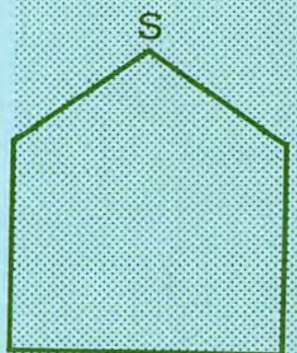


EXPOSURE TESTS FOR ORGANIC COMPOUNDS IN INDUSTRIAL TOXICOLOGY





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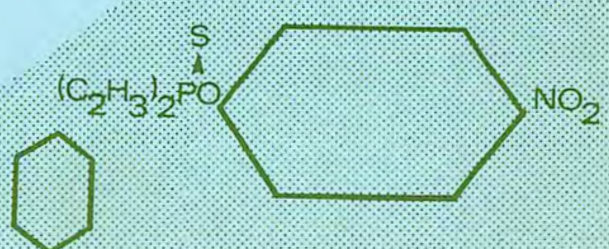

EXPOSURE TESTS FOR ORGANIC COMPOUNDS IN INDUSTRIAL TOXICOLOGY

by

Jerzy K. Piotrowski

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health
Cincinnati, Ohio
September 1977

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402

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DHEW (NIOSH) Publication No. 77-144

PREFACE

This monograph was written in the Department of Toxicological Chemistry, Institute of Environmental Research and Bioanalysis, Medical Academy of Lodz, under the auspices of Polish-American Program 05-516-1.16 financed by the National Library of Medicine, Bethesda, Maryland, USA. It was translated from Polish by Julian Liniecki, M.D., PhD, Dr. Habil.

The National Library of Medicine gave the National Institute for Occupational Safety and Health permission to publish this monograph in the NIOSH Technical Report Series. In preparing the manuscript for publication some editorial rewriting was done to make the text conform with standard English sentence construction and word usage. However, special effort was made not to alter the factual material, the intent, and the conclusions of the author.

Austin F. Henschel, Ph.D.
Editor

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1. INTRODUCTION

The strategies for industrial health protection, being developed in numerous countries today, include more efficiently detecting the subtle, initial symptoms of chronic intoxications and diseases induced by noxious agents. Practical conclusions drawn from surveys attempt to set the permissible levels of exposure sufficiently low so as to prevent the manifestation of pathological changes, even after prolonged exposure lasting for several decades. The capital investment involved in securing such low levels of exposure is usually enormous; and, therefore, attempts to estimate as precisely as possible the real magnitude of exposure, without incorporating excessively wide margins of safety, is fully justified.

Systems for exposure evaluation based on assessing concentrations of toxic compounds in the air of the workplace, and safety criteria based solely on the magnitude of these concentrations, are not always satisfactory. First of all, such systems neglect absorption of toxic substances by routes other than the respiratory tract. Cutaneous absorption is a matter of concern, and its importance becomes all the more appreciated the more thoroughly it is studied. Second, even when substances are considered which are absorbed chiefly or exclusively via the respiratory tract, basing the estimates of real exposure solely on analysis of air concentrations may be inadequate. Movement from place to place while supervising numerous operations in a manufacturing process, spiked concentrations near or inside installations, and lengthy breaks from exposure which occur when workers are temporarily outside of the premises make reasonable interpretation of measured values very difficult. Additionally, in cases where the toxic substance shows a pronounced ability to accumulate in the body, a body burden attained in preceding periods may be high enough to create a serious danger despite an actual present moderate exposure.

The above reasons speak for supplementing air analysis in manufacturing premises with individual exposure tests on workers. Knowledge of the real individual exposure could permit flexible application of various prophylactic measures. These are often less expensive than general reduction of the concentrations in the entire plant, and sometimes are much more effective.

Theoretically, there are two categories of assays that could be applied as exposure tests: a) determination of a toxic substance or its metabolites in biological media sampled from exposed individuals, and b) following the intensity of the biochemical reactions that become manifest under the influence of excessive exposure. In spite of the fact that the latter method would detect an already induced biochemical lesion, review of the literature points to inherent limitations of this technique. For organic compounds two important examples are: inhibition of acetylcholinesterase under the influence of phosphoorganic compounds and carbamates, and methemoglobinemia produced by the metabolities of aromatic nitro- and amino compounds. Without depreciating the usefulness and soundness of these tests, it appears justified to emphasize that their range of applications is much narrower than that of the direct determination of toxic compounds and their metabolites in biological samples–the concept which is to be further developed in this book.

Evaluation of exposure to chemicals is often performed by specialists in industrial toxicology. Such specialists may not be competent in medical procedures (e.g., venopuncture). Because of such reasons as relative urine and blood concentrations, limited availability of frequent blood and tissue samples, etc. the majority of earlier tests were based on analysis of urine, and more recently also on expired air. Thus, exposure tests form a complementary method for the evaluation of exposure in industry, which can be used by those who supervise the air concentration in industrial plants. General knowledge of the situation in a plant makes the interpretation of exposure tests easier. This method, which combines the input from exposure tests with general supervision of occupational conditions, suggests the need for sampling the media to be analysed from workers directly at working place. This approach helps to eliminate instances of misunderstanding that otherwise may be particularly frequent and devastating when interpreting the levels of substances that rapidly turn over in the body. The same methods also may be used by medical personnel in ambulatory tests. Knowledge of metabolic kinetics permits extrapolations of the levels

observed at some point during the day, for instance, to that at the end of work shift, and to make both comparable. However, in general, application of exposure tests in this way has limitations; and the interpretation of results in difficult. Exception must be made here, of course, for the substances with pronounced ability to accumulate in the body, e.g., DDT.

The fate of organic compounds in the animal body became better recognized in the nineteen forties and fifties. The main outlines were described by R. T. Williams in his *Detoxication Mechanisms*. During these same years great progress in pharmacokinetics and analytical chemistry was made. At the same time, the idea of utilizing biological analyses for assessment of individual exposure of humans to industrial organic compounds developed in numerous countries. The approach varied from one author to another. Thus, whereas Elkins seemed to prefer direct industrial experience, Teisinger and his colleagues devoted their main efforts to basic investigations on absorption and elimination of organic compounds in volunteers exposed in controlled experimental conditions. The Polish authors have been trying to combine precision of experimental studies with applicability of the tests to industrial conditions. Examples, among others, for benzene, toluene, xylene, phenol, aniline, nitrobenzene, and carbon disulphide are described in specific chapters in this book.

The exposure tests are not treated at length in textbooks. Some elements of the problem, from various viewpoints, were taken up in the monographs of Teisinger and his coworkers in 1956, and of Elkins in 1959; by the books of Dutkiewicz et al. in 1964, and by Gadaskina and Filov in 1971. Some elements of the theory are contained in papers by Teisinger, Elkins, Truhaut, and others, some of which have been included in the selected general bibliography.

In the present monograph the author has pursued the following goals:

a) to present actual situations and trends in the development of exposure tests for the more important substances widely elaborated in this respect;

b) to review the information about the stage of development of the tests for substances less frequently studied, and at the same time to indicate compounds for which the tests could be applied eventually in practice, if further elaborated;

c) to present a possibly coherent concept of the exposure tests and the methods for their elaboration and interpretation that would render newly acquired data comparable with each other; and

d) to present actual problems in the field calling for, and at the same time permitting, fast experimental solution.

The monograph is not a textbook, and therefore detailed analytical procedures have been omitted. For these the reader will have to go to the original reports. In the general part of this book only those aspects of absorption, metabolism, and pharmacokinetics are discussed which are relevant for the elaboration and application of exposure tests. These chapters were not intended to be exhaustive, either in the treatment of these highly complex problems or in the complete selection of a bibliography. Nevertheless, the author hopes they will provide the reader with a key to specialised original articles where more detailed and complete information can be found. In selecting 15 compounds, the information on which is reviewed in detail, the author was motivated by the scope of existing data, but also to some extent by his own experience and personal engagement in the discussed problems. Thus, in spite of the fact that the selection was somewhat arbitrary, the author hopes it reflects rather adequately the existing body of information.

In spite of the fact that the subject of this monograph is rather narrow, the author had to rely on generous assistance of his coworkers. Gratitude for their valuable help in the bibliography search is expressed to Mr. K. Walczak and Mr. A. Podoski; Dr. W. Kaszper, Dr. Iwona Balcerska, Mrs. Jadwiga Szymanska, Mrs. Elzbieta Komsta-Szumska and Mirs. Honorata Andrzejczak have taken part in editorial work on some of the chapters. The author feels particularly indebted to his secretary, Miss Danuta Poteraj, whose patience, energy, goodwill, and competence have considerably facilitated completion of the work.

The National Library of Medicine greatly assisted the author in obtaining relevant literature by providing bibliographic surveys through the MEDLARS system involving the MEDLINE and the TOXLINE systems. Assistance in obtaining the information through the MEDLARS system, given by the project-officer of the program, Mr. R. Worthington, is most sincerely appreciated. Expressions of gratitude are also forewarded to Dr. Jeanne L. Brand, Chief, International Programs Division, Extramural Programs, who has manifested her enormous goodwill and understanding for the project throughout the total period of its realisation.

2. ABSORPTION ROUTES

The two essential routes of absorption for industrial chemicals are the respiratory tract and the skin. Absorption which takes place through the lungs can be roughly assessed, even in industrial conditions, through air analysis. Results thus obtained can be interpreted by comparison with the TLV values. Absorption via the skin, on the other hand, evades this method of measuring exposure. This is one of the arguments for strongly advocating the use of exposure tests.

ABSORPTION VIA RESPIRATORY TRACT

Absorption of organic compounds may take place both in the upper and in the lower parts of the respiratory tract. Two basic processes may be recognized here, depending on the physical properties of the toxic substance. If the toxic substance is present in air in the form of an aerosol, absorption will be preceded by deposition of the substance in the upper respiratory passages. When very finely dispersed particles are considered, the deposition will be in the alveolar region. For aerosols of organic compounds these processes have not been sufficiently well studied, either from the viewpoint of particle size distribution for different substances and types of aerosol generation, or from the viewpoint of total percentage retained in the human respiratory tract. The same refers to the dynamics of the retention in individual parts of the tract. Until more specific data become available, it seems that application of the general principles of retention dynamics for aerosol particles is in order. These have been worked out for radioactive aerosols (see Task group on lung dynamics, 1966).

A far greater body of data, however, exists for respiratory absorption of substances that are dispersed in air as gases and vapours. The basic absorption mechanism is gas diffusion, and the quantity essential for effectiveness of this process is the partition coefficient between air and blood*. A more detailed discussion of the problem has been presented elsewhere (Piotrowski, 1971); here only the most essential aspects of the problem will be outlined.

Values for the coefficient between air and blood have been estimated for only a few substances; but the coefficients for partition between air and water are available for a far greater number of compounds. These have usually been obtained while working out conditions for absorption of a substance in water with analytical objectives in mind. (The quantity is called the "aeration constant.")

The respective values of the air/water partition coefficients for organic compounds may vary by several orders of magnitude: from about 10^0 for carbon disulphide, through 10^{-3} for acetone, acrylonitrile and nitrobenzene; to 10^{-5} for aniline and toluidine. The yield of pulmonary absorption (i.e., retention of vapours in the lungs) increases with a decreasing partition coefficient for air/blood (water); however variation of the retention is by far less than the variation of the respective partition coefficients. For example, lung retention of aniline vapour (partition coefficient 10^{-5}) amounts to about 90 per cent; of nitrobenzene (partition coefficient 10^{-3}) to 80 per cent; of benzene (partition coefficient 10^{-1}) to about 50-75 per cent, and finally of carbon disulphide (partition coefficient 10^0) to about 40 per cent.

In the course of continuous inhalation exposure to volatile compounds, the concentration of the compound in the blood increases toward an equilibrium between absorption, on the one hand, and metabolism and elimination, on the other. This is accompanied by a decreasing retention. This decrease of retention in the course of continuous inhalation may be observed in practice

* The reciprocal, i.e. blood/air partition coefficient is often used in toxicological literature. In this monograph air/blood partition coefficient is used in accordance with the author's previous monograph (1971) where it was incorporated into a system of kinetic description of elimination processes.

for compounds whose air/blood (water) partition coefficient is of the order of 10^{-3} or greater. Among the compounds discussed in this monograph, decreasing retention (an example in Fig. 2-1) is characteristic for carbon disulphide, tri- and tetrachloroethylene, benzene, toluene and nitrobenzene. Nothing comparable has been observed for aniline (low value of partition coefficient) and styrene. In the case of styrene, analogous to acrylonitrile (Rogaczewska and Piotrowski, 1968), the justification for lack of time-dependent decrease of retention seems to lie not so much in the magnitude of the physical partition coefficient as the reactivity of the vinyl group, which may enter alkylation reactions with blood constituents.

Usually, the retention (R) of organic vapour compounds in the respiratory tract has been studied on human volunteers in chamber-type experiments, where it can be determined directly from the ratio of concentrations:

$$R = \frac{C_i - C_e}{C_i} \qquad (1)$$

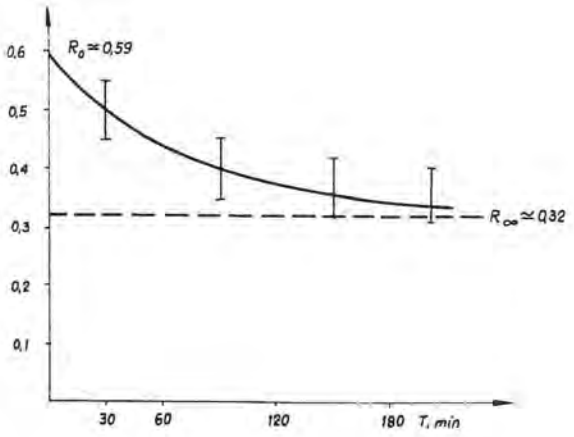

Fig. 2-1. The retention of carbon disulphide vapours in the respiratory tract as a function of time, with continuous exposure (Jakubowski, 1966).

Taken from: Piotrowski J.: *The application of metabolic and excretion kinetics to problems of industrial toxicology*. US-Government Printing Office, 1971. Page 77, Fig. 9

C_i and C_e denote the concentrations in inhaled and exhaled air, respectively. The technique for the determinations varied widely between authors. In older studies some errors, related to adsorption of the substance in question on tubes providing air for breathing, or to vessels or sacks in which it was collected, could not be excluded. To avoid these complications, when classic methods of air analysis by aspiration are applied in the present author's laboratory (see chapters on "Toluene" and "Phenol"), sampling is performed from exhalatory and inhalatory channels directly at the respirator. When gas-chromotographic determinations are made utilizing small air samples, the problem of adequate sampling of the exhaled air assumes particular significance. To estimate the retention (balance), the sample should be representative not just of a specific fraction of the breath (e.g. alveolar air), but of the total exhaled air volume (mean concentration). This may be accomplished by using sufficiently large bags, provided the synthetic material of which they are made has no pronounced adsorptive capacity for the compound in question. Examples of an adequate selection of the material for a given compound may be found in the paper by Smith and Pierce (1970). The most often used one at present is Saran, which is characterized by relatively weak adsorptive capacity. Another solution may be application of a respirator with a solid absorber from which the substance to be determined is eluted and analyzed by means of gas chromatography (Sherwood and Carter, 1970). The extent to which the technique for estimating retention may affect the results can be appreciated from consideration of the wide variation in data for individual substances given in specific chapters of this book.

The total amount of a toxic compound absorbed in the respiratory pathways depends upon its concentration in the air, the duration of exposure, and the pulmonary ventilation rate. The last factor, even if most authors have been conscious of its importance, seems responsible for a lot of misunderstanding in the theory and practice of exposure tests. It is obvious that ventilation rate, which in a practical field assay cannot be measured, depends upon the physical activity of the individual tested (Zenz and Berg, 1970). In chamber-type experiments, in which volunteers are exposed in a sitting position not subject to additional physical effort, the ventilation rate is usually of the order of 0.3-0.4 and

0.4-0.5 m^3/hr, in females and males, respectively. It is reasonable to assume that for people engaged in light work, corresponding to a slow walk, the rate is at least doubled. In calculating the amounts of substances absorbed through the pulmonary tract in industrial conditions, various authors assume different average ventilation rates as typical for workers performing light work: from about 0.8 m^3/h to about 1.25 m^3/h. The latter figure (10 m^3 per 8 hour working shift) is often used as the estimate in the U.S.A.

Taking the above factors into account, the total amount of a substance absorbed in the respiratory tract over a period of exposure would be the product of air concentration, duration of exposure, ventilation rate, and retention rate.

Formulas for calculating the absorbed amounts and air concentrations by extrapolation from chamber exposure studies to field conditions are discussed in the chapter "Elaboration of exposure tests in experimental conditions".

CUTANEOUS ABSORPTION

The role of the skin in the absorption of industrial chemicals is commonly recognized nowadays. For some compounds the skin is the main port of entry under conditions of occupational exposure (e.g. benzidine, parathion); for others this route is roughly equivalent to inhalatory absorption (e.g. aniline, nitrobenzene, phenol); even for the bulk of other organic compounds the contribution of cutaneous to the total absorption cannot be neglected or excluded. For instance, with a great number of phosphoorganic compounds, as studied in animals, intoxication may occur after cutaneous penetration (Kundijev, 1967). In the MAC list compiled in recent years ("Threshold limit values...", ACGIH, 1976), caution as to the possibility that cutaneous absorption exists is voiced for about 1/4 of the organic compounds, and for a significant number of inorganic substances. In cutaneous absorption either transepidermal or transfollicular passage may occur; for organic compounds the transepidermal route seems to be of paramount importance (Djuric, 1966). From the experience gathered so far, it appears that the solubility of a substance both in water and in lipids, reflected in a not too high coefficient of fat/water distribution, is a prerequisite for significant absorption through the skin. This feature may result from structural peculiarities of the skin; a penetrating substance has to pass layers or membranes rich either in protein-water or lipids. From fragmentary experimental and numerous practical observations, it follows that the cutaneous absorption rate of organic compounds increases both with increasing temperature and with increasing moisture of the skin and, therefore, becomes facilitated in warm seasons. The absorption of organic compounds may follow surface contamination of the skin (or of clothes); for some compounds it may occur directly from the gaseous phase.

CONTACT ABSORPTION

Under industrial conditions absorption of a substance from a contaminated skin surface may result in two basic situations:

a) When a job requires continuing repetition of dermal contact, absorption of even relatively volatile substances takes place — substances which otherwise would quickly evaporate from the surface of the skin, precluding significant penetration. The following are examples that may serve here: manual applying a glue containing organic solvents, e.g., benzene and its homologues; manual painting with paints containing the same ingredients; binding broken viscose rayon fibres in the coagulating solution, with carbon disulphide as one of the substances present.

b) Another situation concerns relatively non-volatile compounds that occasionally contaminate the skin, stay on the skin for a prolonged period, and undergo slow absorption. Typical examples here are benzidine and parathion, and to some degree compounds that may be characterized as liquids of high boiling temperature such as aniline and nitrobenzene.

Information on the cutaneous absorbability of these substances after direct contact with the skin comes from various sources which, due to variability of experiments or incidental observations, do not provide a common yardstick for quantitative comparisons. Available data, applicable directly to man, have been usually derived from following sources:

a) Cases of acute intoxication from contamination of a large skin surface (examples in the literature may be found for aniline, nitrobenzene, phenol, parathion, etc.).

b) Observations of urinary excretion of pertinent metabolites in workers in whom inhalatory absorption could be excluded (e.g. parathion).

However, in both situations (a and b) the data do not permit assessment of the absorption rate. The latter may be estimated from experiments on volunteers. In the laboratory of the author the following method has been applied: a known amount of a substance is applied to the skin in a way excluding evaporation, or enabling corrections to be made for losses by evaporation. After a given interval of contact, the amount remaining at the site of application is determined and, from the differences, the absorption rate is calculated. This type of experiment has been performed on animals in the author's laboratory for aniline (Piotrowski, 1957), for nitrobenzene (Salmowa and Piotrowski, 1960), for benzene (Hanke et al., 1961), and for acrylonitrile (Rogaczewska and Piotrowski, 1968). The values, representing upper limits of the absorption rate after application of an infinitely thick layer, were of the order of 1 mg $cm^{-2}hour^{-1}$ for aniline and nitrobenzene, and about 3 times lower for benzene and acrylonitrile. Experiments performed by Dutkiewicz and Tyras (1968), using a similar technique for toluene, xylene, and styrene, yielded considerably higher values. The disadvantage of this type of experiment lies in the technical difficulty in securing, conditions for absorption that eliminate losses due to evaporation. Thus experimental errors usually tend to overestimate the absorption rate. It would be desirable to supplement the results of these experiments with data on the levels of the absorbed compound in blood, or the excretion of its metabolites in urine. This type of internal control was introduced into the cited experiments on aniline and benzene, and proved useful. Technical difficulties arising from the determination of metabolites, or from the estimation of the absorbed amount of a substance, due to lack of knowledge of the efficiency of individual metabolic pathways, could be overcome by using a labelled compound and estimating the absorbed amount from the total activity of the label (^{14}C) excreted in urine, regardless of its chemical form. This type of experiment is sometimes applied in dermatological research (e.g., see: Feldman and Maibach, 1970). However, the conditions of these experiments differ so much from those required by toxicologists as to render quantitative comparisons meaningless. This conclusion is strengthened by the fact that the cited authors classified nitrobenzene as a compound poorly absorbed through the skin.

Cutaneous absorption of organic compounds is the most important factor arguing for the development and application of exposure tests. When determining a given metabolite in urine, one assumes that the efficiency with which it is formed after cutaneous absorption is close to that found after inhalation. Further generalization of this assumption into an hypothesis on the independence of the metabolism of an organic substance from its route of entry is not required because of the minor role of the gastrointestinal tract as a route of entry. However, a study of Dollery et al. (1970) showed differences in the metabolism of compounds administered via the alimentary tract.

With regard to inhalatory and cutaneous absorption, the number of controlled experiments aimed at verifying or discarding the independence of the metabolic pathway from the route of entry hypothesis is too limited to serve as a proof. The efficiency of the metabolic pathway of aniline leading to p-aminophenol as the end-product has been studied in man and found independent of route of absorption, whether dermal or inhalatory (Dutkiewicz and Piotrowski, 1961). In addition, no significant difference was found in humans when benzene was considered and phenol determined in urine as the metabolite (Hanke et al., 1961). In rats, no change in excretory kinetics or ratio of metabolites (p-nitrophenol vs. p-aminophenol) was found when nitrobenzene was administered intravenously or applied to the skin (Piotrowski et al., 1975). On the other hand, fragmentary observations made by Dutkiewicz and Tyras (1969) with regard to some aromatic hydrocarbons pointed to a much lower efficiency* for selected metabolites when skin absorption and inhalatory absorption were compared. In the case of parathion, the independence of metabolism from absorption route has been discarded based on observations that after cutaneous absorption given concentrations of p-nitrophenol in urine are not accompanied by the expected toxic effects (see "Parathion").

CUTANEOUS ABSORPTION OF VAPOURS OF ORGANIC COMPOUNDS

Absorption of some compounds can occur directly through the skin from the gaseous phase. This

* Efficiency is understood here as a ratio of an amount of metabolite excreted in urine to the absorbed dose, calculated from the amount that could not have been recovered from the application site on the skin. The reservations voiced above with respect to this technique of assessing cutaneous absorption-rate should be born in mind.

phenomenon was observed and found to play a significant role for aniline, nitrobenzene, and phenol which otherwise are well-absorbed when applied directly. On the other hand, this type of absorption has not been found significant for benzene and toluene. With respect to the former compounds the issue is of practical importance due to the fact that at given air concentrations amounts absorbed via skin are comparable with those which enter the system through the respiratory tract. An increasing rate was found for cutaneous absorption of aniline and nitrobenzene with increasing ambient temperature, and for aniline with increasing humidity. These are the same factors which are known to favour the contact absorption of the same compounds. It is interesting to note that normal work clothes have only a limited effect upon the absorption rate, lowering it by some 20-30% relative to that in nude persons.

The mechanism of cutaneous absorption of organic vapours is not well-understood. The rate of absorption is roughly proportional to the air concentration of the vapours. The process is accompanied by adsorption of the substances on skin surface, which can be demonstrated by elution. Taking into account the factors affecting both contact and vapour absorption, as well as negative results that have been obtained for highly volatile substances, it cannot be excluded that adsorption on skin surface is a first and indispensable factor in the process. If this should prove true, it could be expexted that absorption from the gaseous phase would occur with all compounds of poor volatility that display good adsorption when in direct contact with the skin.

With regard to compounds for which cutaneous absorption of vapors is significant, this cutaneous absorption should be considered in elaborating the relationship between absorption and excretion (exposure tests). If in the course of test elaboration an exposed subject has spent some time in a toxicological chamber, the absorption through the skin is automatically, even if inadvertently, taken into account. If, on the other hand, as practiced in the author's laboratory, inhalation and dermal absorption are studied separately, it is possible to consider and describe both processes independently in a manner presented in the chapter "Experimental elaboration of exposure tests".

Information on cutaneous absorption of organic vapours, as discussed above, was obtained from experiments on volunteers; and the evaluation of the absorbed amounts was based on exposure tests. All reservations discussed previously, regarding possible differences in metabolic rates and efficiencies when organic compounds are absorbed by inhalatory and dermal routes, are also pertinent here. The experiments were made on resting volunteers; for even if strong dependence of cutaneous absorption of vapours on physical activity seems unlikely, there are no experimental data to preclude such a possibility.

EVALUATION OF CUTANEOUS ABSORPTION IN CONDITIONS OF OCCUPATIONAL EXPOSURE

In occupational exposures quantitative evaluation of the role played by both of the principal absorption routes is possible only by exposure tests. The underlying assumption is that there is no gross difference in metabolism relating to the absorption routes. In practical conditions two basic procedures have been applied:

a) In individuals working at a controlled rate, the absorption is estimated (exposure test) by eliminating totally one of the absorption routes by means of individual protectors: the inhalatory one by application of respirators, and the cutaneous one by means of an impermeable suit. This method, among others, was used in evaluating parathion absorption (Durham, 1963). The disadvantage of this procedure is a possibility of differences in total exposure between the series of investigations; moreover, systemic accumulation of the compound in question from preceding exposure must be taken into account. In addition, protective clothes and gloves made of rubber or synthetics may not completely prevent penetration of a substance into the body (Locati et al., 1968).

b) The study is made under normal conditions of exposure, and total absorption is evaluated from results of exposure tests. The inhalatory absorption is estimated from the concentrations of the test substance in the inspired and expired air over a measured exposure duration. At the same time it should be noted that dermal absorption has two components: vapour and direct (skin) absorption. Total absorbed amount may be expressed by the formula:

$$D(mg) = CT(RV + \alpha) + S \qquad (2)$$

where C = air concentrations of a substance, T = duration of exposure, V = pulmonary ventilation rate, R = retention in the respiratory tract, α = coefficient of cutaneous absorption from the gaseous phase, and S = contact cutaneous absorption. Developing further:

$$D(mg) = CTRV + (CT\alpha + S) \qquad (2a)$$

The second term in equation 2a represents cutaneous absorption. The disadvantage of this method is the fact that the contribution of skin absorption is estimated indirectly from the difference. This procedure was applied for aniline and nitrobenzne, among others, for which it was possible to detect considerable contact absorption.

The potential role of cutaneous absorption can be assessed from data on the extent of contamination of skin and working clothes. Examples of such a procedure can be found in the literature for benzidine, aniline, nitrobenzene, and other substances. One of the methods is a pad-test: a textile or paper pad is attached in a standardized way to exposed skin and the pads analyzed for contamination at the end of the work period. This method has found wide application for pesticides, e.g., DDT and parathion. (Durham and Wolfe, 1962). In this way a comparative measure of dermal exposure may be obtained in individual workers, or when different technology is being applied. It should be emphasized, however, that in no case should "dermal exposure" be identified with the "dermal absorption". As found by Durham (1963) using parathion as an example, the amounts absorbed via the skin were two orders of magnitude lower than those found on the skin (dermal exposure).

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3. BIOTRANSFORMATIONS

The biotransformation of xenobiotics has been a subject of several monographs and textbooks. Among these is the review article by T.E. Gram and I.R. Gillette (1971). Reviews of several more specific subjects may be found in papers by R.T. Williams (1974). D.V. Parke (1972), and J.R. Fouts (1972). Those aspects that are directly relevant to exposure tests* will be briefly reviewed.

INTERSPECIES DIFFERENCES IN THE METABOLISM OF ORGANIC COMPOUNDS

The metabolic pathways of xenobiotics, observed in various animal species, are either identical or very similar. Nevertheless, the rates of the processes and the efficiency of various pathways, as well as the excretion of individual metabolites, may display pronounced quantitative interspecies differences. In particular, examples of such differences may be found for the aromatic compounds.

Thus, for instance, aniline is metabolized in some species mainly via hydroxylation in the para-position (rabbit, rat, man); whereas in others it is metabolized predominantly in the ortho-position (e.g. dog). There are considerable interspecies differences in the efficiency of biotransformation, particularly in the low dose ranges. In rabbits the efficiency of p-aminophenol formation decreases with the increasing dose of aniline, while the reverse is true for man. Nitrobenzene is metabolized in rats and rabbits mainly to p-aminophenol, but in man p-nitrophenol predominates. For the former metabolite the turnover rate in rabbits and rats is high; whereas in man the rate is low, leading to systemic accumulation in repeated exposures. In experimental animals, styrene yields a constellation of metabolites, among which hippuric acid occupies the main position. In man, on the other hand, styrene is almost completely metabolized to mandelic acid and its oxidation product, phenylglyoxylic acid.

The examples listed above are by no means exceptional. In his review, Williams (1974) gave a series of further examples, pointing to a considerable variation in the efficiency with which a given type of transformation proceeds in different species. Such interspecies differences were found for biotransformation reactions of phase I (oxidation, reduction, hydrolysis) for amphetamine, cumarine, biphenyl, quinic acid, and others. For instance, in rats, hydroxylation of amphetamine shows an efficiency of about 80 per cent; whereas in man the same process displays an efficiency of only 5 per cent. Aromatization of quinic acid to benzoic acid is about 60 per cent efficient in man, and below 5 per cent in commonly used experimental animals (rat, rabbit, dog).

Similarly dramatic differences may be found in phase II biotransformations (conjugation). Ninety-five per cent of phenol in cat is conjugated with sulphuric acid; whereas in rat the glucuronide sulphate ratio is close to unity. On the other hand, in the pig, almost all the phenol is excreted in the form of glucuronide. In different species conjugation of aromatic acids shows preference to various endogenous components. Thus, in man, arylacetic acids (e.g. phenylacetic acid) are conjugated almost exclusively with glutamine; whereas in non-primates the conjugation occurs mainly with glycine.

Close similarities in metabolism of xeonbiotics link man and monkeys. The latter, however, are rarely used for these experiments. There are many exceptions to the idea that monkeys parallel man. For quantitative evaluation of human data direct knowledge of the metabolism of xenobiotics in man is indispensable. Quantitative interpretation of the data by comparison with available information obtained from animal experiments is subject to considerable uncertainty.

MECHANISM OF BIOTRANSFORMATION

Studies performed over the last 30 years have demonstrated that, in mammals, many processes by which xenobiotics are transformed *in vivo*,

* The references omitted from the chapter can be found in chapters dealing with specific subjects.

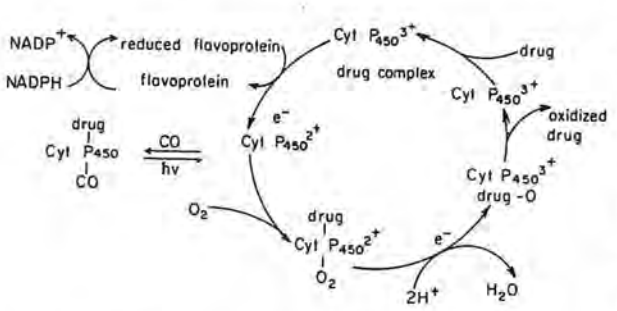

Fig. 3-1. Electron transport in hepatic microsomes (after Parke, 1972).

Taken from: Parke D.V.: *The effects of drugs and steroid hormones on the enzymes of the endoplasmic reticulum.* In **Effects of Drugs on Cellular Control Mechanisms,** B.R. Rabin and R.B. Freedman, Editors. Page 70 — scheme.

take place in the liver. On the subcellular level, these processes are localized in microsomes. The catalyzing enzymes localized in the smooth membranes of the endoplasmic reticulum have been given the name of "microsomal drug metabolizing enzymes". These are not easily identifiable due to their close association with the membranes and the difficulties encountered when solubilization is attempted. On the basis of considerable data it has been accepted that oxidation reactions catalyzed by these enzymes, which lead to incorporation of an oxygen atom into a molecule of a xenobiotic, proceed in the presence of molecular oxygen, and require participation of $NADPH_2$ or $NADH_2$ (in reduced form). One protein catalyzing a reaction of the type:

$$RH + O_2 + 2H \text{ — } ROH + H_2O \qquad (1)$$

is cytochrome P-450. $NADPH_2$ which is the donor of hydrogen atoms for reaction (1) does not transfer them directly into cytochrome P-450, but indirectly by means of the electron transport chain (Fig. 3-1), of which cytochrome C reductase and a nonidentified mediator (x) are components. An essential component of the discussed electron transport chain is also the second of the cytochromes, cytochrome b_5; however, its role and closer localization in the chain are not well-understood. This enzymatic complex is responsible, among other things, for reactions of hydroxylation of aromatic compounds. In this context it appears strange that the same system, and in particular the same enzyme (cytochrome P-450), can catalyze hydroxylation in two different directions (ortho and para) in various animal species. Some light is shed on this problem by observations of spectral changes in cytochrome P-450 that take place in the presence of various xenobiotics.

The name cytochrome P-450 derives from the fact that, upon reaction with carbon monoxide, the reduced form of this cytochrome yields a compound with an absorption maximum at 560 nm, a form that is catalytically inactive. In the presence of various xenobiotics, added to a suspension of microsomes, two types of spectra originate depending on the compound. Type I spectrum (absorption minimum at 419-425 nm, the maximum at 385-390 nm) is obtained if phenobarbital or the inhibitor SKF 525 (β-diethylaminoethyl-diphenyl-propylacetate hydrochloride) are added to the suspension; type II spectrum results from the presence of aniline and of another inhibitor, DPEA (2,4-dichloro-6-phenylphenoxyethylamine). Here the minimum and maximum are observed at 390-405 and 426-435 nm, respectively. From these observations it has been concluded that cytochrome P-450 can react with xenobiotics in two ways: firstly, through a lipoprotein component of the enzyme (type I), and secondly, by forming a complex with the haem-group (type II). The presence of two activity sites in the enzyme could provide an explanation for the catalyzing of two different metabolic reactions by the same compound. Apart from that, it may be legitimately accepted that the protein component of cytochrome P-450 is not a single entity. There are good reasons to suggest that at least 2 distinct forms of the enzyme exist, and this may explain the discrepancies between the observed metabolic reactions in which this enzyme participates. For further review of this topic, a paper by Gram and Gillette (1971) should be consulted.

To maintain the electron transport chain (presented in Fig 3-1) in action, the regeneration of the oxidized form of NADP by means of reduction is necessary. In contrast to the mitochondrial respiratory chain that catalyzes normal biological oxidation in the presence of NAD (oxidized form), the microsomal chain does not contain elements that regenerate the oxidized coenzyme. There must be, therefore, a cooperation between microsomal metabolic processes and other biochemical reactions that are "consuming" the oxidized form of NADP. The process in question here is the pentose cycle of glucose oxidation, catalyzed by NADP-dependent glucose-6-phosphate dehydrogenase.

From the available data it may be concluded that the oxidation mechanism discussed above is engaged in the metabolism of aromatic compounds: oxidation of benzene to phenol; of toluene and xylenes to benzoic acid and toluic acid; of aniline and benzidine to hydroxy derivatives; of styrene to epoxyde; of parathion to paraoxon; etc.

Even if this did not follow directly from the mechanism presented in Figure 3-1, the microsomal enzymes also catalyze a series of other reactions belonging to phase I of biotransformation. Included are reactions of sulphoxidation, desulphuration, O- and N-dealkylation, and some others. The microsomal fraction displays catalyzing ability with regard to reactions of reduction, of which some, e.g., reduction of the nitrogroup, proceed with the participation of cytochrome P-450. In the metabolism of nitrobenzene this system may play a role, as well in the reduction reactions leading to aniline as in hydroxylation of the latter to aminophenols. A reductive dechlorination of DDT, which leads to formation of DDD, also belongs to the reduction reactions taking place in the microsomal fraction. This reaction requires the presence of $NADPH_2$ and can be inhibited by carbon monoxide, which suggests that cytochrome P-450 takes part. The biotransformation mechanisms localized in the liver microsomes are known mostly from animal studies. Evidence exists, however, that similar mechanisms act in the microsomes of the human liver (Ackermann, 1972).

Biotransformation phase I reactions, taking place in the microsomal metabolic system, are not limited to the liver-cells. The liver, presumably with the highest metabolic activity, has been most intensively studied. Other organs have been much less investigated from the viewpoint of ultracellular structure or for the possibility of xenobiotic transformation in various subcellular organellae. Recent data suggest that pulmonary tissue plays a significant role in the biotransformation of xenobiotics. The lung can be fractionated into subcellular elements that are similar to, but less uniform than, those seen in hepatocytes. The microsomal fraction of pulmonary tissue has the ability to catalyze the metabolism of numerous xenobiotics; the mechanism involved seems to be the same as that in liver cells (Bend et al., 1972). The level of cytochromes, both b_5 and P-450, in pulmonary microsomes is much lower than in liver, but the activity of some xenobiotic catabolizing enzymes is similar in both organs; for example, the activity of biphenyl-4-hydroxylase or benzphetamine N-demethylase (Hook et al., 1972). The contribution of microsomal enzymes of pulmonary tissue to the total metabolism of xenobiotics varies from substance to substance. For parathion the contribution is of the order of 1/100 of that of the liver (Neal, 1972). The metabolic activity is not localized in alveolar macrophages, known for their role in the defense of the body against bacteria, viruses, and aerosol particles (Hook et al., 1972). Apart from liver and lung reactions of biotransformation, phase I can be observed in microsomal systems of other tissues, e.g., kidneys or intestines for benzpyrene hydroxylase (Chhabra and Fouts, 1974) and in other tissues for parathion (Poore and Neal, 1972).

While the biotransformation reactions that take place in the liver microsomes have been most extensively studied, there are also metabolic reactions of phase I that are localized in other cellular fractions, for instance: reactions of deamination, oxidation of alcohols and aldehydes, aromatization of alicyclic compounds, reduction of aldehydes and ketones, and various hydrolytic reactions. It is interesting that in the extramicrosomal fraction (on the basis of another mechanism) a reaction may take place that has been known as a microsomal one. Thus, in the human placenta, aniline is hydroxylated not in microsomes but in postmicrosomal fractions as a reaction catalyzed by Hb and MetHb (Juchau and Symms, 1972). Some reactions may also proceed as a result of activity of intestinal bacterial flora, e.g. azoreduction of dyes (Gingell and Walker, 1971).

Reaction products of phase I are usually subject to conjugation. Most common reactions of this kind yield glucuronides of phenols, alcohols, amines, aromatic acid esters of sulphuric acid (mainly of phenols), or hippuric acids that are formed as products of aromatic acids reacting with gylcine. Usually the reactions of conjugation are multistage processes:

a) For the reaction to proceed it is necessary that one of the reacting substances become activated. Thus glucuronic and sulphuric acids must be present in the form of UDPGA (uridine diphosphate glucuronic acid) and PAPS (adenosine-3′-phosphate-5′-phosphosulphate), respectively. To enter the reaction with glycine, benzoic acid has to assume the form of benzoylcoenzyme A.

b) Transfer of the respective group into the

acceptor occurs usually under the influence of the enzyme, a corresponding transferase. Due to this multistep character of a conjugation reaction, it is difficult to localize the process unequivocally on a subcellular level; e.g., enzymes synthesizing the active form of glucuronic acid (UDPGA) are present in the soluble fraction of cytoplasm, whereas the transferases (transglucuronylases) are localized in the microsomal fraction. However, this is not a rule: other transferases may be found in the soluble fraction, e.g. sulphokinases that transfer the sulphate group from PAPS. The enzymes which transfer the methyl group from its active form (S-adenosylmethionine) are localed in the soluble subcellular fraction in most tissues (adrenals, lungs, liver, kidneys). The enzymes responsible for the transfer of aromatic acids from their active forms (e.g. benzoylcoenzyme A) onto glycine as an acceptor can be found in mitochondria of liver and kidney cells. A more detailed review of this problem was published by Gram and Gillette (1971). An essential feature seems to be that the systems responsible for the reaction of conjugation of xenobiotics are relatively ubiquitous. For instance, activity of UDP-glucuronyl transferase, an enzyme that transfers the glucuronide onto p-nitrophenol, has been found in the lungs, intestines, and kidneys of rats at levels not lower than in the liver (Chhabra and Fouts, 1974).

INFLUENCE OF FOREIGN COMPOUNDS UPON THE METABOLISM OF XENOBIOTICS

In general, foreign compounds may accelerate as well as inhibit the reactions involved in the metabolism of xenobiotics. The mechanisms responsible are different in either case. Most intensively studied has been the stimulating effect of alien compounds on metabolic reactions that take place in liver microsomes.

STIMULATION OF MICROSOMAL ENZYMES

The elevation of the activity (induction) of microsomal enzymes under the influence of *in vivo* exposure of animals to various xenobiotics has been extenisvely studied. The basis for assessment of the increased activity is provided usually by measurements of the rate of a model-type reaction of phase I (e.g. hydroxylation of aniline, demethylation of aminopyrine); among conjugation reactions, the effect of induction may be seen in relation to the activity of UDP-glucuronyltransferase (Jansen and Henderson, 1972). The discussed phenomenon occurs only *in vivo* or in an isolated organ or tissue culture; it could not be reproduced by adding the inducing xenobiotic directly to the suspension of microsomes *in vitro**.

A particularly pronounced effect *in vivo* has been observed in very young animals (first few weeks of life) in whom the normal activity of liver microsomal enzymes is very low. To a smaller extent the effect can be also reproduced in adults. The increased activity of microsomal enzymes is accompanied by an elevated protein content of the microsomal fraction, and, in general also, by higher concentrations of microsomal components of the electron transport chain. The stimulatory effect can be abolished by concurrent administration of inhibitors of protein synthesis (actinomycine-D, puromycine). These facts indicate that the stimulatory effect depends upon *de novo* biosynthesis of microsomal proteins.

Induction capability has been demonstrated for numerous xenobiotics that have been divided into two classes on the basis of the underlying mechanisms:

a) Compounds with the type of action demonstrated by phenobarbital, including many other barbiturates and drugs. DDT, and probably other chlorinated hydrocarbons used as pesticides, as well as PCB, may be classified into the same category (Klinger et al., 1973; Villeneuve et al., 1972; Mailman and Hodgson, 1972). Inductors of this group stimulate, in a relatively nonspecific way, the metabolism of numerous organic compounds: e.g., aniline, parathion,

* An exception is formed by so called "biphenyl-2-hydroxylase," which may be stimulated by preincubation of microsomes with a number of polycyclic hydrocarbons such as 3-methylcholantrene, 3,4-benzpyrene and others (Bridges et al., 1973). A similar effect was observed with regard to aniline hydroxylation *in vitro* in the presence of paraoxon (Stevens et al., 1972).

and other phosphoorganic compounds. The stimulatory effect becomes manifest along with an increase in liver weight, protein and phospholipid content, and the concentration of cytochrome P-450, NADPH-cytochrome C and NADPH-cytochrome P-450 reductases.

b) Compounds of which polycyclic hydrocarbons form the best known representation (3-methyl cholanthrene, 3,4-benz-a-pyrene, fluorene, anthracene, and others). These inductors have a much narrower spectrum of activity and display different kinetic features. The inductory effect is already seen a few hours after administration and is accompanied by an increased weight of the liver, and of microsomal protein and cytochrome P-450 content. No enhancement is seen of the activity of NADPH-cytochrome C and NADPH-cytochrome P-450 reductases.

For both types of inductors the effect is transient; and few days after discontinuation of their administration, the activity of microsomal enzymes returns to the normal level. The phenomena are discussed in greater detail in the papers by Gram and Gillette (1971) and Parke (1972).

Usually, the induction of microsomal enzymes leads to nonspecific enhancement of activity of the various enzymes, present in the endoplasmic reticulum, that metabolize foreign compounds. However, cases of quite a selective stimulation are known. For example, urethan stimulates selectively hydroxylation of aniline without affecting the rate of the demethylation processes, which otherwise usually occur. Most likely, this type of stimulation is not based upon the general mechanism of synthesis of microsomal protein and its active components (Schenkman et al., 1974).

From the viewpoint of exposure tests, the induction of microsomal enzymes is of importance because of three circumstances:

a) A worker who is subjected to the test could be under medication, and therefore the metabolic efficiency for a given organic compound may have been altered in comparison with normal . The following drugs have been shown to act as inductors of microsomal enzymes (of the phenobarbital induction type): Phenobarbital, Niketamide, Barbital, Chlorpromazine, Chlorcyclizine, Cyclizine, Glutethimide, Meprobamate, Orphenadrine, Pentobarbital, Phenylbutazone, Tolbutamide, Ethanamate, Aminopyrine, Imipramine (Gram and Gillette, 1971). This list is not complete; in practice a majority of organic drugs may be suspected to act in this way. In relation to many substances the induction-effects have been also observed in animals. Enhanced activity of aniline hydroxylase is one of the classic tests advocated as a proof of the inductory ability of a xenobiotic. The phenobarbital-type effects were seen for the metabolism of styrene (first stage of the pathway, leading to formation of the epoxide) (Ohtsuji and Ikeda, 1971). Phenobarbital stimulates also the metabolism of trichloroethylene; particularly the stage leading from chloral hydrate to trichloroethanol (Leibman and McAlister, 1967).

b) In case of a composite exposure to two or more substances, one of them may possess inductory capability with relation to microsomal enzymes, and, therefore, change the metabolism of a substance to be studied. A typical example is provided by concurrent or alternating exposure to phosphoorganic compounds and chlorinated hydrocarbons. The latter (DDT, Chlordane, Dieldrin, Aldrin, Heptachlor, Hexachlorocyclohexane) possess the ability to induce the microsomal enzymes and may change, therefore, the metabolism of the phosphoorganic compounds. Activity of microsomal aniline hydroxylase increases under the influence of acetone (Clark and Powis, 1974). Composite exposure to this compound and to aniline could therefore affect the metabolism of the aniline. To some extent the problem may be important in smokers (induction due to exposure to polycyclic hydrocarbons) and in individuals who frequently drink alcohol (for discussion, see Parke, 1972).

c) Substances under study may be inductors themselves, and in the course of repeated exposures may stimulate their own metabolism. This problem, which is essential from the viewpoint of exposure tests, has so far attracted little attention. Gram and Gillette (1971) maintain that autoinductory properties are displayed by DDT and benzene among others. The benzene experiments performed on rats in the author's laboratory did not confirm this concept (see also Cornish et al., 1970). Similar studies also seem to exclude a significant autoinduction in the case of nitrobenzene. A slight effect of autoinduction was observed for aniline (Wisniewska-Knypl et al.,

1975), and no effect was demonstrated for trichloroethylene (Ikeda and Immamura, 1973). Some effect is probable for carbon disulphide; however final proof seems to be lacking (Sokal, 1973). Autoinduction can not be ruled out for some phosphoorganic insecticides; this problem, however, is still far from being well-documented (McPhillips et al., 1972). Autoinduction could be important especially with regard to the tests worked out experimentally on volunteers. Usually from single-chamber-type experiments the results (correlation between dose and level of respective metabolite) are extrapolated to industrial conditions, where workers are exposed repeatedly. If a substance has autoinductive properties, the correlation may be rather different.

Due to difficulties in obtaining comparative experimental data for single and repeated, chronic exposure, there has been little direct information available on the degree of real significance this phenomenon might have. Theoretically, one could exptect two kinds of effects:

a) As a result of autoinduction, acceleration of the metabolism along all parallel pathways could take place. In a quantitative sense, an elevated excretion rate of the metabolites would be expected with unaltered general metabolic efficiency (higher urinary levels toward the end of exposure, and more rapid decline of urinary levels after cessation of exposure).

b) Due to autoinduction only one metabolic pathway could be enhanced, and the other parallel ones left unchanged. The expected overall result would include the changes in the dynamics of the increase and decline of the respective metabolite levels, and the alteration of the metabolic efficiency.

It is the present author's opinion that the autoinduction phenomenon is probably of subordinate importance from the viewpoint of its applicability to exposure tests. The supporting argument may be presented as follows:

a) Quantitatively, the induction of microsomal enzymes is of great significance only in very young animals. In adult experimental mammals it is less pronounced, and one might expect that also in adult humans metabolism of xenobiotics would not undergo dramatic changes due to induction.

b) The induction effects have been learned mainly from studies based on isolation of the microsomal fraction of liver cells and *in vitro* assays of the transformation rate of added substrate. *In vivo* metabolic transformations of xenobiotics do not occur only in liver, and the extent to which microsomal induction takes place in other tissues has not been sufficiently studied. It might be presumed, however, that the changes are significantly less pronounced. This opinion seems to be in line with the direct data of Drew and Fouts (1974), who have studied both the liver and lung microsomes.

This might provide an explanation for the fact that in preliminary experiments made in the author's laboratory on adult rats *in vivo*, no serious effect of inductors could be demonstrated upon the transformation rate of several organic compounds (unpublished data). An example of the difference between *in vitro* and *in vivo* situations may be provided by a lack of change in the values of CL_{50} or DL_{50} for benzene in rats under the influence of potent microsomal inductors (phenobarbital, chlorpromazine), in spite of the fact that distinct stimulation of the metabolism of benzene was observed *in vitro* in liver microsomes (Drew and Fouts, 1974).

INHIBITION OF MICROSOMAL ENZYMES

Inhibition of the metabolism of xenobiotics (localized in liver microsomes) has been observed for various compounds; and it may be presumed that different mechanisms were involved. The compound SKF-525A (2-diethylaminoethyl-2,2-diphenylvalerate) is a potent inhibitor of the metabolism of barbiturates, aminopyrine demethylation, hydrolysis of procaine, and glucuronide synthesis, but exerts no effect on hydroxylation rate of acetanilide, dealkylation of phenacetin, or reduction of nitro- and azo-groups. Morphine and codeine inhibit some metabolic reactions in rats, but only in males. Inhibition of microsomal liver enzymes may follow administration of high doses of nicotinamide. As with numerous other enzymatic processes, the inhibitory effect may be seen at high concentrations of heavy metal ions; e.g., repeated exposure to mercury causes an inhibition of 75–90 per cent of the normal values (Cornish et al., 1970). Microsomal inhibition has also been demonstrated after administration of phosphoor-

ganic cholinesterase inhibitors (Rao et al., 1973). Recently, the inhibitory effect of pyridine and some of its derivatives has been demonstrated using inhibition of p-nitroanisol demethylation and hydroxylation of aniline as the test reactions (Jonen et al., 1974). It is postulated that, as demonstrated with relation to other enzymatic processes, the inhibition may be of a competitive character; two compounds metabolised by the same enzymatic system could mutually decelerate their metabolic transformations. The inhibitory effects could be biphasic; the original inhibition may be followed by induction (Gram and Gillette, 1971).

These inhibitory effects upon the microsomal enzyme systems are not well-understood in detail, and the potential importance of these phenomena with regard to interpretation of the exposure tests cannot be assessed at present.

INFLUENCE OF PHYSIOLOGICAL FACTORS ON THE METABOLISM OF FOREIGN COMPOUNDS

The influence of hormones on the metabolism of xenobiotics in microsomal systems has been documented (Parke, 1972). The same, or a similar, microsomal system as that known to be in the liver for the metabolism of xenobiotics has been found in the adrenal cortex, in testicular interstitial tissue, and in the placenta. The system is active in the metabolism of cholesterol and steroid hormones. Biosynthesis of cholesterol in the liver takes place in microsomes, as well as peroxidation of the unsaturated fatty acids (Archakov et al., 1972). Thus, there exists a mutual relationship between the level and intensity of the metabolism of cholesterol and steroid hormones on the one hand, and the metabolism of xenobiotics on the other. For instance, the rate of biosynthesis and degradation of cholesterol is known to be influenced by the inductors and inhibitors of microsomal enzymes discussed in the preceding section. Phenobarbital induction enhances the microsomal metabolism of sex-hormones (testosterone, androsterone, esterone, progesterone, and others).

In rats, the activity of microsomal enzymes, involved in the metabolism of xenobiotics, is higher in males than in females. The difference is related to sex hormones. It is possible to abolish this sex effect by the administration of testosterone to females and of estrogens to males. In males recovery of the activity of microsomal enzymes after intensive whole-body or testicular irradiation can be attained by administration of testosterone (Knott and Wills, 1974). Steroids and xenobiotics form alternative substrates for the same enzymatic systems – thus, the observed metabolic interactions.

The existing data for various species are not sufficienty consistent to allow unequivocal conclusions with regard to sex-related differences in the metabolic rates of various xenobiotics in man. For instance, mice and rats differ in respect to hormonal regulation of the activity of liver microsomal enzymes (Chhabra and Fouts, 1974). Nevertheless, the possible existence of these sex-linked differences in humans should not be overlooked. In experiments aimed at the elaboration of exposure tests, such a possibility is not always taken into account; and often the experiments are performed on groups including individuals of both sexes. Only a few substances are known to display intersex metabolic differences in human beings; for instance the ratio of two trichloroethylene metabolites, trichloroethanol vs. trichloroacetic acid, differs between the sexes (Nomiyama and Nomiyama, 1971).

In rats, the most pronounced intersex differences were observed for hepatic metabolism of hexobarbital and aminopyrine. It is interesting to note that these differences disappear in fasting animals as an effect of depression of activity of microsomal enzymes in males. Similar effects have been observed after adrenalectomy or administration of thyroid hormones (triiodothyromine, thyroxine; for further discussion see Gram and Gillette, 1971). It should be emphasized that these intersex differences in hepatic microsomal metabolism were learned mostly from *in vitro* studies. It seems also relevant that, at least in the rat, the differences may be absent when metabolic reactions catalysed by microsomal enzymes in other organs, e.g., the lungs or kidneys, are concerned. Moreover, in the lungs and kidneys, the sex-related differences may be opposite from those seen for liver microsomal enzymes (Chhabra and Fouts, 1974).

Among the numerous factors that may exert an influence on the normal or induced activity of microsomal enzymes, a deficiency of ascorbic acid should be mentioned. This deficiency depresses the activity of the enzymes for many of the biotransformation reactions (for review, see Wagstaff and Street, 1971). In contrast with this finding, iron deficiency in the diet enhances the activity of microsomal enzymes (Becking, 1972).

DOSE-DEPENDENT VARIATIONS IN THE METABOLISM OF XENOBIOTICS

For the description of the kinetics of biotransformation and excretion of xenobiotics, it is usually accepted that the processes in question follow the first-order kinetics. With this assumption one should not expect any dose-dependent variations in the rate of the metabolic processes, nor in the relative yield of the individual metabolites. Although this assumption is useful for simplified calculations, in reality it is not always fulfilled. An alternative assumption, that the individual partial processes may differ in the type of kinetics, leads to opposite conclusions.

The metabolic processes of xenobiotics are enzymatic in nature. Thus, they should in general follow the Michaelis–Menten kinetics of enzymatic reactions where with a high level of the substrate the reaction rate becomes constant, independent of any further increase of the substrate level (saturation of the metabolic pathway). Since the saturation of various metabolic pathways is reached at different levels of the substrate concentration, the final yield of various metabolites may become variable with changing doses. Let us quote some more typical examples. Variable yield has been found for the excretion of the unchanged substance in animals in the case of aniline, cyclohexane, 4,6-dinitro-o-cresol, carbon disulphide, and fluorobenzene. Also, dose-dependent differences in the excretion yield of individual metabolites were found in animals and/or humans in the case of ethylene glycol, cyclohexane, aniline, nitrobenzene, p-nitrophenol, 2-naphthylamine, and carbon disulphide.

The above findings may have a strong bearing both on the fate of individual xenobiotics, and on the use that is made of the metabolic processes in the exposure tests. For instance, the metabolic pathway of DDT leading through DDD (microsomal reaction) to DDA is accelerated in case of higher doses, contributing to relatively quick clearance of the xenobiotic when the exposure is high. On the other hand, exposure tests are based on a relationship between the absorbed doses and the excreted amount of a given metabolite. A linear dependence is usually accepted which facilitates calculations and extrapolations. It may be of practical importance to realize, therefore, that the metabolic variations in question may be observable even in a relatively narrow range of doses (as in the case of aniline), leading to a curvilinear dependence between the dose absorbed and amount excreted. Such functions, if presented for simplicity's sake in the conventional linear way, give an extremely high error which in random cases may be used as an argument against the exposure test. On the other hand, exposure tests based on curvilinear functions require careful statistical calculations using special methods, especially if the possibility of an extrapolation is considered.

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4. METABOLIC AND EXCRETION KINETICS

INTRODUCTION

In the early period of elaboration of the exposure tests it already became clear that relating levels of a toxic compound, or its metabolite, in biological media to the magnitude of exposure (dose, concentration in air) was possible only if the rate of metabolism and excretion were considered quantitatively. The first attempts to systematize the concepts and principles of computation for purposes of exposure tests (Soucek, 1952) were made in the period of the development of the kinetic theory of metabolism and excretion that followed application of radioactive tracers in biological studies. This development has lead to the creation of the method of compartmental analysis (Solomon, 1953). At present numerous authors apply the principles of kinetics for processing the results of experiments aimed at the elaboration of exposure tests, and for the interpretation of the data. Attempts to systematize the relevant concepts for purposes of industrial toxicology, together with the review of possible practical applications, were made by the present author (Piotrowski, 1971). In this chapter the concepts and principles of calculations that seem particularly useful for developing exposure tests will be discussed.

KINETIC MODELS AND COMPARTMENTAL ANALYSIS

The grounds for kinetic considerations and resulting concepts are provided by observations of the time-course of the blood level or urinary excretion of a substance and its metabolites after intravenous administration. After the single introduction of a foreign substance, the level of it or of its metabolite in biological media changes curvilinearly with time in a linear coordinate system. As with other applications of chemistry and physics, the search for laws governing the process is facilitated by a system of coordinates in which the process may be depicted by a linear function, or an algebraic sum of several linear functions. This condition is fulfilled by the semi-logarithmic system of coordinates, which is very useful in kinetic studies, in that the time-course of processes in question may be usually depicted by one of the functions as presented in Figure 4-1. The most simple case is represented by curve I. If [S] = the level of a substance in the system (body), t = time of observation after administration of the dose, k = rate constant of disappearance of the substance (in t^{-1} units), the slope of the line $\alpha = 0.4343\ k$, the curve may be ascribed a mathematical form:

$$\log[S]_t = \log[S]_o - \alpha t \qquad (1)$$

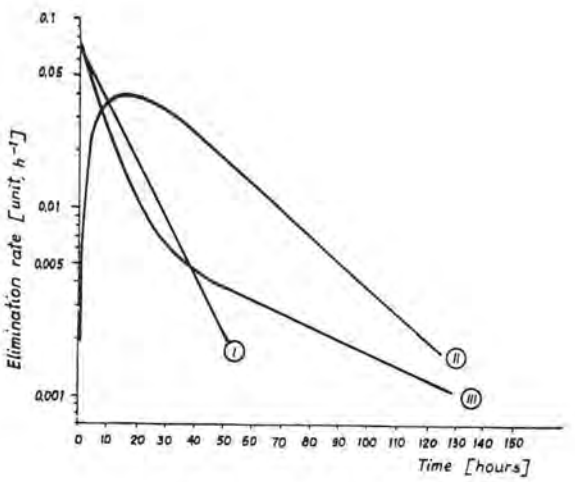

Fig. 4-1. Three basic types of elimination curves following single instantenous exposure, in a semilogarithmic system of coordinates (Domanski and Piotrowski, 1971).

Taken from: Piotrowski J.: *The application of metabolic and excretion kinetics in industrial toxicology*. US Government Printing Office, 1971. Page 144, Fig. 1ii.

or

$$[S]_t = [S]_0 e^{-kt} \quad (2)$$

where e = the base of natural logarithms. Equation (2) is the most common of kinetic equations. This equation could also be written in the form:

$$[S]_t = [S]_0 10^{-\alpha t} \quad (2a)$$

However, the original form (2), that makes use of the commonly applied elementary function e^{-x}, is more communicative and secures easier comparability with the results of many studies made so far.

Function III (Fig. 4-1) may be graphically depicted into two contributing linear functions and may be written in the form:

$$[S]_t = C_1 e^{r_1 t} + C_2 e^{r_2 t} \quad (3)$$

In formula (3) C_1 and C_2 represent the intercepts of both lines with the ordinate (at t = O), where-

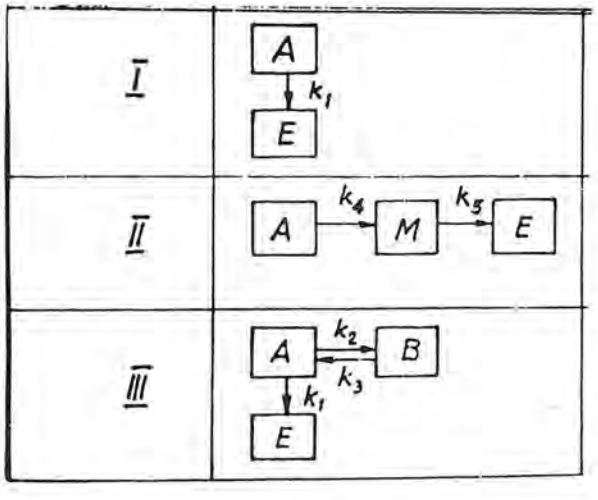

Fig. 4-2. Three basic kinetic models (Domanski and Mikolajczyk, 1971).

Taken from: as in Fig. 4-1, Page 132, Table 1i.

as r_1 and r_2 (negative values) provide the respective slopes.

A similar function can be obtained by graphical analysis of curve II. In this case the function may be expressed by a difference between two linear functions of different slopes but equal intercepts ($C_1 = C_2$). The function is usually applied for metabolite M:

$$[M]_t = C_2 e^{r_1 t} - C_1 e^{r_2 t} \quad (4)$$

The equations (2, 3, 4), as presented above, reflect three different kinetic models, which are presented in Figure 4-2. These models will be referred. to as follows: I — single compartment model, II — metabolic model, and III — two compartment open model. In all these models it is assumed that partial transfer and excretion processes are of the first order, which means that the rates represented by arrows are directly proportional to actual contents (concentrations) of the substance (or metabolite) in a given compartment. Thus, change in the systemic content [S] (Fig. 4-2 I) may be described by the most simple differential equaltion:

$$-\frac{d[S]}{dt} = k[S] \quad (5)$$

the solution of which (the integral) is provided by equation (2), derived previously from purely empirical observations. From the models II and III systems of differential equations may be deduced. For instance, the kinetic description of the processes taking place in model III, is of the form:

$$\frac{d[S_A]}{dt} = k_1 + k_2[S_A] + k_3[S_B]$$

$$\frac{d[S_B]}{dt} = k_2[S_A] - k_3[S_B] \quad (6)$$

$$\frac{d[S_E]}{dt} = k_1[S_A]$$

The solution (the integral) of this system of differential equations, in relation to the content of

the substances in the first, rapid exchange compartment A (which is composed of blood plasma, etc.) is given by the general formula of equation (3).

In the most simple case, when substance S was introduced into the system by means of single intravenous injection (into the compartment A), the integration constants C_1 and C_2 appearing in equation (3), equal:

$$C_1 = \frac{r_1 + k_3}{r_1 \quad r_2}\left[S_A\right]_o \qquad C_2 = \frac{r_2 + k_3}{r_1 - r_2}\left[S_A\right]_o \tag{7}$$

where the coefficients r_1 and r_2 may be derived in a complex way from individual kinetic coefficients k_i:

$$r_{1,2} = -\frac{(k_1+k_2+k_3) \pm \sqrt{(k_1+k_2+k_3)^2 - 4k_1k_3}}{2} \tag{8}$$

Similarly, solution of the system of differential equations for the model II may be obtained in the form:

$$[M]_t = \frac{k_4}{k_5-k_4}[S]_o\left(e^{-k_5t} - e^{-k_5t}\right) \tag{9}$$

which represents the explicit form of empirical equation (4).

The above reasoning points to the possibility of using empirical kinetic curves for reproduction of the model and its coefficients. In most situations, however, when exposure tests are considered, this procedure is not necessary. The interesting information and calculations may be deduced directly from the general equations (2, 3, 4), which can be obtained by graphical analysis of empirical kinetic data.

The three kinetic models as discussed above represent only the most simple situations. In reality, kinetic models applicable for the purpose in question may be much more complicated, either due to existence of parallel metabolic and excretory pathways or because there are, in fact, numerous metabolic compartments. An example is the complex metabolic model postulated for trichloroethylene (see chapter on "Trichloroethylene") that has not yet been solved. The existence of more than 2 compartments presents a serious complication, for the complete solution of such a model is possible only by using a computer. On the other hand, parallel pathways do not introduce very serious computational complications. In Figure 4-3, examples are given of models with parallel pathways for which solutions have been developed (Piotrowski, 1971). For practical purposes, it is convenient to use a coefficient of loss that denotes a sum of all coefficients of parallel metabolic degradations of a substance and its excretion (ΣK_{EM}).

REAL MEANING OF KINETIC PARAMETERS

The possibility, mentioned above, of decoding a model with its coefficients from purely empirical kinetic curves implies that some biological meaning could perhaps be attributed to kinetic parameters and concepts.

For organic substances, the number of systematic compartments essential from the kinetic viewpoint may be usually limited to two. In such a case, one of these will be defined as a rapid, and the other as a slow, exchange compartment. The

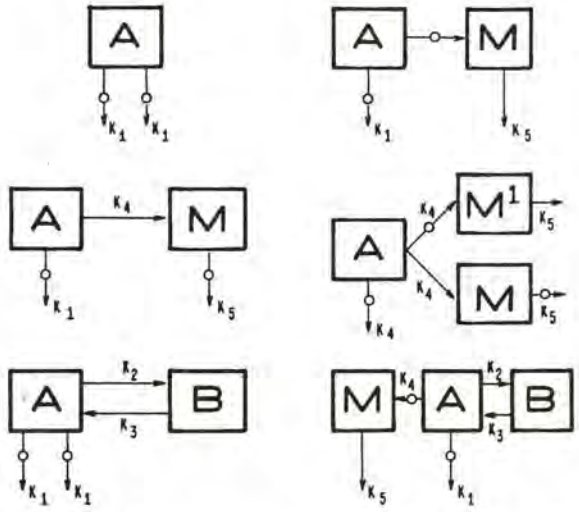

Fig. 4-3. Simple kinetic models allowing for parallel processes (Domanski and Piotrowski, 1971).

former (A) always includes blood plasma, usually all extracellular fluids, and perhaps the intracellular fluids as well. In the case of organic compounds, the slow exchange compartment (B) is usually identified with the adipose tissue; evidence in favour of this contention can be found for numerous substances. Agreement between reality and kinetic theory would require, however, full applicability of the concepts that follow from the partition of the substance in question between two phases, and, in particular, the consistency between coefficients of distribution in adipose tissue plasma and the value which can be deduced from coefficients of the model k_2 and k_3. However, the data in direct support of this are rather meager. Moreover, an analysis made using carbon disulphide as the compound (Bartonicek, 1959) has shown some problems with the concepts, reflected by differences in the desorption rate of the substance deposited in various kinds of adipose tissue. A discussion of this subject, published earlier (Piotrowski, 1971), has pointed to the complexity of the problem and justified reluctance to attribute precise biological meaning to the kinetic concepts.

Similar complications arise when the biological significance of the kinetic coefficients characterizing biotransformation of alien compounds is analyzed. These metabolic reactions are usually enzymatic processes, the course of which in general should obey the Michaelis-Menten law. The general equation would apply:

$$u = \frac{a[S]}{b + [S]} \qquad (10)$$

where u = biotransformation rate, and a and b are constants. The above function (10) may become transformed into the first order equation postulated by the metabolic and excretion kinetics only when concentration of substrate, which is proportional to the content of the substance in the body [S], is very low. Then, for $[S] \ll b$ the function (10) becomes:

$$u = \frac{a}{b}\,[S] = k[S] \qquad (11)$$

For many situations considered in industrial toxicology this condition is certainly fulfilled because of low exposure levels, predetermined by the maximum permissible concentrations in the air of industrial premises. Nevertheless, the assumption, as it stands, is being accepted unproven in the kinetics of metabolism and excretion and may fail in some cases.

SIMPLIFICATIONS OF KINETIC MODELS

Kinetic concepts and methods are often disregarded even by experienced toxicologists because they are not familiar with the rather complex mathematical procedures. On the other hand, the degree of understanding of the real biological meaning of a complex mathematical model as applied to a concrete situation is often limited. In practical problems related to the exposure tests, it seems justified, therefore, to present the models in the most simple fashion. It is important, however, that the simplification introduced has no serious practical bearing on the final calculations.

A typical simplification is often applied to organic compounds when their metabolites are being determined in the biological media. Model II (Fig. 4-2) should find application here, and in the respective equations that follow (e.g. 4). However, such a procedure is indispensable only if the maximum of the excretion rate of the metabolite is considerably delayed with regard to the end of the exposure interval. If, as often happens, the shift is small, or even undetectable, at the usual time pattern of urine collection (every 2-4 hours), the process may be adequately described by means of equation (2), characteristic of the one-compartment model I. At sufficiently long collection intervals, the first term of equation (4) approaches zero, and the whole process may be described by the remaining term of lower decay constant "r". In some cases similar simplification may be applied to the two-compartment open model, on condition that the contribution of the first term of the equation is relatively small.

A calculation often used for the characterization of the rate of processes in a single compartment model is the half-time of disappearance (elimination). If the rate constant of disappearance k is known, the half-time may be calculated from equation (2), assuming from the definition that the half-time $T_{1/2}$ is related to the decay of the substance in the system to half of its initial content. Thus we obtain:

$$T_{1/2} = \frac{0.693}{k} \qquad (12)$$

In tables 4-1 and 4-2 the simplified kinetic data for some organic compounds are assembled.

RHYTHMS OF EXPOSURE

Kinetic equations (2, 3, 4) and their unfolded forms may be applied under the assumption that a foreign substance was introduced into the system as a result of a single exposure of short duration (i.e., directly into the blood–rapid exchange compartment). To obtain the relation between the equations and the absorbed dose, it is sufficient to substitute a magnitude of the single dose Q for $[S]_0$ or $[S_A]_0$. For instance, equation (2) will then assume the form:

$$[S]_t = Qe^{-kt} \qquad (13)$$

However, for industrial toxicology the exposure of interest takes place continuously over several (usually 8) hours, and repeatedly, separated by periods off work (lasting usually 16 hours). The problem of cumulation and the related question of weekends with no exposure, will be discussed separately below.

Limiting the problem at first to a description of the process that takes place on one (the first) day, it seems useful to start with continuous exposure, which forms the first and principal fragment of the daily cycle.

CONTINUOUS EXPOSURE

To obtain the appropriate description of the process of continuous exposure, the models presented above (Fig. 4-2) have to be solved under the assumption of continuous absorption of the substance in question into the rapid exchange compartment. Let us assume, that the absorption is characterized by the absorption rate q, constant in time. The scheme on Figure 4-4 applies to model I. The respective differential equation will be:

$$\frac{d[S]}{dT} = q - k_1[S] \qquad (14)$$

where T = duration of exposure from the onset of absorption. Assuming, that at the beginning, when T = 0, foreign substance is absent from the body $[S]_0 = 0$, the solution of equation 14 will be of the form:

$$[S]_T = \frac{q}{k_1}\left(1 - e^{-k_1 T}\right) \qquad (15)$$

and respectively, the elimination rate "u" will be given by:

$$u = q\left(1 - e^{-k_1 T}\right) \qquad (16)$$

The initial run of the function, presented in Figure 4-5, may serve as an example. For the more complicated kinetic models, the shape of the functions will be similar, but they will be more complex. For instance, for model III (Fig. 4-2)

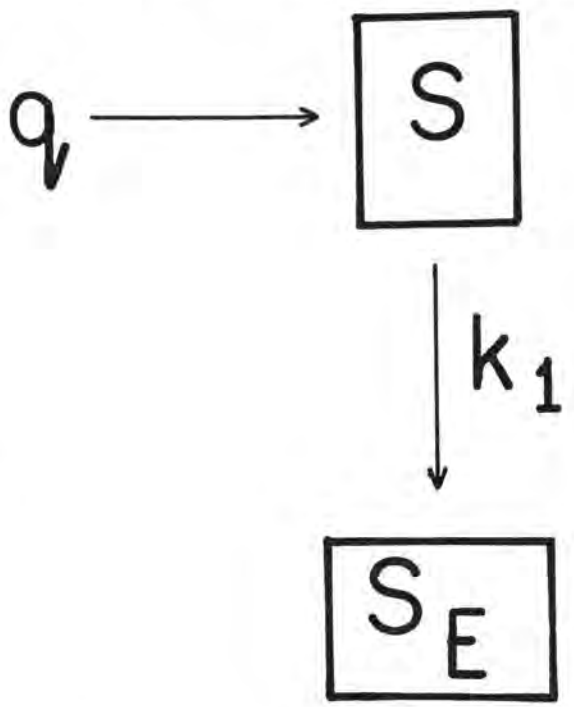

Fig. 4-4. Continuous exposure related to one-compartment model.

Substance	Animal	Routes of loss from the organism	Medium of determination	Σk_{EM}
Methyl chloride	rats	respiration air, metablism	expired air	2.8
Methyl chloride	dogs	do	blood	2.5
p-nitrophenol	man	urine, metabolism	urine	0.7
Carbon disulfide	rats	expired air, metabolism	expired air	0.5
Do	man	do	do	0.8
Polyhydroxy-alcohols (1,2-propandiol, 1,2-butandiol 1,2,4-butantriol)	rabbit	urine, metabolism	blood, urine	0.26-0.37
Benzene	man	expired air, metabolism	blood, expired air	0.23
Benzidine	rabbit	urine, metabolism	urine	0.035
Do	dog	do	do	0.12
Benzidine	man	urine, metabolism	urine	0.13
Do	do	do	do	0.1
2,6-dinitro-o-cresol	do	metabolism	blood	0.005
Do	rat	do	do	0.04
Do	rabbit	do	do	0.1
Methanol	man	metabolism, urine, expired air	urine	0.1
Fluorobenzene	rabbits	metabolism expired air	expired aid	0.08

Table 4-1. Examples of simplified kinetic data for organic substances. Coefficient of loss calculated from determinations of the parent compound (from: Piotrowski, 1971).

Substance	Animal	Investigated metabolite	Coefficient of loss Σk_{EM} (hr. $^{-1}$)
Carbon disulfide	man	Metabolite catalyzing iodine-azide reaction	0.5
Toluene	man	Benzoic acid	0.35
Aniline	man	p-Aminophenol	0.24
Methanol	man	Formate	0.3
2-Naphthylamine	dog	2-Amino-1-naphtol	0.16
2-Naphthylamine	rabbit	Sum of C^{14}	0.5
Cyanides	rat	Thiocyanates	0.07

Table 4-2. Examples of simplified kinetic data for organic substances. Coefficient of loss calculated from determinations of the metabolites (from: Piotrowski, 1971).

the solution will be given by the following formula:

$$\left[S_A\right]_T = \frac{q}{k_1}\left(1 - \beta_1 e^{r_1 T} - \beta_2 e^{r_2 T}\right) \quad (17)$$

where β_1 and β_2 are constants depending upon the coefficient of elimination k_1 and the complex coefficients r_1 and r_2. The similarity of the functions (17 and 15) is readily apparent.

If continuous absorption takes place over a sufficiently long period ($T = \infty$), differences between individual models would disappear; and for all of them we would obtain:

$$[S]_A = \frac{q}{k_1} \quad (18)$$

and accordingly, the elimination rate would equal the absorption rate:

$$u_\infty = q \quad (19)$$

Unfortunately, as a rule, in the course of a relatively short daily exposure, which is characteristic of industrial situation, the steady-state will not be attained, and the excretion rate will show a rising trend throughout the whole exposure interval. Nevertheless, a continuous pattern of absorption favours acceptance of the simplified single compartment model for substances for which the urinary metabolites are determined. It may be shown that the time required to reach maximum excretion rate, measured from termination of continuous exposure, becomes shorter the longer the continuous exposure (Piotrowski, 1971).

INTERRUPTED EXPOSURE

For a full description of systemic levels and excretion throughout the day, initiated with exposure of a few hours' duration, the following reasoning may be developed.

The time-duration of a continuous exposure may be denoted by T, and the whole time of observation, starting with the onset of continuous exposure, by t. The time elapsed since termination of the exposure will be given by the difference t - T. For the time period of continuous exposure

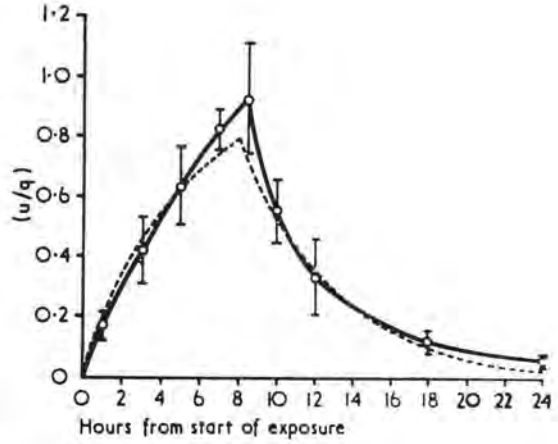

Fig. 4-5. Excretion rate of phenol as a function of time of exposure, and after its termination, expressed as a fraction of absorption rate. Dotted line — theoretical curve for k = 0.2 hr (Piotrowski, 1971).

Taken from: Piotrowski J.K.: *Evaluation of exposure to phenol.* **Brit. J. Industr. Med. 28,** 172-178, 1971. Page 175, Fig. 4.

there are valid rules as discussed earlier; and for the one-compartment model, function (16) applies.

Beginning with the discontinuation of exposure, the values of function (16) cease to rise any more and start to decline exponentially, in accordance with the magnitude of rate constant k_1. Over the entire period of observation, the excretion function will be described by the equation:

$$u_t = q\left(1 - e^{-k_1 T}\right)e^{-k_1 (t-T)} \quad (20)$$

Figure 4-5 presents the urinary excretion of phenol after inhalatory absorption; the curve represents the typical time course of function (20). The figure at the same time provides information about the agreement that can be obtained between the theoretically expected curve and empirical data, represented by mean values from various experiments on volunteers.

By analogy to equation (20), respective functions have been calculated for models II and III (Fig.

4-2) (Piotrowski, 1971). However, it seems they have not so far found practical application.

CUMULATION OF FOREIGN SUBSTANCES IN THE BODY

Cumulation of a substance in the body is understood as a process in which the level of the substance in question increases with the time of duration of exposure. It applies both to continuous and to repeated exposure. From the standpoint of exposure tests, the cumulation is taking place when rising levels of a substance occur in the analyzed media (urine, blood, expired air). In particular cases, increase of the concentrations in adipose tissue may also be of interest.

The cumulation, if it occurs, results from a slow turn-over of the substance in question. Thus it may take place under conditions of every kinetic model, provided the decay-constant is low (long half-time). From theoretical considerations it follows that the highest value of the decay constant (single compartment model) at which cumulation may take place may not exceed 0.1 hour^{-1}. For substances that are eliminated mainly unaltered in the breath, the cause of slow turn-over may be the low partition coefficient of air/blood; for substances excreted by the kidney, a low clearance may result from poor glomerular filtration, or intensive tubular reabsorption, or both.

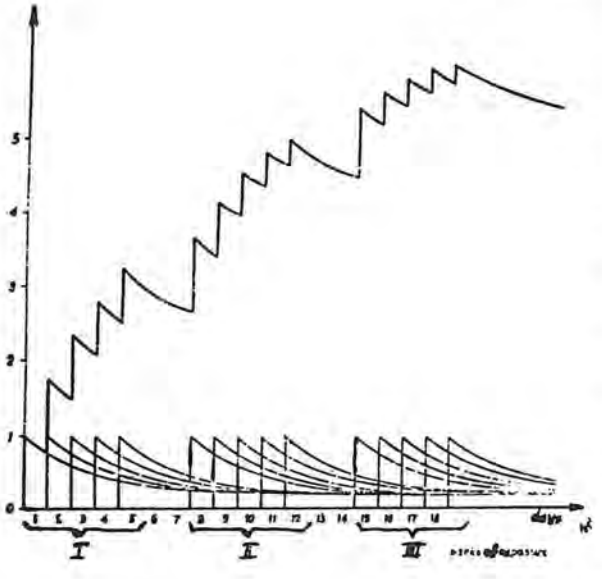

Fig. 4-6. The principle of graphic summation assuming regular weekly periods free of exposure (Piotrowski, 1971).

Taken from: as in Fig. 4-1, Page 107, Fig. 26.

In practical situations two further circumstances may be of significance, namely: slow biotransformation (when excretion takes place in metabolized form) and deposition in adipose tissue. These factors may act in combination, as is the case for nitrobenzene or DDT.

When exposure tests are considered, the problem of cumulation manifests itself most clearly in the following situation. Classically, the elaboration of a test is based on single exposure of volunteers to the compound of interest. As the result of absorption taking place over several hours, for a given level of exposure, an excretion function is obtained. In the case of a substance undergoing cumulation in the body, the function obtained after a single exposure will differ from that in people exposed previously for a longer period. An empirical solution, through a search for a steady-state level of excretion at a given exposure, is rarely feasible. The laboriousness of such a procedure is extreme; and the studies, if performed, are limited in the number of subjects and the range of exposure levels, which in turn limits the credibility of the results obtained. In principle, in addition to the levels at steady-state situation, the kinetics of attaining these levels may be calculated theoretically, if only the kinetics of the process are known from single exposure experiments. The underlying assumption is that subsequent doses of a given foreign substance do not modify the rates of their own metabolism and excretion. However, it should be mentioned in this place that this assumption may fail when substances are considered that are capable of inducing microsomal enzymes, or if one or more metabolic routes become saturated.

The mathematical concept of calculation of the time-course of cumulation is based on the principle of summation of the curves describing the levels of a substance (or its metabolites) after a single exposure over time intervals that are adequate for the situation considered. In the field discussed here, the summation of curves is made over intervals of days with weekend intermissions. The summation itself may be done graphically, as shown in Figure 4-6. When the period considered is long, the graphical procedure is tedious and mathematical summation is preferred. The principle of such a summation is explained below, taking the most simple single compartment model as an example and assuming for sake of simplicity daily instantaneous exposure of magnitude Q.

The systemic content of a substance after single administration changes as a function of time according to the formula (13)

$$[S]_t = Qe^{-rt}$$

Immediately after administration (t = 0) the body burden will equal Q. One day later it will be Qe^{-24r}; two days later Qe^{-48r} etc. If at all these intervals repeated doses Q are introduced into the system, the body level at subsequent days will be represented by a sum consisting of "fresh" dose Q plus the amounts that have been retained from the previous doses, and on subsequent days we shall obtain following values: Q; $Q + Qe^{-24r}$; $Q + Qe^{-24r} + Qe^{-48r}$ etc. This series may be expressed by the equation:

$$[S]_n = Q\sum_{1}^{n} e^{-24(n-1)r} = Q\,\frac{1 - e^{-24nr}}{1 - e^{-24r}} \qquad (21)$$

where n = number of the consecutive days of exposure. At sufficiently long observation ($n \rightarrow \infty$) the limit of this function will be given by

$$\lim_{n \to \infty} [S]_n = Q\,\frac{1}{1 - e^{-24r}} \qquad (22)$$

For more complicated models the same principles of calculation apply, and solutions are obtained of a similar shape but only more complex mathematically. For each of the models discussed here, the function can be calculated also for daily repeated exposures of several hours' duration. Interested readers may consult another monograph of the present author (Piotrowski, 1971).

The functions describing trends of cumulation in the period of repeated exposure (formula 21) reach asymptotically the limit which denotes equilibrium between daily absorption and elimination. This regular trend is disturbed by the weekend breaks in exposure. Then the body burden or metabolite levels in biological media drop. The steeper the slope, the faster is the turnover of a substance; and after the return to exposure the next week, they continue to rise again.

The rising trend is most pronounced in the first week of exposure. From theoretical considerations, it follows that for substances with a very slow turn-over, after a very long exposure period, the upward trend within the week may be very slight or even inconspicuous due to the usual biological scatter of the data. An example of upward trends in the first and in a remote week of exposure for substances of an assumed elimination constant (0.1 day^{-1}) is presented in Figure 4-7. The trends displayed by body burden or urinary excretion of substances, or their metabolites, in weekly cycles may be calculated theoretically; however, the resulting formulas are quite complex and have not been so far verified experimentally (Piotrowski, 1971).

The concept underlying calculation of the time-course of cumulation as presented above, proposed in principle by Soucek and Pavelkova (1953), has its adherents, among them Filov (see Goulebev et al., 1973). It should be emphasized, however, that the volume of experimental data that could be found for verification of the method is limited. For substances that are considered in some detail in this monograph, such verification has been obtained for nitrobenzene. For other substances for which the problem of systemic cumulation is pertinent (trichloroethylene, tetrachloroethylene, parathion, DDT, etc.), limited kinetic data for repeated expsoures are available. However, they have been confronted with results representative of single exposure, in the manner postulated here. Bearing in mind the limited scope of experimental data, wider application of the concepts recommended here must

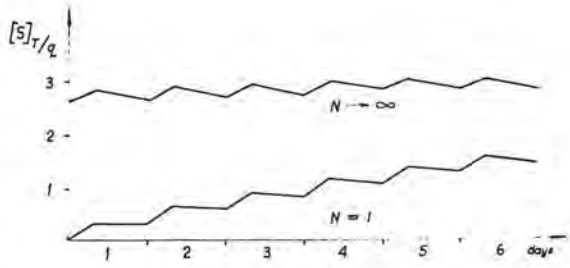

Fig. 4-7. Increasing trends of systemic levels of a substance in the first week (N = 1) and in an infinitely distant week (N→∞) of exposure. Calculated for the single-compartment model with coefficient of loss 0.1 day^{-1}. (Piotrowski, 1971).

Taken from: as in Fig. 4-1, Page 109, Fig. 27.

be left to the discretion of individual investigators, and their confidence in the kinetic methods of description for the observed regularities.

SYSTEMIC CUMULATION AND EXPOSURE TESTS

Elaboration of an exposure test, in fact, may be reduced to the assessment of exposure level (E) and the determination of the corresponding level of the foreign substance, or its metabolite, in the biological medium (I). Determination of the latter must take place at a constant time relative to the end of the daily exposure period. In the most simple case, the reading of the test (I) is directly proportional to the level of exposure, in accord with the equation:

$$I_1 = a_1 E \qquad (23)$$

where subscript 1 denotes first day of the exposure cycle (e.g., an experiment on individuals that have not been previously exposed). For substances that do not undergo substantial systemic cumulation, the proportionality coefficient "a" will not vary with consecutive days of exposure ($a_1 = a_\infty$, and $I_1 = I_\infty$, at E = constant). These conditions are fulfilled by such substances as phenol, aniline, carbon disulphide, etc. For substances which undergo cumulation in the body, the relationship (23), obtained from measurements in individuals not exposed previously, will not hold under conditions of repeated exposure. For further discussion it is convenient to assume that a worker is observed during a week infinitely remote from the onset of exposure, when the relationship would be expressed by the formula:

$$I_\infty = a_\infty E \qquad (24)$$

Thus, for interpretation of the experiments performed in toxicological chambers, it is essential to estimate coefficients that would permit deduction of equation (24) from equation (23). In fact, this is possible when kinetic data are considered which may be obtained additionally from a properly designed chamber experiment. From the latter we have obtained the time-curve of levels of a substance (e.g., urinary excretion rate as a function of time) that, for period $\tau = t - T$ after discontinuation of exposure, may be expressed by a general formula:

$$\frac{\mu_1(\tau)}{q}(1) = \mu_1 e^{r_1 \tau} + \mu_2 e^{r_2 \tau} \qquad (25)$$

where μ_1/q is the reading of the test (excretion rate) standardized against absorption rate (or absorbed dose) in a constant exposure period T. Coefficients μ_1 and μ_2 may be obtained empirically as intercepts of the two terms of the excretion curve (model II or III; in the model I, $\mu_2 = 0$) for $\tau = 0$.

For an infinitely remote week of exposure the following equation holds:

$$\frac{\mu_\infty(\tau)}{q} = \mu_1 L_{1s} e^{r_1 \tau} + \mu_2 L_{2s} e^{r_2 \tau} \qquad (26)$$

where L_{1s} and L_{2s} denote borderline values of cumulation coefficients for both terms of equation (30).

Exposure is usually measured toward the end of the daily exposure period, at the end of the week. For a chamber experiment this corresponds to the equation in which $\tau = 0$, and therefore equation (23) will assume the form:

$$I_1 = (\mu_1 + \mu_2)E \qquad (27)$$

Assuming further that in industry the measurment takes place on the fifth day of an infinitely remote week, directly after cessation of exposure, the equation corresponding to equations (24) and (26) will be obtained in form:

$$I = \mu_1 L_{1s}(5) + \mu_2 L_{2s}(5) \qquad (28)$$

where $L_{is}(5)$ is an asymptotic value of coefficient L_i, calculated for the fifth day of an infinitely remote exposure week. Through respective comparison of equations (27) and (28), and respecting the general form of equations (23) and (24), we obtain:

$$a_\infty = a_1 \frac{\mu_1 L_{1s}(5) + \mu_2 L_{2s}(5)}{\mu_1 + \mu_2} \qquad (29)$$

Coefficients L_{is} in this equation, estimated for $r = 0$, are given by equation:

$$L_{is} = \frac{1}{1-e^{7r_i}} \cdot \frac{1-e^{(7-m)r_i}}{1-e^{r_i}} e^{(j+m)r_i} + \frac{1-e^{jr_i}}{1-e^{r_i}} \qquad (30)$$

where coefficients L_i (L_1 or L_2) correspond to the terms of the equation (excretion) determined by coefficients r_i (r_1 or r_2), j = number of a consecutive day in a given week of exposure, m = number of weekend days (1 or 2, depending on whether there are 5 or 6 working days in a week). Further details concerning formula (30), deduced by Domanski and Mikolajczyk, were given in the previous monograph of the present author (Piotrowski, 1971). This monograph should also be consulted for practical advice regarding kinetic calculations from experimental data.

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5. WORKING OUT THE EXPOSURE TESTS ON VOLUNTEERS IN EXPERIMENTAL CONDITIONS

INTRODUCTORY REMARKS

Experiments made directly on humans in controlled conditions, aimed at elaboration of the exposure tests, are usually a final stage of longer preparatory studies that have been performed earlier. Due to difficulties which are generally encountered in finding a large number of volunteers, these experiments are designed as a rule to provide answers only to some selected problems, or aspects of a problem, that could not have been solved either by means of animal experimentation, or by studies of biological material sampled from individuals exposed in industry.

One usually starts working out an exposure test by experiments on humans only when the following conditions have been fulfilled:

a) there is a practical need for the test in question for the evaluation of the workers' exposure in industry;

b) a general outline of the metabolism of the compound is known from experiments on animals;

c) there is a sufficiently sensitive method for determining the compound or its metabolite in biological material; and

d) it has been proven in industrial conditions that the available method yields positive results in exposed people.

The experiments on human volunteers are usually conducted in several stages. The first of these may have the objective of obtaining data on the quantitative relations between the metabolites in man and the kinetics of their elimination. It is not exceptional that elucidating the human metabolism of an industrial poison leads to the selection of a metabolite, for the basis of a test, whose value for such a purpose would not have been recognized, if the results of animal experiments alone had been considered. Among the compounds, treated in detail in this monograph, this has happened for ethylbenzene, styrene, and nitrobenzene. Nitrobenzene exemplifies pronounced differences in metabolic kinetics between experimental animals and man. The next step consists usually of a basic quantitative experiment in which, on basis of applied inhalatory exposure, a correlation is established between the absorbed amount (air-concentration) and the eliminated amount of the substance or of its selected metabolite. The third stage may be of a complementary nature and may include, according to the needs, evaluation of cutaneous absorption and, perhaps, studies of the degree of systemic cumulation under conditions of repeated exposure. In practice it seldom occurs that the stages are followed in the logical sequence as given; this can be worked out and introduced into practical use at an early stage of toxicological reconnaissance when basic toxicity is evaluated, MAC values postulated, or methods worked out for the determination of a substance in air. The tests are, as a rule, developed for substances whose toxicity and potential hazards are rather well known, and for which the main practical problem lies in adequate control of the magnitude of exposure. Moreover, taking due account of the fact that elaboration of a test is a lengthy and expensive procedure, and that serious motivation must exist for undertaking any experiments on human beings, working out the tests is undertaken mostly for substances that create a serious health hazard in industry. The degree of health hazard may result either from the seriousness of biological effects (e.g. benzene, benzidine), or from widespread application and size of the exposed population (for example: toluene, styrene, carbon disulphide, DDT). A most obvious motivation for the elaboration of an exposure test seems to exist for those substances for which dermal exposure is of significance (e.g. benzidine, nitrobenzene, aniline, parathion). If several motives exist, an exposure test becomes highly desirable.

TOXICOLOGICAL CHAMBERS.

The chambers used in experiments on volunteers are relatively simple and can be easily constructed in most larger laboratories. The chamber it-

self consists of a small room (or part of it) of such a volume as to accommodate in a comfortable standing, sitting, or lying position at least one volunteer. The usual volume would thus be 10-20 m^3. The chamber should be in the direct vicinity of a laboratory; the contact should be close enough to render easy service of the champer possible (taking the samples and performing analyses). The chamber is usually divided into two spaces: the first one is where preparation of the air to given parameters takes place (i.e., dossage of test substance, mixing and conditioning of the air); the second space is occupied by the volunteer and provides air with predetermined concentrations of the test substance. A diagram of a typical toxicological chamber, designed by Dutkiewicz (1960), which has been used by the present author, is presented in Figure 5-1. The design allows for controlled variation of flow-rate, temperature, and humidity of the air. A capability for maintaining predetermined parameters of the microclimate is particularly relevant for experiments on dermal absorption of vapours of organic compounds.

The dosage of a test substance needed to attain programmed concentrations can be based on various principles, in the same way as it happens in chambers for animal experiments. In the author's laboratory where liquid non-decomposing compounds have been studied (aromatic hydrocarbons and their derivatives, chlorinated aliphatic hydrocarbons, carbon disulphide) a simple method proved useful: namely, a technique based on evaporation of a liquid from a vessel of a constant surface. The increasing concentrations are easily obtained by raising the temperature of the vessel, and keeping it constant by means of a thermostatically controlled heater.

Apart from large chambers, enabling studies both on inhalatory and cutaneous absorption, minichambers are used sporadically for inhalation studies. One variant, designed by Senczuk and Orlowski (1974), consists of a helmet covering the head of the subject. In another type this chamber forms an air-tight blouse, attached to a dosing mechanism in such a way that investigation of inhalatory absorption becomes possible. These little chambers seem particularly useful in small laboratories, and, also, for preliminary *ad hoc* estimations of absorption of a given compound in the respiratory tract.

Concentrations of test substances in the exposure chambers may be measured either in a classical way by aspiration and determination in a solution by one of the available methods, or directly in the gaseous phase using physico-chemical techniques. UV-absorption is one of the methods of choice; as an example, a simple Mercury Vapour Concentration Meter (Bardodej, 1964) may be quoted. A system, particularly suitable for analysis of expired air by UV-absorption measurements was described by Bocek and Nemecek (1970). Infra-red absorption measurements are also used, mainly in gas-cuvettes of the multi-reflex type, enabling a light beam pathway of several meters to be obtained. Finally, the most universal method – gas chromatography – is commonly used at present. The latter may be combined with aspiratory sampling of air or with direct injection of the air sample into the column of the instrument. The latter technique has

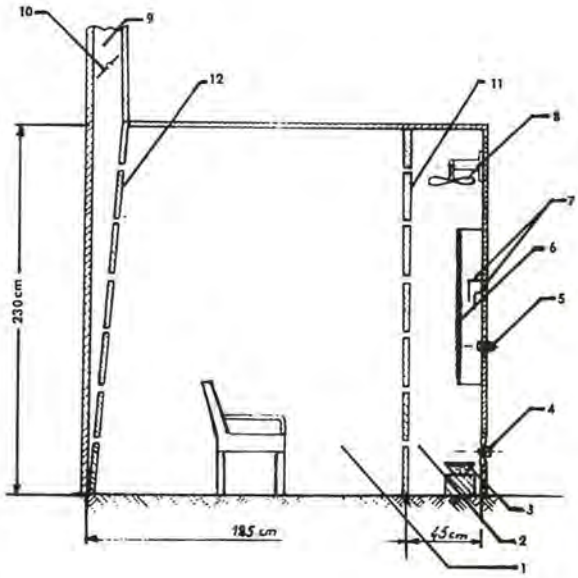

Fig. 5-1. Example of a chamber designed for experiments on volunteers (Dutkiewicz, 1960).

Legend: (1) main chamber space, (2) mixing chamber, (3) vapour-generating device, (4) air flow meter, (5) steam inlet, (6) electric heater, (7) contact thermometers, (8) pendulat ventilator, (9) suction channel, (10) valve, (11) and (12) division walls with regulated slits. Maintaining of desired temperature and humidity automatic through (5), (6), (7). Air flow regulated mechanically through (10). Vapour generation by heating of the liquid substance in a temperature yielding the desired concentration.

Taken from: Dutkiewiz T.: *Toksykologiczna komora doswiadczalna.* **Medycyna Pracy 11,** 117-124, 1960.

been used extensively by R.D. Stewart; the references to this work are in the chapters on "Styrene", "Trichloroethylene", and "Tetrachloroethylene".

PURPOSE AND DESIGN OF THE EXPERIMENTS

The experimental design (including the number of persons exposed concurrently), the range of air concentrations, the frequency of urine and breath sampling, etc., depends on the purpose of the experiment and the facilities available.

Experiments of the first reconnaissance stage aim usually at estimating the respiratory retention, as well as, the amount of excreted metabolities. The experiment, therefore, should incorporate analysis of the inspired and expired air, and measurement of its volume. Collection of urine may be performed according to any desired scheme, up to termination of the measurable excretion. For methodological reasons, as a rule, it appears useful to apply a relatively large dose of a substance, and therefore starting the study from a series of self-experiments is desirable. This preliminary experiment permits orientation as to how the kinetics of metabolite excretion look. A series of examples of earlier experiments of this type have been assembled in the monograph by Teisinger et al. (1956).

Systematic experiments have to have more precisely defined objectives, namely: a) establishing a relationship between exposure (dose) and urinary level of a metabolite; and b) derivation of kinetic parameters enabling interpretation of the data for repeated exposures. In the author's laboratory the following experimental design has been accepted as the most useful one:

a) The experiment is conducted for air concentrations in a range below and above the accepted TLV*, incorporating as a rule a series of 3 to 4 concentrations with an interval of a factor of 2. For each concentration 3 to 5 experiments are made on different individuals. The total number of volunteers amounts on the average to 5-10. The duration of exposure has been adapted to the length of a working shift. When for a given substance two daily exposure intervals (6 and 8 hr) are met in industry,** the experiment is for 8 hours, and the design allows for interpretation of the results with regard to both situations. In the course of the experiment one or two 1/2 hour breaks are made for meals.

b) Urinary samples are collected every 2 hours over the exposure period, and for the rest of the day; the night urine is collected as a single portion. If collection over the next day appears necessary, it is instituted at liberal intervals. The principle to be observed, however, is to collect for analysis complete portions of the urine excreted within an interval, with precise notification of the voiding time; this permits calculation to be made of the excretion rate. In all samples, concentration of a metabolite and specific gravity or concentration of creatinine are measured. These parameters permit analysis of the correlation for various variants of an exposure test.

INHALATORY EXPERIMENT

In the author's laboratory the inhalatory experiments are performed in such a way that exposed subjects stay outside the chamber and breathe the air from the chamber through a respirator with two one-way valves. Such a procedure precludes dermal absorption during the experiment. The sampling method of the inhaled air is essential for an appropriate calculation of the absorbed amount of the substance under study. Simple measurements of the concentrations in the chamber air cannot be applied in the calculations because of the adhesion of the substance to the tubing; true concentrations in the inhaled air are lower than those in the chamber. For practical purposes, a satisfactory solution would be such that secures sampling of inspired and exhaled air directly from the tubing in the proximity of the subject's face. This principle has not been commonly adopted by all laboratories; in most inhalation experiments the exposed individuals are placed inside a chamber which does not exclude cutaneous absorption.

In these experiments the measure of exposure is the amount of a substance retained in the system in the course of the experiment. To some extent this allows elimination from consideration of one source of variation of the results that would occur if the test were based merely on concentrations in the chamber air. These variations depend on individual differences in lung ventilation rate. The necessary step from the doses to concentrations is made in computation, assuming a likely

* Threshold limit value.

** In Poland for highly toxic substances the permissible daily duration of exposure is 6 hr; in the rest of cases 8 hrs; in equivocal situations both lengths of daily exposure are encountered in various branches.

ventilation rate in industrial workers, according to the formula:

$$D = CTVR \qquad (1)$$

where D = the absorbed dose (in mg), C = concentration in the inspired air (mg/m³), T = duration of the exposure (hr), V = ventilation rate (m³/hr), R = retention of a substance in the respiratory tract, expressed as a fraction (dimensionless). While comparing the doses absorbed by workers with recommended or legally obligatory values of TLV a ventilation rate typical for light work can be assumed – namely 0.8-1.25 m³/hr. This procedure has not been generally accepted; moreover, experimental designs dominate in which direct concentrations in the air in chamber are taken as the measure of exposure. Examples of both approaches may be found in the chapters devoted to specific compounds.

When the experiments have been completed for a series of concentrations, the results may be diagramatically presented as in Figure 5-2. In the course of exposure, the concentration of a metabolite (excretion rate) rises, then declines thereafter with the cessation of absorption. The elimination curves obtained for various doses (air-concentrations) are usually proportional to the doses. For practical purposes values are relevant

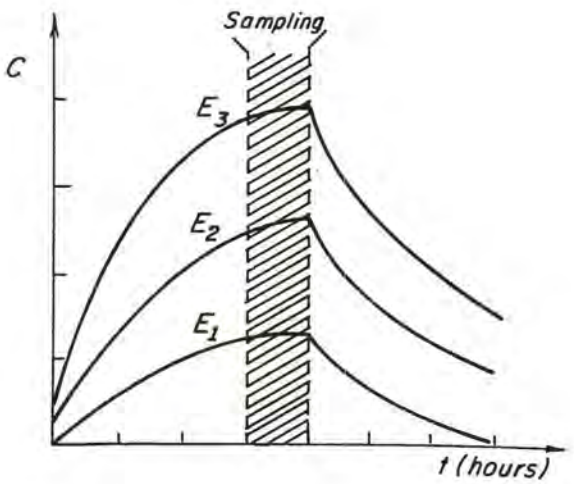

Fig. 5-2. Schematic presentation of the time-course of concentrations in biological media (C), as a function of time, at various exposure levels (E).

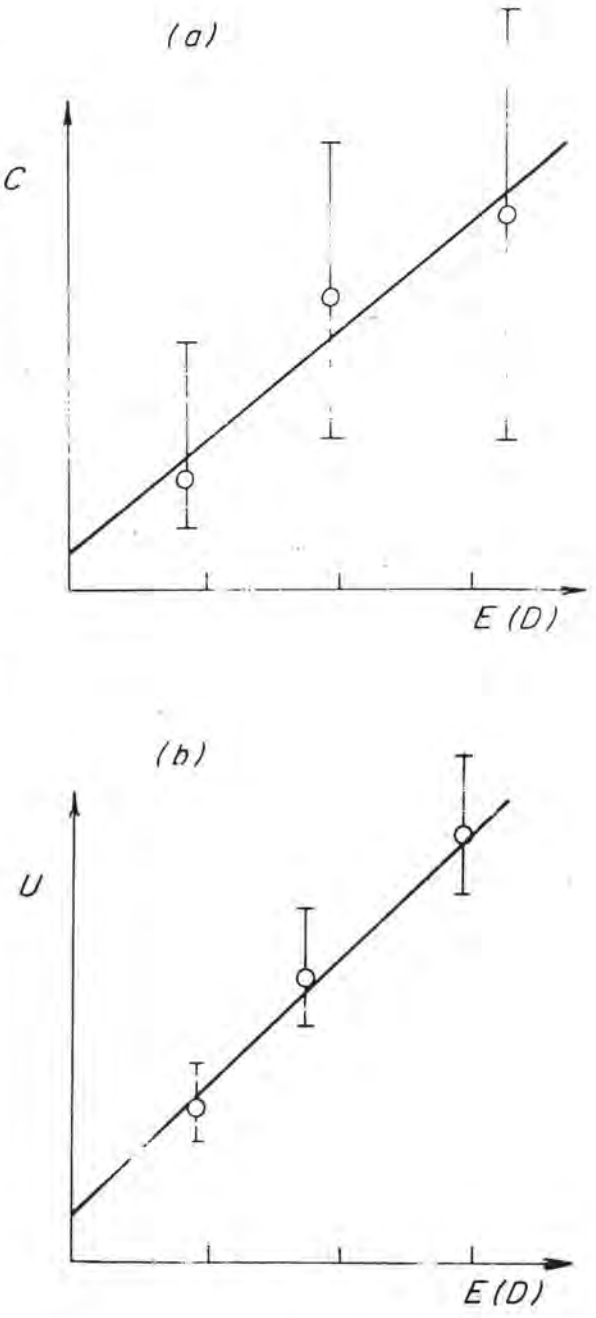

Fig. 5-3. Schematic presentation of the level of substance (or metabolite) in urine collected toward the end of exposure (see Fig. 5-2), as dependent on the exposure level (E) or dose absorbed (D); (a) concentrations uncorrected (C); (b) concentrations corrected resp. excretion rate (u).

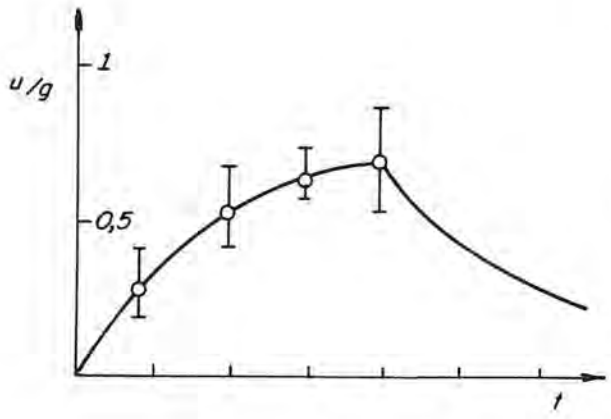

Fig. 5-4. Excretion rate of a metabolite expressed as a fraction of the absorption rate of the parent compound (u/q) observed during exposure and after its cessation.

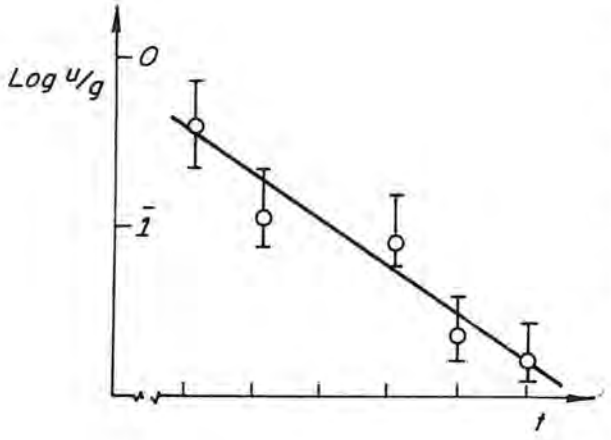

Fig. 5-5. Excretion rate (expressed as a fraction of the absorption rate) as dependent on the time-lapse after cessation of exposure, in a semi-logarithmic system of coordinates.

at a constant point in time, usually those observed in the last two hours of exposure. The levels of metabolite in this interval (shaded area) are correlated with absorbed doses in a way presented in Figure 5-3. Two variants of a test are presented on the same measurements; the graph (b) shows the concentrations in urine as measured directly, and graph (a) presents the same data normalized to standard specific gravity, average creatinine content, or excretion rate. Accuracy of the correlation and precision of the assessment of the absorbed dose may be estimated statistically for each of the variants. As a rule more precise results are obtained if the excretion rate of a metabolite is taken as the variable. This rate is a product of the concentration of the metabolites and the excretion rate of urine:

$$u = cd \qquad (2)$$

where u = excretion rate of a metabolite (mg/hr); c = concentration of the substance in urine (mg/ml), and d = excretion rate (mg/hr). Differences in precision of the variants of the test were most pronounced when absorption of phenol has been assessed from urinary excretion of the substance. If direct urinary concentrations were taken as the dependent variable, the absorbed dose could be estimated with precision of about ± 80 per cent only; this could be improved to about ± 40 per cent when the results are normalized to the standard specific gravity of urine = 1.024, and reached the unusual value of ± 6 per cent for the urinary excretion rate of phenol. A similar trend, even if less pronounced, was observed when the tests were worked out for aniline, nitrobenzene, and toluene.

The experimental design, as outlined above, allows for calculation of the kinetics of the process from the experimental data. In the most simple case, in all experiments in which the measured values over a given time interval were analytically satisfactory, the data could be presented in the form:

$$u/q = f(t) \qquad (3)$$

where u = excretion rate of a metabolite (mol/hr), q = absorption rate of a parent substance (mol/hr) (Fig. 5-4). In this arrangement, if metabolic efficiency were independent of the dose, the curve would in turn be independent of air concentrations of the substance. Over the exposure period the value u/q tends to reach an asymptote, determined by the metabolic efficiency. In the most favourable case, the value of the latter equals unity (e.g. phenol). The results obtained for the descending slope of the curve, depicted in the semilog coordinates, yield the half-time and excretion constant (Fig. 5-5) that is required for final interpretation of a test (see chapter "Kinetics"). For substances whose elimination constants

exceed 0.1 hour $^{-1}$, no cumulation should be expected at repeated industrial exposure; if the vapours of a substance are not absorbed through the skin, the test is complete. Interpretation of the test may be then based directly on the graph (Fig. 5-3) or on a formula of the type:

$$U_t = U_o + aD \quad (4)$$

where Ut = excretion rate of a metabolite (or its concentration) at a constant time relative to the period of exposure, e.g., the last 2 hours of exposure; D = the dose absorbed during the experiment; a = regression coefficient as estimated (usually by means of least squares); U_o = mean background level, assessed in the period preceding the experiment. When the relationship appears curvilinear, the formula may be calculated by means of one of the nonlinear regression functions. If the elimination constant is less than 0.1 hr $^{-1}$ in the final formula that expresses quantitative interpretation of the test, a due correction has to be introduced (see chapter "Kinetics"). If the cumulative trend is pronounced, the correction can be considerable and its experimental verification in daily repeated exposures may be useful.

The above principles, outlined earlier by Piotrowski (1970) in their basic form, seem also to apply to the tests based on analysis of the breath. Essential difference does not depend here as much on the change of analytical technique, as on the difference in the dynamics of behaviour of volatile substances eliminated in the expired air relative to that inherent for excretion of metabolites by the kidneys. As far as the analytical procedure is concerned, it is essential to assure reproducible and well-defined sampling of exhaled air. There seem to exist two possibilities: analysis of alveolar air (an isolated sample collected toward the end of expiration), or analysis of the total exhalation. For the latter alternative a useful technique of sampling was described by Sherwood and Carter (1970). Details of sampling and interpretation of measurements in alveolar air were given by Bocek and Jandrova (1970). However, it seems that detailed principles regarding the time of sampling have not been precisely formulated. Difficulties in making a decision about sampling time result from the very fast decline of the concentrations. It appears that sampling in the first few minutes post-exposure should be avoided as desorption of a substance from the upper respiratory passages is still taking place. On the other hand, too long a delay in sampling can be inconvenient due to the considerable decline of the concentrations. Furthermore, the decision becomes even more complicated in industrial conditions because the time of sampling cannot be scheduled with sufficient precision relative to the moment when exposure was discontinued. It seems, therefore, that optimal sampling time should be selected in such a way as to minimize the error that would result from minor shifts in time.

There are also difficulties in the interpretation of the data that derive from different ways in which the concentrations in expired air increase as compared with the concentration of metabolites in urine. Usually the latter increases almost linerly with time of exposure and thus the measurements depend not only on the absorption rate but also on the time of exposure; in other words, they reflect the total absorbed amount (dose). On the other hand, concentrations of volatile substances in the breath increase rapidly at the onset of exposure with slow changes afterwards. Therefore, measurements of elimination in the expired air reflect more likely the actual absorption rate than the accumulated dose. This problem has not been studied in sufficient detail. Of interest is the proposal of Sherwood and Carter (1970), who suggested with regard to benzene that for assessment of systemic deposits of the substance the expired air should be analyzed as sampled on the next day after exposure, before the start of work. At that time the elimination rate depends more on the systemic deposits than on the recent absorption rate of the previous day.

CUTANEOUS EXPOSURE

From the point of view of exposure tests, experimental evaluations of dermal absorption are particularly essential if the substance in question is absorbed through the skin directly from the gaseous phase. In such a case it has to be presumed that the TLV for the concentration in air was established on the assumption that absorption takes place both via the skin and the respiratory tract. This has obvious bearing on the relationship between the allowable concentration and the allowable absorbed dose.

The only possibility of quantitatively assessing dermal absorption from the gaseous phase rests in the utilization of the previously elaborated exposure test, based on the inhalatory penetration of a substance into the system. Determination of a metabolite after dermal exposure permits calculation of the absorbed dose from a previously established graph or from a proper mathematic relationship. There is of course the underlying

assumption involved that the efficiency of metabolization, as well as the dynamics of excretion, are not essentially different for both routes of absorption. For further discussion of this issue see the chapter "Absorption routes".

Technically the experiment is conducted in such a way that the volunteer is inside the toxicological chamber but breathes clean air from the outside through a respirator and required tubing. Microclimatic conditions play an essential role in such experiments and should, therefore, be thoroughly controlled. In the subsequent series of experiments the following issues are usually studied: a) influence of the concentration of the substance under study on absorption rate, b) influence of temperature and humidity, and c) effects of clothing. The data permit an assessment of how much the variable conditions in the working environment affect cutaneous absorption from the gaseous phase.

Of basic importance for the quantitative interpretation of the test is the relationship between the concentration of the substance in air and the dermal absorption rate. In the simplest case it can be linear, and then the absorbed amount would be directly proportional to the concentration C and duration of exposure T:

$$D = \alpha CT \qquad (5)$$

where α = a proportionality coefficient. If the dose D is expressed in mg, time in hours, air-concentration in mg/m^3 then the dimension of the coefficient α would be m^3/hr. This provides information about the volume of air "cleared" through dermal absorption of the substance per unit of time. This coefficient is directly comparable with the coefficient RV (equation 1) for the inhalatory exposure.

When both cutaneous and respiratory absorption occurs, the relationship between the absorbed dose and air concentration would be expressed by the formula:

$$D = CT(RV + \alpha) \qquad (6)$$

in which the magnitude of both coefficients reflects the relative role of the two processes in total exposure. Examples have been given in the chapters "Phenol", "Aniline", and "Nitrobenzene".

For evaluation of the routes of absorption, quantitative experiments are sometimes made of dermal absorption from direct contact of a substance with the skin. If it is possible to eliminate the losses by evaporation, or to assess quantitatively the loss, the amount absorbed may be calculated from the reduction of the substance at the site of application (for examples see the chapters: "Aniline", "Nitrobenzene", "Benzene"). The technique is rather subtle and subject to numerous reservations whose detailed discussion falls outside the scope of present monograph. Basically speaking, however, this is one of the possible ways to test the hypothesis of the independence of the metabolism of a substance from the route of absorption. Another alternative and indirect possibility is provided by a study of the ratio of two different metabolites after administration of a substance by two different routes. In such a case it appears desirable that the metabolites be formed along two independent metabolic pathways. An example is given in the chapter "Nitrobenzene".

Quantitative data on the cutaneous absorption rate of a liquid or solid substance in direct contact with the skin are of comparative significance. The values do not enter the equations upon which the interpretation of exposure tests is based. On the other hand, in industrial conditions it is possible to estimate the intensity of dermal absorption. The principle is based on a precise comparison of the effective concentration to which a worker has been exposed during the work shifts with the absorbed dose which is estimated by means of an exposure test. The amount exceeding that given by formula (6) is attributed to cutaneous absorption resulting from direct contact of the substance with skin.

EXPERIMENTAL REPEATED EXPOSURE

Simulation of daily repeated exposure in experimental conditions would be highly desirable because of the scarcity of such human data. This is particularly true when the degree of expected cumulation in the body is considerable, and there exist reasons to suspect that using the coefficients calculated from kinetic equations may lead to serious errors.

Generalizations based on the few existing experiments seem premature; the remarks given below should be considered only as suggestions.

The basic objective of such experiments is acquisition of the data characterizing the steady-state situation. Therefore, the experiments should be continued until the moment when the daily excreted amount of a metabolite equals that ex-

pected from the metabolic efficiency* which had been determined from a single inhalatory experiment. Observations of the dynamics of the increasing urinary levels of a metabolite in subsequent days provide basic data to answer the question concerning which day of the week the samples should be taken for evaluation of exposure.

Due to easily understandable difficulties in attracting volunteers for experiments of this type, the number of studied individuals and of discrete experiments will be more seriously limited than those of single exposure assays. This means that collecting data for various levels of exposure is particularly difficult, and in practice one is left with direct proportionality extrapolation. Therefore, the data on levels of metabolites at a given magnitude of exposure cannot be presented with very great precision. Moreover, since systemic cumulation of organic compounds is determined largely by their deposition in adipose tissue, individual variation in amounts of body fat may affect the magnitude of the cumulation. In addition any factor that mobilizes a substance from adipose deposits could elevate the urinary levels of the metabolites, to the point that they exceed by far those amounts that could be attributed to the actual absorption rate at a given metabolic efficiency.

Therefore, it may be suggested that experiments with repeated exposure should be treated mainly as controls for kinetic extrapolations. If there is a statistically insignificant difference between the two procedures, the choice of appropriate values for interpretation of a test forms a dilemma that must be left to the intuition of the observer.

PHYSIOLOGICAL OR "BACKGROUND" LEVELS OF METABOLITES

The relationship given by equation (4) indicates that for the exposure tests the significance of the absolute level of a metabolite after exposure is less than the significance of the increase above the normal "physiological" or "background" level. The increment is attributed to the absorption of the substance in question. The precision of the test decreases sharply at low levels of exposure when physiological levels contribute significantly to the total level observed. This seriously limits the application of the tests to low levels of exposure. Examples are given in specific chapters, mostly those dealing with toluene and carbon disulphide.

Direct assessment of the physiological level and of the increment is not possible in the same sample; evaluation of the physiological or background level always bear the stigma of an extrapolation. The error of extrapolation is directly related to the magnitude of oscillation of the physiological level. The observer's influence upon the dispersion of the physiological values is certainly limited; however, applying due corrections (for specific gravity, creatinine content, or diuresis similar to those discussed above for the very tests themselves) may considerably reduce the variation in some cases. In the author's laboratory the issue of determining a true physiological level is usually considered in a separate preliminary experiment in which the urine of non-exposed individuals is sampled in exactly the same way as in the experiment itself. In addition, each person volunteering for exposure experiments collects his or her urine over a complete 24 hour period preceding the experiment, and the mean value of the substance under study is later used as a physiological reference level.

In some cases (toluene, carbon disulphide) physiological levels of the metabolites display season- or nutrition-dependent variations. Such a possibility should be investigated in preliminary studies for an exposure test.

PRECISION OF EXPOSURE TESTS

The precision with which absorption of toxic compounds in industry can be assessed by means of an exposure test is debatable. Some light on the subject has been shed by the data from inhalation experiments on human volunteers.

The precision of a test may be assessed from the results of an inhalatory experiment only if criteria of the magnitude of exposure have been set adequately. Dispersion of results for various individuals may be more apparent than real if they are related directly to mean concentrations in the chamber, disregarding individual variations in retention and ventilation rate. Thus, an earlier opinion of Teisinger and his group (Teisinger et al., 1956), based upon consideration of extreme variations, appears now too pessimistic. From the present author's own experience, if the absorbed doses are taken as the independent variable, a well-controlled test can, as a rule, be

* This may become difficult in presumably rare situations, when autoinduction or "autoinhibition" could distort the picture; for fuller discussion see the chapter "Biotransformations".

characterized by a precision of ± 20 per cent. Exceptional cases were found in the exposure test for phenol which, due to the simple metabolism and high excretion rate, displayed a precision of below ±10 per cent. On the other hand, the precision of the test for nitrobenzene, due to complex biotransformation and to cumulation of the substance in the adipose tissue, could not be obtained below ± 30 per cent. A usually attainable precision of ± 20 per cent seems satisfactory as no other method for the evaluation of exposure yields better or even equivalent results. Nevertheless, assuming that 20 individuals will have absorbed 100 mg of a substance each, the statistics lead to the conclusion that by means of an exposure test, 12 out of 20 will be estimated to lie in the range from 80 to 120 mg, and most of the rest between 60 and 140 mg; 1 individual will fall outside this range. To summarize, in a group of twenty people differences in metabolite levels that vary by a factor of two does not form evidence for a real difference in exposure. To reduce the dispersion a so-called "collective" test was proposed (Teisinger). This concept limits discussion to the estimation of the average exposure of a group of individuals engaged in the same type of work. Too rigorous adherence to this concept, however, would preclude identification of individual cases of excessive exposure. Another variant of the same concept was suggested by the present author, who proposed to assess the mean daily dose of nitrobenzene absorbed by a given worker on the basis of several measurements performed on separate days. The concept underlying this proposal accounted for both the unsatisfactory precision of the test (± 30 per cent) and the systemic cumulation of the substance. Due to the latter factor the results of the test provide insight into the absorption over the last few days, rather than to that on any one day.

Despite the above reasoning, the question of the precision of exposure tests in industrial situations remains open. In chamber type experiments the rhythm of exposure is constant, and this factor certainly reduces the dispersion of the results. Introduction into the body of the same dose, but at various time intervals (for instance mainly in the first or in the last hour of exposure), may enhance considerably the variation of results, and make the precision much worse than that seen in controlled experiments. However, quantitative data with regard to this issue do not seem to be available.

SAFETY PROBLEMS

The exposure tests for toxic substances are developed for low doses predetermined by the accepted TLV's. Assuming that the TLV's are intended to be safe for persons exposed daily for the working lifetime, and that they incorporate significant safety factors, the chance of endangering the health of volunteers by a single exposure seems remote. In spite of this, taking into account the possibilities of hypersensitive volunteers, unidentified diseases, and finally possible, even if highly unlikely, experimental errors, the experiments on volunteers must observe all possible principles of prudence. In the author's laboratory the following principles are routinely observed:

1) All individuals who may participate in the experiments are medically examined in a clinic specializing in occupational diseases and intoxications. The examining physician is informed about the nature and magnitude of exposure. Selection of the volunteers is based on the same principles that are applied for admission to work with exposures to the substance to be studied.

2) In the course of the experiments there is ready access to a physician, who is notified in advance about the nature of the hazard.

3) In a preparatory period of the investigation, concentrations of the substance to be studied in the chamber are determined by two independent methods in parallel. When the experiment is in progress, the concentrations are determined at least once every hour.

4) All preparatory experiments, as well as changes in exposure parameters (concentration, duration of exposure, temperature, humidity, absorption route), are preceded by auto-experiments. The investigators who have not been given medical consent for auto-experiments for health reasons are not permitted to participate in experiments on volunteers.

5) The volunteers are thoroughly informed about the nature and magnitude of exposure, and about symptoms of poisoning with the substance in question.

6) In the course of the experiments the chief investigator or his assistant are always present, and continuously observe the behaviour and reactions of the volunteers.

7) The experiment must be discontinued at

once when a volunteer so wishes, regardless of the reason.

The strict application of the above principles, over twenty years of research on exposure tests, has resulted in not having a single case of suspicion of poisoning in a volunteering individual.

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6. BENZENE

ABSORPTION

Under industrial conditions, absorption of benzene occurs mainly via the respiratory tract. Penetration through the skin may also be of some importance in cases of direct contamination with the liquid.

Retention of benzene vapours in the respiratory tract decreases with the duration of the exposure. At the onset and toward end of an exposure of several hours' duration, the values found amounted to 70-80 and 50 per cent (Srbova et al., 1949), or 75 and 62 per cent (Dutkiewicz, 1971), respectively. Absorption of the vapours through the skin is negligible. Contact of the liquid with the skin leads to significant absorption; the maximal rate of absorption was estimated at about 0.4 mg $cm^{-2}hour^{-1}$ (Hanke et al., 1961).

METABOLISM AND ELIMINATION

The metabolism of benzene has been studied in experimental animals and in man. In rabbits some 43 per cent of the administered dose of benzene was eliminated unaltered in the expired air. Sulphates and glucuronides of phenol (24%), hydroquinone (5%), pyrocatechol (2%), and hydroxyhydroquinone (0.3%) were found in urine. A small percentage is excreted after cleavage of the aromatic ring (muconic acid, about 3%). Less than 1 per cent is excreted in the urine in the form of phenylmercapturic acid. Carbon dioxide from degradation of benzene (experiments with ^{14}C-labelled substance) appears in small amounts (1.5%) in the expired air (Porteous and Williams, 1949; Parke and Williams, 1953).

The metabolism of benzene in animals is rather fast, and almost all of the phenol produced is excreted within the first 24 hours after administration. Excretion of hydroquinone and pyrocatechol continues somewhat longer; hydroxyhydroquinone appears even longer in the urine (Porteous and Williams, 1949). Hydroxylation, the basic process in the metabolic transformation of benzene, is an enzymatic process; and arylhydroxylase, localized in the microsomes of the hepatocytes, is the responsible enzyme. The metabolism of benzene, however, is not limited to the liver (Parke, 1968). The rate of the process may be modified both by inhibitors and by inductors of microsomal transformations (Gut, 1971; Lustinec et al., 1969). However, chronic exposure of rats to benzene does not seriously alter the original rate of benzene hydroxylation, as measured in rat liver homogenates *in vitro* (Wisniewska-Knypl et al., 1975).

Humans, exposed experimentally to benzene by inhalation, eliminate part of it in an unaltered form in the expired air (12-16 per cent) and traces of it in the urine (0.1-0.2 per cent) during the desaturation period. The metabolites found in the urine were: phenol, 30 per cent; pyrocatechol, 2.9 per cent; and hydroquinone, 1.1 per cent of the absorbed amount (Teisinger et al., 1952; Teisinger et al., 0956). In other laboratories a somewhat higher fraction of the chief metabolite (phenol) was found; about 40 per cent (Dutkiewicz, 1971) or 50-87 per cent (Hunter and Blair, 1972). From experiments on volunteers about 50-90 per cent of the retained amount may be accounted for; the fate of the rest remains obscure.

The rate of the metabolic transformation of benzene in man has been estimated on the basis of the kinetics of its disappearance from the blood and expired air, and from a similar disappearance rate of phenol in the urine. Srbova et al. (1949) found that the disappearance of benzene from the blood and exhaled air occurred with the decay coefficient of 0.23 $hour^{-1}$. Sherwood and Carter (1970), on basis of preliminary observations, favour the opinion that in the exhalation of benzene two phases may be distinguished, with half-times of 1 hour and 1 day. Hanke et al. (1961) and Sherwood (1971) established the rate of disappearance of urinary phenol following exposure to benzene at approximately 0.1 hr^{-1}. More complete equations are at least biphasic, as given by Dutkiewicz (1971) where the respective coefficients of disappearance were 0.35 and 0.077 hr^{-1}. From these data, a two compartment open model could be postulated (Piotrowski, 1971) in which the rate of the irreversible loss (expiration and metabolic transformation) is represented by a coefficient of 0.18

hr^{-1}. The transfer of unaltered benzene from the rapid exchange compartment into the adipose tissue, and its return, would occur with respective rate coefficients of 0.08 and 0.19 hr^{-1}. A more sophisticated, tricompartment model was proposed recently and solved using an analogue computer (Fiserova-Bergerova and Cettl, 1972).

From the existing data it follows that, under conditions of industrial exposure to benzene, one should not expect a significant rise of phenol in the urine in consecutive days of the work week. However, there is very little systematically collected data to support this contention. Thus, Sherwood (1972) has found somewhat elevated phenol levels in the morning urine, even after the weekend break, in one worker exposed to high concentrations of benzene vapours. On the other hand, the possibility of some benzene cumulation in adipose tissue and in the bone marrow may not be excluded. Elimination from these tissues might cause a rising trend of benzene concentrations in the expired air which is collected before the onset of work on consecutive days (Sherwood and Carter, 1970).

DETERMINATION OF BENZENE AND ITS METABOLITES

At present, from the point of view of exposure tests, only the determinations of benzene in expired air and of total urinary phenol appear of importance.

DETERMINATION OF BENZENE IN EXPIRED AIR

In earlier studies, the determinations of benzene in the air in experimental chambers, in the work environment, and in the exhaled air were usually made by absorbing the compound in a nitrating mixture. Subsequently, the resulting m-dinitrobenzene could be determined by means of polarography (Teisinger et al., 1956) or colorimetrically with butanone, or acetone, and alkali (Schrenk, 1935; Piotrowski, 1954).

In the more recent reports, gas chromatography is recommended for benzene determination in the expired air; and two different techniques of sampling are used:

(a) A sample is collected into a small glass vessel, coated with a layer of synthetic polymer that reduces the adhesion of the benzene to the walls. The expired air is passed through the vessel during an entire expiration phase and the final fraction, corresponding to the alveolar air, is retained in the vessel for analysis.

(b) The expired air is collected using a respirator in which an absorbent (silica gel) is mounted beyond the outlet valve. In this case the benzene eliminated in a given time interval (e.g. 10 min.) is collected. By means of this technique, the elimination rate of benzene may be determined. For concurrent evaluation of the concentrations, measurements of the volume of air would appear necessary; however, an indirect estimate is possible due to the fact that the humidity of the exhaled air is constant. The weight increment of the absorber (25 mg per liter of air) may substitute for a gasometric measurement. The concentration obtained using this technique is lower by a factor of 1.5 when compared with the concentration in the alveolar air (Sherwood and Carter, 1970).

The final gas chromatographic determination is made by introducing directly the gas sample or alcohol eluate of the silica-gel absorbent on the column. The convenience of gas-chromatograph determinations of benzene is supported by several reports (e.g., Graziani et al., 1970; Angerer et al., 1973).

DETERMINATION OF PHENOL IN URINE

See the chapter "Phenol".

OTHER METHODS

In the search for an appropriate exposure test, determinations of benzene have been performed in blood (Fabre and Fabre, 1948; Angerer et al., 1973) and in urine. There have also been attempts to utilize the so called "sulphate ratio" in urine (Yant et. al., 1936). This test, however, due to its low sensitivity and poor precision, is only of historic interest. The methods used in the urine test can be found in the older literature (Teisinger et al., 1956), Gadaskina and Filov, (1971). Determinations of pyrocatechol and hydroquinone in urine (Bergerova and Skramovsky, 1952; Teisinger et al., 1956) have not been utilized as exposure tests.

EXPOSURE TESTS

The test for phenol in urine in its original version, as developed by Teisinger and Fiserova-Bergerova (1955), was based on experiments made on volunteers in a toxicological chamber. The determinations of phenol (after distillation from an acid medium that enabled hydrolysis of conjugated phenol) were made according to Gibbs, as described in the chapter "Phenol". Interpretation of the test data presented in graphical form made it possible to assess, from urinary excretion of phenol in the daily urine correspond to an effecin the air to which a subject had been exposed. However, due to the low precision of the evaluation (-25 to +35%), only a crude evaluation of exposure could be attained. At a urinary phenol level below 40 mg/day, average concentrations of benzene in the air were assumed to be below 100 μg/l, even though on the average 100 mg of phenol in the daily urine corresponded to effective concentration of 100 μg/l in the air. As a 24 hour collection of urine was necessary for the test, it has not been used in industrial practice.

Dutkiewicz (1963, 1965) introduced two substantial modifications: (a) basing the test on collection of "spot" urine samples toward the end of the exposure period, and (b) acceptance of the dose of benzene absorbed by the inhalatory route, and not the air concentration, as the measure of effective exposure. The values given in Table 6-1 refer to the increment in the phenol level in urine above the physiological values (see "Phenol"), and may be used in relation to any particular MPC value. For instance, at the MPC of 20 mg/m^3 that has been accepted recently in the USSR, assuming pulmonary ventilation of 7 m^3 per 8 hours' shift, and retention of benzene vapours at 70 per cent, the amount of the substance absorbed into the system would be about 100 mg, and the increment in phenol concentration at the end of 8 hours' exposure would equal about 45 mg/l. The precision of the test, estimated for the range of doses applied, is of the order ± 20-25 per cent.

Further studies have not introduced essential changes in this interpretation: benzene concentrations have been accepted again as the independent variable, and most of the data have been derived from field investigations in which precise assessment of exposure seems particularly difficult; some data have been based on single human subjects. The most common methods of interpretation are assembled in Table 6-2, where the data have been normalized in such a way as to reflect exposure to a constant concentration of benzene in air at 88 μg/l or 25 ppm. The proposal of Sherwood (1971, 1972), not based directly on experimental data, differs from field studies of Bardodej (1960), Walkley et al. (1961), Docter and Zielhuis (1967), as well as from the well-controlled experiments of Dutkiewicz (1963). Rainsford and Davies (1965) proposed a simplified method for phenol determination in urine as a screening test; however, their accuracy was far below that of the other tests discussed above.

The present author recommends:

(a) Application of the Fiserova-Bergerova method of phenol determination in urine as modified by Hanke et al. (1961) based on the Gibbs reaction, or a gas-chromatographic method in a version yielding still lower physiological phenol levels and equally high efficiency of hydrolysis of the conjugated phenol;

(b) Assessment of the exposure from the excretion rate of phenol in urine, collected at the end of exposure, as related to the absorbed dose of benzene (Tab. 1). This circumvents the difficulties resulting from the variation of phenol concentration depending on the intensity of diuresis, as the excretion rate is independent of the latter (Bardodej et al., 1962). Using the absorbed dose as the yardstick of exposure renders assessment of the latter independent of pulmonary ventilation, which is connected in turn with intensity of the physical effort. Variation of physical activity may cause considerable differences in phenol levels at the same air concentration (Benes et al., 1962).

(c) Relating the results to exposure on the day of examination, regardless of which day of the working week it is made.

In spite of the above recommendations the present author is of the opinion that the phenol test should be reinvestigated on volunteers under strictly controlled conditions, in a manner similar to that reported by Dutkiewicz (1963), but for much lower concentrations of benzene in the air. The existing experimental data were obtained at relatively high concentrations above 50 mg/m^3. The proposed range of concentrations should reach down at least to the MPC, accepted currently in the USSR, i.e. 5 mg/m^3. This should permit an assessment of the limits of applicability of the test.

It has to be mentioned that, in contrast to higher exposure levels, at these extremely low air concentrations the increment of urinary phenol

Variants of the test based on:	Formula of benzene is estimated	Precision with which the absorbed amount
Phenol concentration in urine collected between 6th and 8th hour from onset of absorption (mg per liter)	x 1.96y	24%
Urinary excretion rate of phenol between 6th and 8th hour from onset of absorption (mg per hour)	x 27.4y	23%
Amount of phenol in daily urine 24 hours collection; mg)	x 2.27y	16%

x - doze of benzene absorbed into the system mg
y - excretion of phenol in dimensions given

Table 6-1. Variants of the benzene exposure test.

Author(s)	Method of determination; the physiological level	Type of studies	Concentration of phenol in urine mg/l
Dutkiewicz, 1963	Gibbs, version of Fiserova-Bergerova (1955) modified by Hanke et al. (1961); physiol. conc. 9 mg/l	Experiment on human volunteers, assumed Ve 0.85 m^3/hr	200
Bardodej, 1960, 1962	With 4 amino-antipyrine results correspond to those by Gibbs' method	field studies	180
Walkley, et al., 1961	Theis and Benedict (p-nitroaniline; p-cresol interfers) physiol. conc. 30 mg/l	field studies	210

Table 6-2. Assembled data on interpretation of urinary phenol determinations, normalized to air concentrations of benzene of 88 μg/l (25 ppm). The data refer to samples of urine collected toward the end of exposure lasting 8 hours.

above the physiological level will be relatively low. Thus, individual variations of urinary phenol (normal background) will be the factor which determines the lower limit of the test.

BENZENE IN EXPIRED AIR

Based on earlier experiments, Teisinger et al. (1956) and Gadaskina and Filov (1971) did not attribute too much significance to quantitative interpretation of free benzene in blood, urine, and exhaled air. The idea prevailed that these determinations are of merely qualitative value, supplementing the less specific phenol test.

A renewed interest in the analysis of expired air is part of a more general phenomenon related to the development of gas chromatography. In the case of benzene, there is more data on experimental techniques than on interpretation of the results. Sherwood and Carter (1970) and Sherwood (1972) believe, on a basis of limited data, that directly after exposure (in a sitting position) to benzene vapours at a concentration of 25 ppm (88 μg/l) for 4.5 hours (115 ppm-hours), the concentration of the substance in exhalatory air amounts to about 2 ppm (1.5 ppm when a respirator, and 2.8 ppm when an alveolar air collector, is used). Sixteen hours later (in the morning of the next day) the concentration drops to about 0.2 ppm. The data cited by Sherwood and Carter (1970) are not consistent with the above information.

Results of more systematic experimental studies were published by Dutkiewicz (1971) from his early experiments, where classical colorimetric methods were still used for the determination of exhaled benzene. Results, obtained for various time intervals following discontinuation of exposure, were expressed in terms of exhalation rate; thus comparability with the data quoted above is difficult. It is of interest that, when relating the results to the dose absorbed over a 6 hour inhalation period, the precision of the evaluation was worse as compared with urine analysis (± 32 per cent, vs ± 20 per cent, Fig. 6-1).

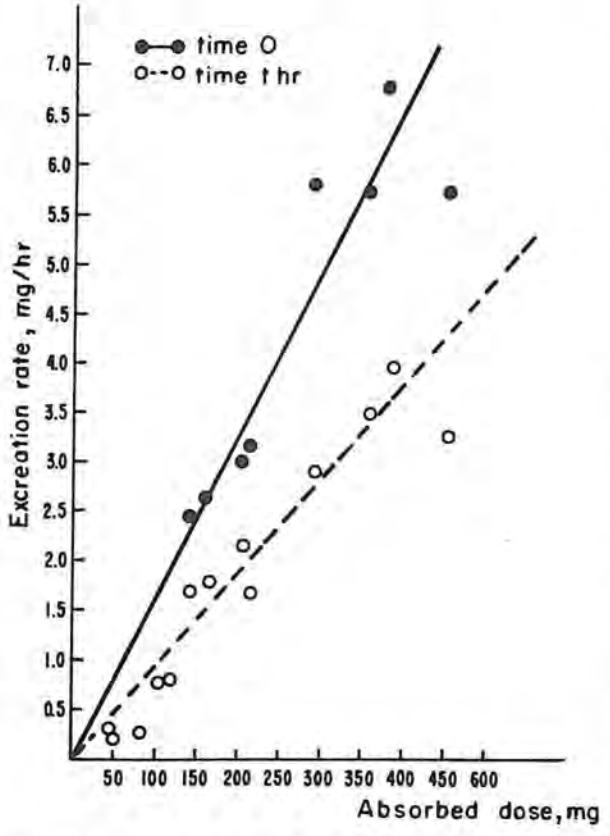

Fig. 6-1. Excretion rate of benzene with expired air following inhalation of benzene vapour, as dependent on dose retained (Dutkiewicz, 1971).

Sherwood (1972) reported that the exhalation kinetics of benzene could have been described using two exponentials with half-lives of 2.5 and 22 -32 hours. He drew the conclusion that determinations performed early after discontinuation of the exposure reflect mainly the absorption over the last 1-2 hours of inhalation, whereas the measurements made on the next day, before the onset of further exposure, provide information about the accumulated amount of benzene in the body. Thus early morning values of benzene in expired air should display a rising trend over consecutive days of an exposure week, reaching roughly a factor of 2 on Friday or Saturday above Monday. This postulate seems consistent with expectations (see "Kinetics"). However, it still awaits experimental verification.

At present, the determination of benzene in the exhaled air does not contribute significantly to the value of the evaluation of the exposure by means of the urinary phenol test. This subject, however, warrants further studies because of the existing tendency to lower the MPC for benzene. With decreasing concentrations of benzene in the air, the value of the phenol test diminishes rapidly, most likely because of the interference of the physiological urinary levels of phenol (Dutkiewicz et al., 1964).

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

Due to the serious health consequences of excessive exposure to benzene and the common existence of control measures, there must exist a great body of data on industrial exposure, the bulk of which, however, remains unpublished. Relatively abundant data have been published in Czechoslovakia (e.g. Kindrichova, 1958; Vlasak, 1959; Bardodej, 1960; Jindrichova, 1974), where interest of the authors concentrated mainly on the practical value of the phenol test. In Poland, where the phenol test is being used routinely for control of benzene in the industrial environment, the published data are scarce (Dutkiewicz, 1965; Mikulski et al., 1972). The phenol test has been used also in the USA (Walkley et al., 1961) and in the U.K. (Rainsford and Davies, 1965); in the U.K. it was combined with analysis of the expired air (Sherwood, 1971, 1972). Preliminary data were reported also from the USSR (Kanner, 1971). The reports listed above have come from various branches of industry (shoe-manufacturing, production of telecommunication equipment, production-plants of aromatic compounds, ship painting, crude oil pumping stations, etc.). The over-all correlation of urinary phenol concentrations with benzene concentrations in the air was relatively good; the magnitude of exposure varied, of course, with the technological processes and the time over which the determinations were made.

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7. TOLUENE

ABSORPTION

Under industrial conditions, toluene enters the body mainly via the respiratory tract. According to Srbova and Teisinger (1952), the retention of the vapours amounts on the average to 53 per cent (41-63.5%); Piotrowski (1967) found 72 per cent at the onset of inhalation, and 57 per cent at the steady-state (average about 60%). Vapours of toluene do not penetrate the skin (Piotrowski, 1967); however, the liquid applied directly does (Dutkiewicz and Tyras, 1968).

METABOLISM AND ELIMINATION

In man, of the amount of toluene retained in the body, about 16 per cent is exhaled unaltered and a trace (0.06%) is found in urine. The chief metabolite is benzoic acid, accounting for 72 per cent of the retained toluene (Srbova and Teisinger, 1953). Another report estimated this fraction at 62 per cent (Piotrowski, 1967). Of the urinary benzoic acid, 10-20 per cent is conjugated with glucuronic acid and the rest is eliminated in the form of hippurate (Srbova and Teisinger, 1953). In rabbits about 75 per cent of the administered toluene is excreted in the hippurate form (El Masri et al., 1956). It may be presumed that the fraction of benzoic acid conjugated with glycine should decline somewhat with an increasing dose of toluene due to the limited pool of easily accessible glycine in the body (Arnstein and Neuberger, 1951; Bray et al., 1952). Nevertheless, Ogata et al. (1970) found that hippuric acid accounted for 68 per cent (on a molar basis) of the absorbed toluene, with linear proportionality between the hippurate excreted over 24 hours and exposure up to 1500 ppm x hours (i.e. up to 200 ppm = 0.8 mg/l). From animal experiments it is known that the yield of urinary hippuric acid may be depressed by simultaneous exposure to trichloroethylene (Ikeda, 1974). No human data are available with respect to the influence of other xenobiotics on the process.

After inhalation of toluene vapours the excretion of conjugated benzoic acid is rapid; the excretion half-time is 2-3 hours (Piotrowski, 1967; Ogata et al., 1971). Therefore, 24 hours after exposure, levels of the metabolite decline to control values; and breaks in the continuous inhalation (alternating 1 hour exposure and 1 hour off) do manifest themselves in the excretion rate of hippuric acid in urine (Ogata et al., 1971). In the case of an 8 hour inhalation exposure, 55-60 per cent of benzoic (hippuric) acid is excreted within the exposure interval (Ogata et al., 1970).

DETERMINATION OF TOLUENE

Toluene has been routinely determined (after aeration) in the blood and urine, as well as in the breath. The Czech authors (Srbova, 1952) used a method based upon nitration to dinitrotoluene with subsequent polarographic measurement; Fabre et al. (1955) measured the same product colorimetrically after reaction with alkali and butanone, and Piotrowski (1967) after nitration to trinitrotoluene and reaction with alkali and alcohol.

At present, in the analysis of blood, air, and particularly of breath, gas chromatography with flame ionization detection is used (Ogata et al., 1970; Mikulski et al., 1972; Angerer et al., 1973; Astrand et al., 1972).

DETERMINATION OF BENZOIC AND HIPPURIC ACID IN URINE

In the studies of Srbova and Teisinger (1952, 1953), Teisinger and Srbova (1954) and Piotrowski (1967) a titrimetric method (Kingsbury and Swanson, 1921) was used in which urine was hydrolyzed in an alkaline medium, and the chloroform extract was titrated with sodium alcoholate. In earlier monographs this method was recommended for exposure tests (Teisinger et al., 1956; Dutkiewicz et al., 1964). Average physiological levels of benzoic acid in the urine, as determined by this method, were as follows: 750

mg/24 h (Teisinger et al., 1956), 600 (830) mg/l in winter and 750 (970) mg/l in summer (the numbers in parentheses correspond to concentrations normalized to the specific gravity of urine = 1.024). The excretion rate was about 39 mg/h independently of the season (Piotrowski, 1967). Consumption of plums increased substantially the excretion of benzoic acid.

Flek and Sedivec (1971) proposed that total benzoic acid (after alkaline hydrolysis) be determined by means of gas chromatography in the form of methyl ester, obtained from a reaction with diazomethane. Preliminary data suggest that the physiological levels, obtained in this way, may be lower (20-25 mg/h).

The original proposal was related to hippuric, and not to benzoic, acid (Elkins, 1954); the former was titrated after precipitation by the method of Quick (1931). This version had low sensitivity and was useful for analysis of urine only after heavy exposure. At present, a return to the determination of hippuric acid may be noted, and two principal groups of methods are used:

a) Colorimetric procedures by which the total hippuric acid (together with methylhippurates, if present — see "Xylene") is determined in a small sample of urine (1 ml or less). These methods stem from studies of El Masri et al. (1956) and Umberger and Fiorese (1963), and are applied in several versons. Ogata et al. (1969) used, with some modifications, a method based on the extraction of the metabolite (ether-ethanol or ethyl acetate), and subsequent evaporation and colour reaction with p-dimethylaminobenzaldehyde in the presence of acetic anhydride (plus perhaps $FeCl_3$). The product, azlactone, shows maximal absorbance at 460-470 nm. In addition, Ogata et al. (1969) described an alternative method with benzenosulphonyl chloride (absorption maximum - 380 nm). Both methods are characterized by good recovery (94-100 per cent) and similar sensitivity. Burkiewicz and Zielinska (1972) reported a procedure in which hippuric acid is extracted with chloroform, and color is developed in the presence of acetic anhydride and pyridine. The obtained product, oxazolone, has an absorption maximum at 460 nm. All these methods are sensitive with good precision, and those of Mikulski et al., and of Burkiewicz and Zielinska are, also, very simple. The physiological level of hippuric acid varies from report to report: 790 ± 430 mg/l or 554 mg/l (Mikulski et al., 1972) when corrected for specific gravity of urine and noncorrected, respectively. Burkiewicz and Zielinska (1972) reported an excretion rate of 23 ± 6.2 mg/h. These values are similar to the existing data for benzoic acid. Sensitivity of the discussed methods is similar for hippuric and methylhippuric acid, and thus they may be used for approximate evaluation of simultaneous exposure to toluene and xylene (Mikulski et al., 1972).

b) Chromatographic methods (paper and thin-layer chromatography) permit selective determination of hippuric and methyl-hippuric acid when both are present in the urine. Application of these methods is particularly indicated in case of exposure to xylene; accordingly, they are discussed in the chapter "Xylenes". Ogata et al. (1969, 1970), using these methods, found physiological levels to be reasonably low about 300 ± 100 mg/l. The reasons for this discrepancy with the data discussed above is not clear. Apart from the better specifity of the method, differences in nutritional habits of the subjects studied may have contributed to the low levels.

Other methods for the determination of benzoic and hippuric acids have also found application in the evaluation of toluene exposure. Pagnotto and Lieberman (1967) described a method for hippuric acid measurement based on ultraviolet spectrophotometry (230 nm) of an urine extract in an i-propanol-ether mixture; the method has been criticised for lack of specificity in the presence of uric acid and other substances (Ogata et al., 1969). Nevertheless, the physiological level, as determined by this method, seems consistent with other data, and amounts, on the average, to about 800 mg/l (Pagnotto and Lieberman, 1967; Kaucka et al., 1973).

Recently, Buchet and Lauwerys (1973) proposed the determination of both the hippuric and m-methylhippuric acids using gas chromatography after transformation to methyl esters with diazomethane.

EXPOSURE TESTS

Relatively exhaustive information is available regarding determination of total benzoic acid for the purpose in question. Teisinger and Srbova (1954) have investigated, in experimental conditions on volunteers, the relationship between toluene concentration in the air, in a range from 100 to 1000 mg/m³, and daily excretion of ben-

zoic acid. Due to the high level and great scatter of the physiological excretion rate (0.5-1.5 g/day), confidence limits of the correlation were very wide. The authors concluded that the test may be used only at relatively high toluene concentrations in the air (of the order of 200 ppm = 800 mg/m³), and then only as a "collective test" (see chapter 5, "Working out exposure tests"). In this version the test has not found wide practical application. Piotrowski (1967), also in a human experiment (chamber exposure), has based the test on the analysis of a urine fraction, collected toward the end of 8 hours' inhalation exposure, and recommended the evaluation of the absorbed dose of toluene (D, mg) from the excretion rate according to the formula

$$V = 39 + 0.09D \qquad (1)$$

where V = excretion rate of benzoic acid over the last 2-4 hours of the exposure (mg/hour), 39 = mean physiological level of benzoic acid (mg

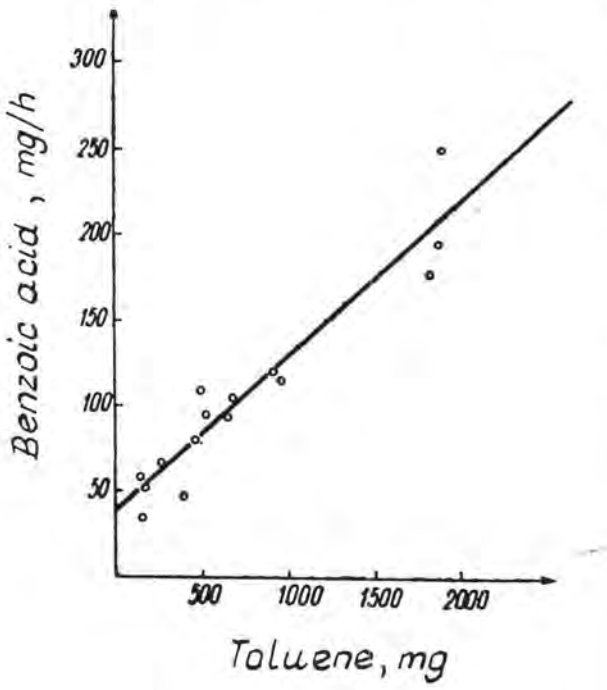

Fig. 7-1. Excretion rate of benzoic acid (mg/h) in urine collected toward the end of 8-hrs exposure, as dependent on the absorbed dose of toluene (mg/h) (Piotrowski, 1967).

Taken from: Piotrowski J.: *Ilosciowa ocena wchlaniania toluenu u ludzi,* **Medycyna Pracy 18,** 213-223, 1967. Page 221, Fig. 8.

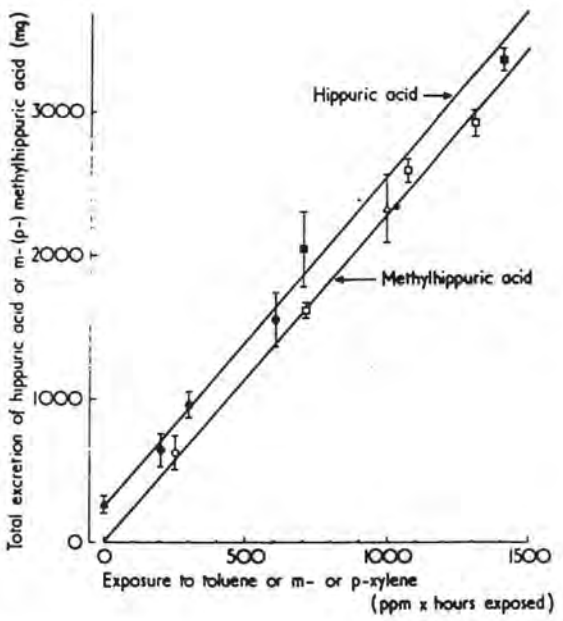

Fig. 7-2. Relationship between total exposure (ppm × hr) and total excretion of urinary hippuric acid in volunteers (Ogata et al., 1970).

hour), 0.09 = coefficient of regression (hour^{-1}); (see Fig. 7-1). In 18 separate experiments on 6 individuals each, at air concentrations of 100-800 mg/m³, a precision of evaluated absorbed dose of ± 180 mg was attained. This means that the test is burdened with an admissible error of ± 20 per cent, at doses of 900 mg toluene and more; this corresponds to air concentrations above 200 mg/m³ (40 ppm).

With regard to determinations of urinary hippuric acid, the first data provided an example of the amounts excreted in urine, collected over the total interval of exposure (9 hours) to various concentrations, from about 0.4 to 3 mg/l. The excreted amounts of hippuric acid were in the range from 700 to 5000 mg.

Recently, Ogata et al. (1970) reported data from experiments on volunteers exposed to concentrations of 100 and 200 ppm (400 and 800 mg/m³). The data enable a relatively precise correlation to be made (Fig. 7-2) of the exposure with 24 hours' excretion of hippuric acid. For practical purposes, however, of most significance is the relationship obtained by these authors between air concentrations and the excretion in urine collected over the last 4 hours of a days' exposure, with a one hour break in the middle of the interval (Fig. 7-3). These data may be com-

pared with those reported by Piotrowski (1967) on the basis of equation (1). For this purpose a pulmonary retention of 0.6 and lung ventilation rate of 0.8 m³/hour may be assumed, and a correction coefficient of 0.7 introduced to account for differences in the molecular weights of benzoic and hippuric acids*. The data of Ogata et al. when recomputed yielded a formula:

$$V = 12 + 0.08D \qquad (2)$$

This equation differs from equation (1) by: a) the lower physiological excretion rate (12 mg/hour) and b) the regression coefficient of 0.08 instead of 0.09. The ratio of the latter two values may reflect the efficiency of conjugation of benzoic acid with glycine (90 per cent). From the above discussion it follows that the test of Piotrowski (1967), based on titrimetric determination of benzoic acid, and that of Ogata et al. (1970), based on colorimetric determination of hippuric acid, yield similiar results.

* Excretion rate was recalculated from mg per min. to mg per hour.

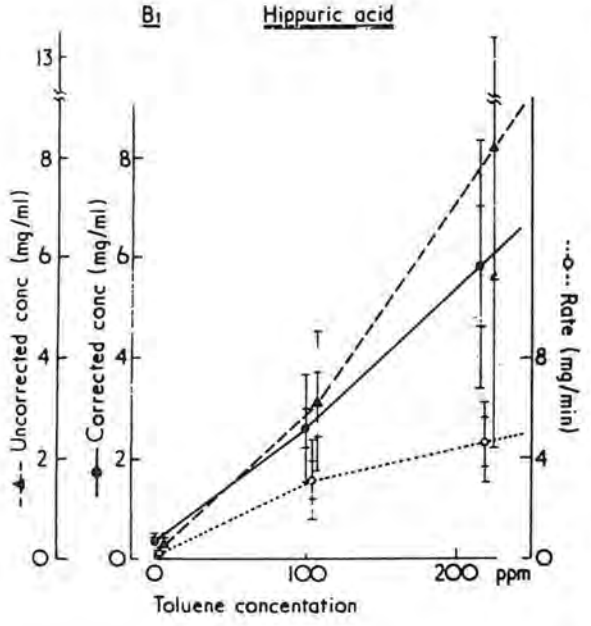

Fig. 7-3. The level of hippuric acid in urine collected between 4-8 hrs of exposure, as dependent on the concentration of toluene in air (Ogata et al., 1970).

Figures 2 and 3 taken from: Ogata M., Tomokuni K., Takatsuka Y.: *Urinary excretion of hippuric acid and m- or p-methylhippuric acid in the urine of persons exposed to vapours of toluene and m- or p-xylene as a test of exposure.* **Brit. J. Industr. Med. 27,** 43-50, 1970. Page 46, Fig. 4 and Page 47, Fig. 5-B¹.

For evaluation of exposure to a lesser degree, the determination of unaltered toluene in blood and expired air has been used. Gerarde (1960) presented a graph relating the blood concentration of toluene following 8 hours exposure vs. the concentration in the air at the working place. The graph embraces high levels, from 750 to 3000 mg/m³ of air; the corresponding blood concentrations vary from 5 to 20 μg/ml. Teisinger et al. (1956) presented graphs depicting the kinetics of the disappearance of toluene from the blood and expired air after cessation of the exposure. When the fastest elimination in the breath was completed, the decay of concentrations both in the breath and in the blood was characterized by a half-time in the order of 2 hours.

Recently, the use of toluene determinations in the expired air and blood has gained new adherents (Angerer et al., 1973; Szadkowski et al., 1973; Astrand et al., 1972). The background level of toluene in blood, when determined by gas chromatography, in nonexposed people, has not exceeded 0.15 ppm (Szadkowski et al., 1972). In short-time exposure it has been shown that, both in alveolar air and in arterial blood, a steady-state is rapidly reached, usually within 30 minutes. At the air concentration of 100 ppm, the concentration of toluene in alveolar air and in arterial blood was about 18 ppm and 1 ppm, respectively. At 200 ppm in air, values twice as high were obtained. These values, characteristic of the end of a thirty minute exposure in subjects at rest, showed further rise if, in the following period, exposed volunteers were also subjected to exercise (300-900 kpm/min). There was a close linear correlation between the toluene concentration in alveolar air and in arterial blood, irrespective of the type and magnitude of exposure (Astrand et al., 1972). However, the above data seem still inadequate for routine toluene determination in expired air or blood, when evaluation of industrial exposure is the objective.

The present author proposes to use, for purposes of current control of the exposure, either the test of Piotrowski (1967) or that of Ogata et al. (1970). On the other hand a low physiological

level of hippuric acid, as seen by Ogata et al., would call for clarification as to whether it was due to the particular specificity of the method, or to the dietary habits of the studied individuals. If the former is true, the method of Ogata et al. (1969) should have priority over all other methods used so far for determination of benzoic and hippuric acid in urine.

Even at the low physiological levels of hippuric acid, reported by Ogata et al., the applicability of the test is limited to moderate and high toluene concentrations in the air. At concentrations equal to or lower than 50 mg/m³ (12 ppm), none of the published tests will provide acceptable quantitative data for individual measurements. Studies seem warranted to investigate further the usefulness of blood and breath analysis for toluene by means of gas chromatography.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

Although exposure tests based on hippuric and benzoic acid assays are routinely applied in some countries for exposure control, published data on this topic are scarce. Recently published data on occupational exposure may be found in the following reports. Pagnotto and Lieberman (1967) studied the exposure of workers employed in leather finishing and rubber coating plants, where average toluene concentrations in the air ranged from 53 to 112 ppm. Using spot samples of urine collected at the end of working shifts, these authors found a high correlation between the air concentration of toluene and urinary level of hippuric acid. Exposure to 100 ppm was reflected by high hippuric acid concentration of about 4 mg/ml. Cappellini and Alessio (1971) found in industrial conditions somewhat lower values at comparable exposure levels. Ogata et al. (1971) studied the correlation between urinary concentrations of hippuric acid and air concentrations of toluene in industrial conditions with rather poor results (r = 0.67). Mikulski et al. (1972) assessed the exposure of workers engaged in ship painting (benzene, toluene, xylene) and found high levels of hippuric acids (methylhippuric inclusive), on the average from 1800 to 5500 mg/l depending on working conditions. The correlation between the sum of toluene and xylene air concentrations in ppm and concentrations of hippuric acids was high (r = 0.81).

Kaucka et al. (1973) used the test (hippuric acid, method of Pagnotto and Lieberman) for evaluation of worker exposures at concentrations of toluene of the order of 100-500 mg/m³; and found high urinary concentrations, up to 5000 mg/l.

Szadkowski et al. (1973), studying the exposure of printing workers, found no correlation between the concentrations of toluene in the air and of hippuric acid in the urine; however, a correlation between the former and the toluene concentration in blood did exist.

These observations are, however, of limited value regarding the evaluation of exposure tests, since the basis for comparisons (effective exposure) cannot be properly estimated in industrial conditions, especially if skin absorption cannot be excluded. Moreover, poor correlations are to be expected because of the differences in lung ventilation in various individuals. Additionally, taking the urinary concentration (instead of the excretion rate) as the measure of the metabolite level reduces significantly the accuracy of the evaluation (see "Working out of exposure tests").

From personal information collected by the present author it follows that, in Poland, practical application of the test meets difficulties resulting from the low level of exposure found in industry.

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8. XYLENES

Xylene, when used as an industrial solvent, is usually composed of three isomers with m-xylene accounting for 75-85 per cent of the total.

ABSORPTION

It appears that absorption of xylenes in humans has not yet been subjected to systematic studies. By analogy with toluene, it is believed that under conditions of industrial exposure the respiratory tract forms the main route of entry (Gadaskina and Filov, 1971). Experimental data of Ogata et al., (1970) indicate that retention of m-xylene vapours in the respiratory system reaches about 87 percent and is greater than that of toluene. The investigation of Orlowski (1972) showed a retention of 70-87 per cent at the beginning of exposure, with a slight downward trend after several hours of inhalation (65 to 85 per cent). With direct application to the skin, xylene is absorbed through this route also (Dutkiewicz and Tyras, 1968).

METABOLISM AND ELIMINATION

In the body, xylene undergoes oxidation leading to the formation of toluic acids, and, to a smaller degree, of xylenols. The m- and p-toluic acids are coupled to glycine, forming m- and p-methylhippuric acids, whereas o-toluic acid undergoes conjugation with glucuronic acid. The oxidation of m- and p-xylenes to toluic acids is highly efficient, in the order of 90 per cent with all the toluic acid bound to glycine (Bray et al., 1949, 1950; Fabre et al., 1960). The excreted m-methylhippuric acid has been found to account for 72 per cent of the absorbed m-xylene when studied on volunteers; and the metabolic efficiency for m- and p-xylene remained unchanged, up to the exposure product of 1500 ppm x hours. Data reported by Orlowski (1972), based also on the observation of human beings, point to an efficiency of m-xylene transformation into m-methylhippuric acid of about 90 per cent. The excretion rate was similar to that of toluene, followed concurrently, indicating a half-life in the range of 2-3 hours (Ogata et al., 1970). Senczuk and Orlowski (in press) showed that the dynamics of m-methylhippuric acid excretion in humans, during exposure to m-xylene and after its discontinuation, may be satisfactorily described using a single rate coefficient of $k = 0.5\ hour^{-1}$; this corresponds to a half-life of about 1.5 hours.

METHODS FOR DETERMINATION OF XYLENE AND ITS METABOLITES

The methods applied originally for the determination of xylene were similar to those used for toluene. Fabre et al. (1960) determined xylene in biological materials by means of nitration and color reaction with butanone and alkali. Methods used at present for analysis in expired air, both for xylene and toluene, are based on gas chromatography with flame ionization detection (Ogata et al., 1970; Senczuk and Orlowski, 1974). These determination methods have not been used so far in exposure tests.

Of the metabolites of m- and p-xylenes, the respective methylhippuric acids are determined in urine. They may be determined directly in urine extracts, similarly to hippuric acid; however, this procedure does not seem satisfactory due to the interference of high and variable levels of hippuric acid in the urine of non-exposed people (see Toluene). Therefore, the exposure tests for m- and p-xylenes have become more attractive since the chromatographic separation of their metabolites have been worked out.

In an original method of Gaffney et al. (1954), paper chromatography is applied with the colour product of hippuric acid being obtained directly on the paper. Ogata et al. (1969) found this procedure poorly reproducible, and proposed a modification of the thin layer chromatography technique using Silica Gel G: the ethylacetate extract of urine applied to the slab as the solvent is developed using toluene. acetic acid, and water in the proportion of 100, 50, 2.3. The colour reaction is produced by spraying the chromatogram with p-dimethylaminobenzaldehyde; the spots are eluted with ethanol; and absorption is measured

at 460-470 nm. The molar extinctions of azolactones, obtained from the hippuric, as well as m- and p-methylhippuric acids, are similar and amount to 1.5×10^4 and 1.4×10^4, respectively.

The paper chromatography technique has been refined, and after selection of optimal parameters related to drying and colour development gives reproducible, quantitative results (Orlowski, 1974).

EXPOSURE TESTS

Ogata et al. (1970) performed chamber experiments on volunteers exposed to vapours of m-xylene (100 and 200 ppm) and p-xylene (100 ppm) for 3 and 8 hours. Air determinations were made using gas chromatography; the methylhippuric acids in the urine were determined by means of paper chromatography. The total excretion of methylhippuric acids (in 24 hrs. urine) was found to be directly proportional to the product of air concentration and duration of exposure (see also "Toluene"). For practical purposes, relevant results can be obtained from exposures of 8 hours' duration and from fractions of the urine collected over the last 4 hours of the exposure. Figure 8-1 shows that for both isomeres the results were relatively well comparable for concentrations normalized to a urine specific gravity of 1.024, and for the excretion rates. For m-xylene the proportionality between these values and the air concentration is clearly apparent.

Senczuk and Orlowski (in press), on the basis of human experiments, proposed to assess the absorbed dose from urinary excretion of the metabolites over the last 2 hours of exposure. The excretion rate (u, mg/hour) may be related to the absorbed amount of m-xylene (D; mg) by a formula

$$D = 4.9u$$

INTERPRETATION OF THE TEST FOR SIMULTANEOUS EXPOSURE TO XYLENES AND TOLUENE

The chromatographic method, as proposed by Ogata et al. (1969) renders separate determination possible for hippuric and methylhippuric acids. The efficiency with which these metabolites are formed from toluene and xylenes is similar (see "Toluene"), as are the values obtained from analysis of urine collected toward the end of daily exposure to both compounds at equal concentrations. If the maximum allowable concentrations of toluene and xylenes are equal (e.g. in USSR, see "Sanitary Standards"), the measured total concentration of a mixture in the air may be compared with that calculated from the urinary excretion of the metabolites. In such a case, a common method for determination of total hippuric acids without separation may be applied (Mikulski et al., 1972).

On the other hand, if the TLV's for the compounds are different, the air concentrations of toluene and xylenes have to be calculated from the levels of hippuric and methylhippuric acids in the urine. For the interpretation of the results, the principle of summation should be applied as used for mixtures in the air (Ogata et al., 1970).

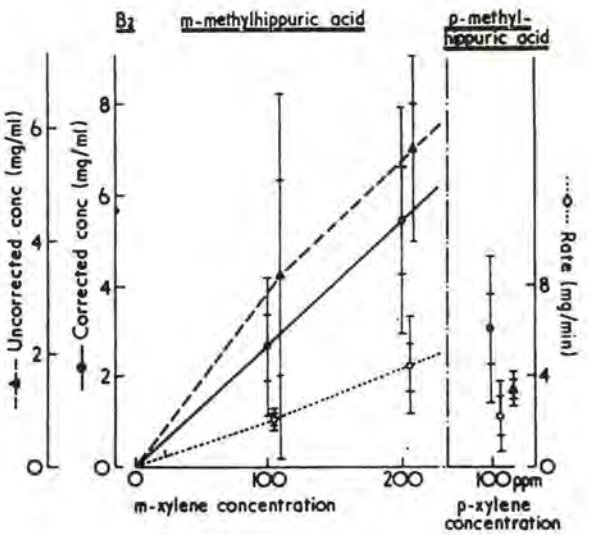

Fig. 8-1. The concentration of m-methylhippuric acid in urine as dependent on the concentration of m-xylene in air (Ogata et al., 1970).

Taken from: Ogata M., Tomoluni K. and Takatsuka Y.: *Urinary excretion of hippuric acid and m- or p-methylhippuric acid in the urine of persons exposed to vapours of toluene and m- or p-xylene as a test of exposure.* **Brit. J. Industr. Med. 27,** 43-50, 1970. Page 47, Fig. 5-B-2 (including table with numerical values).

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9. ETHYLBENZENE

ABSORPTION

Under conditions of industrial exposure, respiratory absorption forms the main route of entry for ethylbenzene. Retention of the vapours in the respiratory tract of volunteers, in controlled experiments at concentrations in the range of 100 to 810 mg/m^3, amounts on the average to 60-64 per cent (Bardodej and Bardodejova, 1966a, b). In an earlier study by the same authors (1961) the retention had been estimated at 45-50 per cent. The authors express the view that absorption of ethylbenzene vapours through the skin is not significant, and consider appreciable cutaneous absorption of the liquid substance as unlikely. However, Dutkiewicz and Tyras (1967) were able to demonstrate that liquid ethylbenzene is absorbed to a significant degree through the skin, if applied directly.

METABOLISM AND ELIMINATION

The data at hand allow the conclusion that almost all the ethylbenzene absorbed into the system is metabolised. Only a minor amount is eliminated unaltered in the urine and exhaled air (Bardodej and Bardodejoba, 1966a). Summarizing the earlier animal studies, the metabolic transformation of ethylbenzene occurs via two parallel metabolic pathways that start with oxidation of the carbon atom in position 1 or 2 of the side-chain. The metabolites of the first pathway are phenylmethylcarbinol glucuronide and hippuric acid (30-35 per cent each), with a minor amount of mandelic acid (1-2 per cent) as well. The other pathway eventually reaches phenaceturic acid (15-20 per cent) through phenyl acetate. Hydroxylation of the aromatic ring, yielding p-ethylphenol, only accounts for traces of the metabolite pool (0.3 per cent; Bakke and Scheline, 1970). The metabolism in man is somewhat different. Thus Bardodej and Bardodejova (1966a) suggest that the second pathway, which starts with oxidation of carbon atom 2, is of no significance. The first pathway, on the other hand, starts with phenylmethylcarbinol (excreted as glucuronide in small amounts of the order of 5 per cent), and ends up with phenylglyoxylic and mandelic acid, both probably in mutual redox equilibrium. The latter acids form the main metabolites of ethylbenzene in man, and account for some 64 and 25 per cent of the amount absorbed into the system, respectively. No hippuric acid (one of the main metabolites in animals) has been detected in man and the same may be said of mercapturic acid and phenylacetylglutamine. The balance, as presented by the authors, leaves no free space for appreciable amounts of other metabolites. The metabolism of ethylbenzene in man is relatively rapid, the half-time of the excretion rate of mandelic acid being close to 5 hours (Bardodej and Bardodejova, 1966a). The metabolism in man is schematically presented in Figure 9-1. The same authors discussed all the aspects summarized above in a review article in 1970 (Bardodej and Bardodejova, 1970).

DETERMINATION OF ETHYLBENZENE AND ITS METABOLITES

Determination of ethylbenzene in air and in breath has been performed directly by UV absorption, applying the "Mercury Vapour Concentration Meter" (Hendrey Relays), or spectrophotometrically in ethanol or heptan solutions at the wavelength of 262 nm (Bardodej and Bardodejoba, 1966a).

For determination of the metabolites (mandelic and phenylglyoxylic acids) see "Styrene".

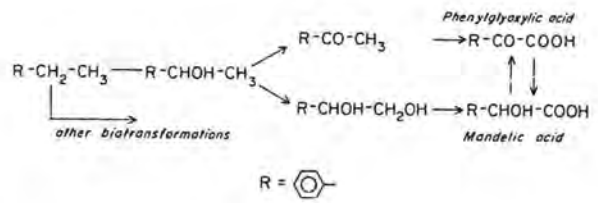

Fig. 9-1. Metabolic pathways of ethylbenzene in humans (Bardodej and Bardodejova, 1966).

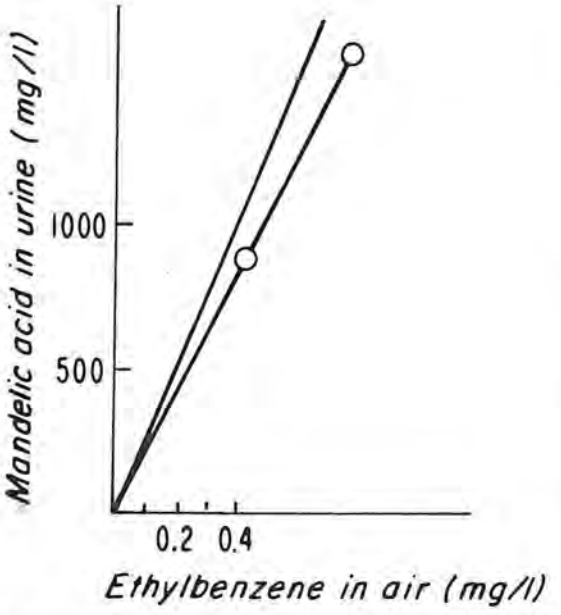

Fig. 9-2. The concentration of mandelic acid in urine as dependent on the concentration of ethylbenzene in the air (Bardodej and Bardodejova, 1961).

tilation rate of 7.6 l/min, the numerical values are presented in graphic form in Figure 9-2. The assessment of exposure is based on measurements of the concentration of mandelic acid in samples of urine collected in the two last hours of the exposure lasting for 8 hours. In the earlier paper (1961), these authors suggested a straight-line relationship in which the urinary concentration of 900 mg/l corresponded to an air concentration of 400 mg/m³. These data as shown suggest a trend to reduced efficiency of mandelic acid formation with increasing concentrations of ethylbenzene in air.

Due to higher ventilation rates in working subjects, the authors propose, for interpretation of field determinations (in industry), a relationship in which the amounts of mandelic acid corresponding to ethylbenzene air-concentrations are higher than those displayed in Figure 9-2. The 200 mg/m³ (46 ppm; ethylbenzene) should be represented by about 1000 mg of mandelic acid per liter of urine, when the urine is normalized to a specific gravity of 1.024; or, if expressed as a ratio to creatinine concentration, 0.7 mg per mg creatinine.

EXPOSURE TEST

The basis for the interpretation of mandelic acid in urine was worked out by Bardodej and Bardodejova (1961). They relied upon data from human experiments when the subjects were exposed in a toxicological chamber for 8 hours to inhalation of ethylbenzene vapours at concentrations of 100-400 mg/m³. With an average ven-

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

In studies of urinary excretion of mandelic acid in workers exposed in chemical plants, it was found that the concentrations were below 1500 mg/l, or 1.2 mg per mg creatinine.

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Dutkiewicz T. and Tyras H.: *A study of the skin absorption of ethylbenzene in man.* **Brit. J. Industr. Med. 24,** 330-332, 1967.

10. STYRENE

ABSORPTION

Respiratory absorption is probably the main route of entry for styrene into the body. Data on the retention of styrene vapours are divergent. According to Bardodej et al. (1961), the retention reaches 45-50 per cent; however, Bardodej (1964), as well as Fiserova-Bergerova and Teisinger (1965), have reported a figure of 60 per cent. Stewart et al. (1968) obtained data for retention in the range of 70-75 per cent. Fiserova-Bergerova and Teisinger observed that, in contrast with other volatile organic compounds (e.g. benzene, toluene, trichloroethylene), the retention of styrene in the respiratory tract remained constant throughout the whole exposure time. After direct contact of fluid styrene with the skin, systemic absorption was observed by Dutkiewicz and Tyras (1968). It seems that cutaneous absorption of the vapours has not been studied.

METABOLISM AND ELIMINATION

Almost all of the absorbed styrene is metabolized. In man, the proportion of unchanged substance eliminated in the expired air is very small. Fiserova-Bergerova and Teisinger (1965) were unable to find any exhaled styrene after cessation of exposure; Bardodej and Bardodejova (1966) found only traces; and Stewart et al. (1968), applying gas chromatography, estimated the share of its elimination by this route at somewhat more than 1 per cent of the retained dose. This would conform with earlier information on styrene metabolism in animals in whom elimination of the unaltered substance in exhaled air had been estimated at 2-3 per cent (Danishefsky and Willhite, 1954; El Masri et al., 1958). Practically, metabolic changes of styrene affect only the side chain: the amount of detected phenol (4–vinylphenol) accounted for only 0.1 per cent of the administered dose (Bakke and Scheline, 1970). The scheme of the metabolic pathway of styrene is still controversial. In Figure 10-1 the metabolic scheme is presented as proposed by Ohtsuji and Ikeda (1971); it should be of assistance while further discussing the subject.

In experimental animals hippuric acid is the main metabolite; this was stressed particularly in earlier reports (Spencer et al., 1942; Carpenter et al., 1944; El Masri et al., 1958). Bardodej and coworkers (1961) demonstrated that mandelic acid is the principal, and perhaps the only, metabolite of styrene in man. In later studies mandelic acid has been found in significant amounts also in experimental animals. Thus Vrba et al. (1964) found that in rats the excreted mandelic acid could account for 21-28 per cent of the styrene dose; and in the same animals Ohtsuji and Ikeda (1971) found that the amount of this metabolite, excreted over the first 20 hours of observation, corresponded to 15 per cent of the dose. In rabbits 20 to 30 per cent of the administered styrene was excreted in the form of mandelic acid (Rubinskaja, 1965). When ether extracts of rats' urine were analyzed (Vrba et al., 1967) by means of gas chromatography, apart from mandelic acid, phenylglyoxylic acid and four other metabolites were found. The latter were not identical with

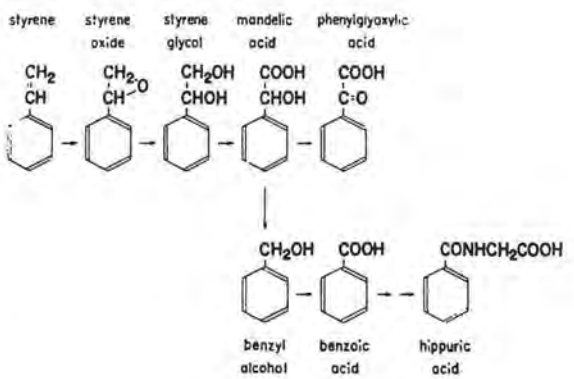

Fig. 10-1. Metabolic pathways of styrene (Ohtsuji and Ikeda, 1971).

Taken from: Ohtsuji H. and Ikeda M.: *The metabolism of styrene in the rat and the stimulatory effect of phenobarbital.* **Toxicol. Appl. Pharmacology 18,** 321-328, 1971. Page 326, Fig. 1.

those known already from the earlier studies. The results of recent investigations by Bardodej et al. (1971) demonstrated that in rats both phenylglyoxylic and mandelic acid may be taken as the principal metabolites of styrene. In addition to mandelic acid, phenylglyoxylic acid was also demonstrated in man (Bardodej, 1964; Bardodej and Bardodejova, 1966; Huzl et al., 1967). These observations were subsequently confirmed by Ohtsuji and Ikeda (1970). No appreciable elevation of benzoic or hippuric acid levels has been found in humans, either in earlier or in more recent studies (Bardodej et al., 1960; Stewart et al., 1968; Ohtsuji and Ikeda, 1970). Ohtsuji and Ikeda (1971) presume, therefore, that the metabolic pathway leading to mandelic acid is common for man and for most experimental animals. In the former, the efficiency of mandelic acid decarboxylation, responsible for further metabolization to hippuric acid (Fig. 10-1), is low. Therefore, mandelic and phenylglyoxylic acids probably form the only metabolic end-products of styrene in man.

Ohtsuji and Ikeda (1971) also demonstrated that phenobarbital stimulates styrene metabolism in rats in a manner typical for microsomal induction. They further found that it is the first stage of the metabolism which is thereby stimulated, leading to the formation of a more toxic epoxide (styrene oxide). It seems that the kinetics of styrene metabolite excretion in man have not been systematically studied. Bardodej (1964), as well as Gadaskina and Filov (1971), reported that after styrene inhalation, the half-time of excretion of mandelic acid was 7 hours; whereas after administration of the mandelic acid itself, it amounted only to 2 hours.

METHODS FOR DETERMINATION OF STYRENE AND METABOLITES

Styrene: For the determination of styrene in the air, breath, and blood a spectrophotometric method has been applied, with the final measurement being made in pentane at 245 nm (Filov and Rusin, 1960; Bardodej et al., 1961; Bardodej, 1964). The measured concentrations in the final solution were within the range from 0.5 to 10 μg/ml. For automatic analysis of air in an experimental chamber, Bardodej (1964) applied, among others, the "Mercury Vapor Concentration Meter" (Hendrey Relays, Ltd.); whereas Stewart et al. (1968) applied, for the same purpose, continuous measurements with an infra-red spectrophotometer at 11.0 μm wavelength directly in the gas medium in a ten meter path length gas cell. The latter authors applied gas chromatography (hydrogen flame ionization detector) for the determinations of styrene in breath and blood, after extracting the compound with carbon disulphide.

Mandelic acid in urine: Bardodej and Bardodejova (1961) determined mandelic acid following oxidation to benzaldehyde and distillation. For analysis of the distillate three methods were porposed: a) titrimetric–the excess of iodine will be titrated using 0.01 or 0.1 n thiosulphate depending on the concentration of benzaldehyde, b) polarographic (described later in detail by Bardodej et al., 1964)–the range to be determined 0–1000 or 0–5000 mg mandelic acid, c) spectrophotometric–measurements are made in a water solution at 260 nm. Bardodej (1964) proposed a simpler method, consisting of ether extraction, oxidation with $FeCl_3$, and light absorption measurement at 405 nm.

Ohtsuji and Ikeda (1970) proposed a spectrophotometric determination of mandelic acid at 450 nm following ether extraction of urine, evaporation of the extract, and reaction with formaldehyde in sulphuric acid (El Masri et al., 1958). The range of values to be determined varies from 40–400 μg in the sample.

Slob (1973) described a method based on a gas-chromatographic assay of a derivative which may be obtained from reaction of mandelic acid with N,O-bis(thiomethylsilyl)acetamide; the introductory step of the analysis consists of extracting the acidified urine with ethyl acetate and separation of the fraction by means of paper chromatography.

Phenylglyoxylic acid in urine: Bardodej (1964) proposed a spectrophotometric determination at 380 nm of a product of the colour-forming reaction with dinitrophenyl hydrazine, following extraction with ethylbenzene and reextraction. Due to the interference of pyruvic acid (which enters the same reaction) at low exposure values, a preliminary separation of phenylglyoxylic acid by means of paper chromatography was proposed. Bardodej and Bardodejova (1966) applied polarography for the determination of phenylglyoxylic acid. Ohtsuji and Ikeda (1970) made use of the same procedure as that applied for mandelic acid, having only shifted the wavelength to 350 nm. Whereas phenylglyoxylic acid does not interfere with the determination of mandelic acid, the reverse is not true, and due corrections are required. The authors recommend concurrent determination of both metabolites by means of nor-

mal spectrophotometric analysis of a mixture of two substances, applying a system of two algebraic equations.

PHYSIOLOGICAL LEVELS

Bardodej et al. (1961) reported that the concentration of mandelic acid in the urine of nonexposed individuals was relatively constant and did not exceed 5 mg per liter; in 1964 Bardodej reported concentrations of about 20 mg/l; whereas Gadaskina and Filov (1971) present the opinion that readings in the urine of nonexposed people, obtained via benzaldehyde, are due to the interference of other compounds, which correspond to concentrations of mandelic acid on the average from 14 to 18 mg/l. Applying Bardodej's (1964) method, Hinkova (1972) found a mean physiological level of mandelic acid of 31.5 mg/l; Bolanowska and Sapota (in press) reported a mean value of 25 mg/l (± 35%). Using their own method Ohtsuji and Ikeda found a mean level of 92 (47-178) mg/l in nonexposed persons; these values seem to exclude their method from assays when evaluation of low-level exposure is the objective.

Applying gas chromatography for the purpose in question, Slob (1973) has observed physiological levels of 0.6-7.2 mg/l, or 0.3–0.8 mg per 8 hours.

For phenylglyoxylic acid, with the polarographic method, Bardodej and Bardodejova (1966) reported physiological levels below 50 mg/l; applying the spectrophotometric method of Bardodej (1964), Bolanowska and Sapota obtained a mean concentration of 18 mg/l (± 81%); spectrophotometric determinations made by Ohtsuji and Ikeda (their own method) yielded similar values with a mean of 19 mg/l (range 11-34).

EXPOSURE TESTS

Bardodej et al. (1961) proposed a test for styrene using human volunteers, under controlled conditions, and based on the determination of mandelic acid in urine. The subjects were exposed to relatively high concentrations of styrene, 550 and 1000 mg/m³ for 8 hours; as a dependent variable the concentration of mandelic acid in the fraction of urine collected toward end of the exposure interval was chosen. At the lower and higher exposure levels mean concentrations in the urine of 6 subjects amounted to 1340 (1015–1750) and 2760 (2330-3180) mg/l, respectively (Fig. 10-2). It must be born in mind, however, that the data, as reported by the authors, lead to the conclusion that 100 per cent of the systemically absorbed styrene was metabolised to mandelic acid, leaving no room for phenylglyoxylate.

An attempt to use the test for lower concentrations of styrene in air was made by Hinkova (1972). The study was performed similarly to that of Bardodej, but at concentrations of 5 and 50 mg/m³; the exposure was also shorter, lasting 6 hours. The mean levels found in urine were 75 and 145 mg/l (corrected for specific gravity), respectively. These values should be viewed in relation to the rather high mean physiological concentration of 31.5 mg/l.

As a measure of the internal consistency of the results reported in these two papers, the mean ratios of concentrations in urine (U = mg/l) and in the air (A = mg/m³) may be applied. The U/A ratio of the means for the higher and lower concentrations were respectively, 2.3 and 8.6 (Hin-

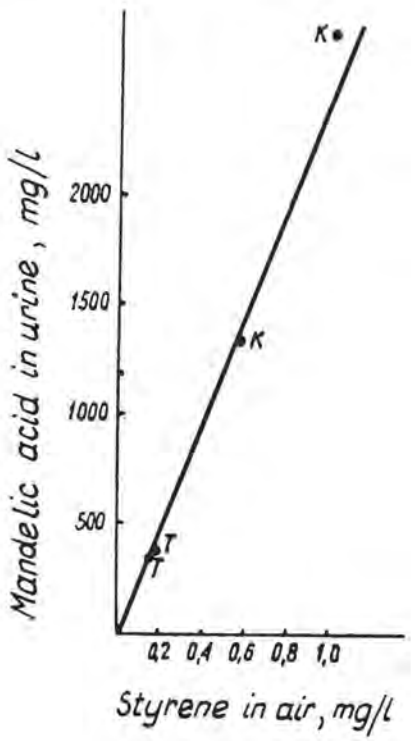

Fig. 10-2. The concentration of mandelic acid in urine collected toward the end of exposure, as dependent on the styrene concentration in the air (Bardodej and Bardodejova, 1961).

Taken from: Bardodej Z. and Bardodejova E.: *Hodnota a pouziti expozicnich testu. Exposicni test pro ethylbenzen.* **Cesk. Hygiena 6,** 537-545, 1961. Page 544, Fig. 8.

kova, 1972). From this comparison it follows that the test, at least in its general form, may be applied to air concentrations of styrene down to about 50 mg/m³; for lower concentrations the test is still not applicable. The present author is of the opinion that the cited experimental data may form only a tentative basis for the interpretation of urinary mandelic acid concentrations. Thus more complete elaboration of the test, under experimental conditions and for a satisfactorily wide range of the concentrations, is still needed.

In view of the lack of feasibility of using the increment of hippuric acid* in the urine as a measure of exposure to styrene, Stewart (1968) proposed to base the test upon styrene determination in the breath, even if the fraction eliminated this way is rather minute. In 6 volunteers, who were exposed to air concentrations of styrene of about 100 ppm (425 mg/m³) for 7 hours, he found a concentration in the breath of 2.5 ppm immediately after discontinuation of inhalation, and it decreased rapidly thereafter. After an initial rapid decay (over 1 hr), the concentration fell to about 0.7 ppm, diminishing over the next 5 hours to about 0.3 ppm. From experiments in which other styrene concentrations were applied for 1 or 2 hours, it seems to follow that the concentration in the breath directly after cessation of the inhalation reflects the final, preceding absorption rate rather than total absorbed dose of styrene. The reviewed data do not permit as yet a conclusion as to whether, and under which conditions, the method could be applied as an exposure test.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

Bardodej et al. (1961) presented the results of exposure assessment in several factories where laminates were manufactured. When two plants with negligible exposure were eliminated from consideration, mean urinary concentrations of mandelic acid fell within the range of 130 to 1650 mg/l. Mean U/A** ratios ranged from 2.3 to 4.7, with a general mean of about 3.0.

Simko et al. (1966) found mean urinary concentrations of mandelic acid from 685 to 1330 mg/l in 4 groups of workers (6-23 people each) exposed to styrene while manufacturing laminates. These data, representing a cross-section for the years 1961-65, are not adequate for a correlation analysis with air concentrations. However, approximate U/A ratios were within the limits of 1.4-3.4. The same authors provided information on the dynamics of the increase in the urinary concentrations in course of a work shift. Mean values in the 27 persons studied at the beginning, in the middle, and at the end of the shift were about 360, 600, and 1300 mg/l, respectively. Similar observations were made recently by Burkiewicz et al. (1974). The starting level, approximately 1/4 of the final value, indicates a possibility of a significant systemic cumulation of styrene. However, no increasing trend was found within the week's exposure period (Burkiewicz et al., 1974).

Huzl et al. (1967) have evaluated the exposure accompanying the production of laminates and observed urinary concentrations of mandelic acid of the order of 200-400 mg/l, the U/A ratio being about 1.0.

Goetell et al. (1972) determined the mandelic and phenylglyoxylic acid in the urine of workers exposed to styrene concentrations ranging up to 300 ppm (1300 mg/m³). In the group of lower exposure (up to 150 ppm), mandelic acid concentrations were below the limits of 3000 mg/l (mean $\Delta U/A$ ratio 4.3) and the phenylglyoxylic up to about 500 mg/l ($\Delta U/A$ 0.6). In the groups exposed to lower concentrations an increase of both metabolites was observed that was proportional to the concentration of styrene in the air. In those, however, who were exposed to concentrations above 200 ppm, an unexpected drop of metabolite concentration was found with an increase of styrene concentrations in the air.

Hinkova (1972) determined the mandelic acid in the urine of people working in contact with polyester paints used for furniture finishing. In winter and summer, the mean air concentration of styrene was 4.8 and 47 mg/m³, respectively. The corresponding mean urinary concentrations of mandelic acid were 38 and 55 mg/l, at the physiological reference level of 21 mg/l.

Ohtsuji and Ikeda (1970) performed a determination of mandelic and phenylglyoxylic acids in the urine of workers employed in the manufacture of plastic tanks, at moderate air concentrations of styrene. In groups exposed to styrene in the range of concentrations of 10-30 ppm (40-120 mg/m³), 7-20 ppm (30-80 mg/m³) and 1-20 ppm (4-80 mg/m³), the corresponding mean uri-

* The author did not cite the work of Bardodej, who introduced the test based upon mandelic acid determination; earlier attempts of Bardodej et al. (1960) to base the test upon excretion of benzoic acid had also yielded negative results.

** Calculations were made by the present author for 5 factories, where more than 6 air determinations were made.

nary levels of mandelic and phenylglyoxylic acids were: 875, 473 and 310 mg/l (approximate U/A ratios 8-4), and 381, 287 and 201 mg/l, respectively.

Bolanowska and Sapota studied the urinary excretion of mandelic and phenylglyoxylic acids in the workers of several industrial plants where average concentrations of styrene vapours were in the range of 11 to 130 mg/m³. Mean mandelic and phenylglyoxylic acid concentrations were from 65-650 mg/l (mean U/A ratios 3.5-9) and from 60 to 200 mg/l, respectively. The ratio mandelic acid : phenylglyoxylic acid varied from 1.1–1.3 in those with low-level exposure to 3.2 in those exposed to the highest concentrations.

From the above review, and mainly from the variable U/A ratios reported, it may be concluded that the results are still internally inconsistent and warrant further studies, mainly in the range of low-level exposure.

An attempt to make use of the determination of styrene in the breath has been described by Goetell et al. (1972). In subjects exposed to styrene concentrations in ranges of 17–32 ppm, 88–139 ppm, and 235–239 ppm, the styrene concentrations in the breath measured immediately after discontinuation of exposure were 0.7, 4.5, and 9.0 ppm, respectively. In 5 hours they dropped to about 0.2, 0.3, and 0.9 ppm, respectively.

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11. PHENOL

ABSORPTION

In industrial conditions phenol is absorbed mainly via the respiratory tract and through the skin. Retention in the respiratory tract is highly efficient; about 70 per cent on the average, starting with 80 percent at the beginning and declining to about 68 per cent toward the end of an 8 hours' inhalation period.

The phenol vapours are also absorbed directly through the skin. For persons in working denims the amount absorbed into the system over 6 hours ranged from 5 to 12 and from 30 to 65 mg, at average concentrations of 5 and 25 mg/m^3, respectively. On the average the cutaneous absorption rate in exposed persons equalled that of an air concentration of 0.35 m^3. The working clothes did not reduce the cutaneous absorption rate of the vapours as judged from comparisons with a group of naked persons (Piotrowski, 1971).

The possibility of direct cutaneous phenol absorption was pointed out by numerous investigators who studied incidental poisonings in human beings after contact of the substance solely with skin (Watorski, 1950; Wroniewicz, 1953; Duverneuil and Ravier, 1962).

METABOLISM AND ELIMINATION

The absorbed phenol is distributed in a relatively uniform fashion in the blood and other tissues (Oettingen, 1949; cited by Gadaskina and Filov, 1971). The metabolism of phenol is quite simple: in rabbits, for instance, a small fraction undergoes hydroxylation to hydroquinone and pyrocatechol, 10 and 1 per cent, respectively. The phenol and diphenols are also conjugated with sulphuric and glucuronic acid; at low doses coupling mainly with the former, and at higher doses with the latter acid. Phenol combined with these acids is excreted in the urine with an efficiency exceeding 90 per cent of the absorbed dose (Parke and Williams, 1953). Low doses of phenol are excreted totally in a combined form; at toxic levels a substantial part of the free compound is excreted. In human volunteers, at doses of the order of 20-60 mg absorbed by the inhalatory route in well-controlled experimental conditions, the efficiency of total phenol urinary excretion was close to 100 per cent (Piotrowski, 1971). High efficiency of absorption and elimination of phenols is also supported by approximate estimates of Ohtsuji and Ikeda (1972) for field conditions. Administering ^{14}C-phenol (0.01 mg/kg) orally to volunteers, Capel et al. (1972) found that the urinary metabolites were present mostly in form of the phenylsulphate (77%) and phenyl glucuronide (16%).

KINETICS OF PHENOL EXCRETION

In animals, small doses of phenol are excreted completely within 24 hours; at higher levels, 2–5 per cent may be still detected in the body after this interval (Porteous and Williams, 1949). In man after inhalation and systemic absorption, the kinetics of phenol excretion can be adequately described using a simple one compartment model with an elimination constant of 0.2 hour^{-1}. It may be concluded, therefore, that in man, significant cumulation of phenol should not occur under conditions of daily repeated industrial exposure to this compound (Piotrowski, 1971). This conclusion is also consistent with the data of Ohtsuji and Ikeda (1972), who were unable to demonstrate unequivocally an elevation of morning, preexposure phenol concentrations in urine collected at the end of a working week compared to those seen on the first post-weekend morning.

METHODS FOR DETERMINATION OF METABOLITES

The exposure test is based on the determination of total urinary phenol (Piotrowski, 1971). To assess the exposure to phenol, the procedure usually recommended for its determination in urine is based on distillation from the acid medium with subsequent colorimetric measurement of the product of this reaction with Gibb's reagent—2,6-dibromoquinone-chlorimide. A practical version of the method is the one derived from a procedure developed for animal urine by Porteous

and Williams (1949), and modified later for lower phenol concentrations in human urine by Fiserova-Bergerova (1954). This version combines acid hydrolysis of the coupled phenol with its distillation in one short procedure. The method is recommended by Teisinger and coworkers (1956). With slight modifications consisting of increased amounts of the reagent (Hanke et al., 1961; Forster and Malinakova, 1961), the method has been recommended by Dutkiewicz et al. (1964), by Gadaskina and Filov (1971), as well as by the author of the present review.

Other methods of phenol determination in urine, reported in the literature, differ widely in all possible parameters and procedures, namely those of: a) hydrolysis, b) separation, and c) final determination of the phenol.

The procedure of hydrolysis, as proposed originally by Porteous and Williams, forms a separate part of the analysis and is quite drastic; its conditions could be justified by difficulties encountered while hydrolyzing the coupled compounds of phenol. Ikeda (1964), who had been hydrolyzing the material in sealed glass ampoules, has shown that the use of 10 n acid does not lead to higher readings than does the use of 0.25 n. Buchet and coworkers (1972), who have applied hydrochloric acid for the purpose in question, were unable to demonstrate any significant influence of the HCl concentration, or duration of the procedure, upon efficiency of the hydrolysis. Controlled analyses performed in the laboratory of the present author show that the combination of hydrolysis with steam distillation, as proposed by Fiserova-Bergerova (1954), assures complete hydrolysis of the coupled phenol. Van Haaften and Sie (1965) reported that heating urine with phosphoric acid (1:1) directly in the heated section upstream of the chromatographic column of GLC apparatus provides a method for quantitative hydrolysis.

Ikeda (1964) and Ohtsuji and Ikeda (1972) have substituted ether-extraction for steam distillation of phenol from an acid medium. It should be mentioned, however, that the high physiological levels of phenol in urine reported by these authors are likely to be due to the presence of other unidentified phenolic compounds extracted concurrently.

Final determination of phenol is sometimes performed also by the method of Theis and Benedict (coupling with diazo-p-nitroaniline), or using the Emerson colour reaction with 4-aminoantipyrine (Emerson, 1943; Kukachova and Naus, 1956; Bardodej, 1960; Bardodej and Krivucova, 1961). In the presence of p-cresol which is found in physiological urine, the former reaction is non-specific. This refers to both reactions relative to o- and m-cresol, if present. Gas chromatography is most specific due to the nature of the process utilized by the method. However, following some procedures a preliminary preparation of a urine high "background" may be seen, resulting from interference of substances which had not been separated from urinary phenol at the preliminary stage of the procedures. (Van Haften and Sie, 1965; Buchet et al., 1972).

The method recommended above for use in the exposure test is characterized by the lowest physiological level for phenol: below 20 mg/day (Teisinger and coworkers, 1956), or 16 mg/l mean: 7 ± 3 mg/l - Dutkiewicz et al., (1964); more systematic investigations of Piotrowski (1971) yielded the following physiological values: 8.7 ± 2 mg/day or 9.5 ± 3.6 mg/l of urine standardized to specific gravity of 1.024.

OTHER METHODS

Determination of phenylsulphuric and phenylglucuronic acids may not serve as a basis for evaluation of exposure of a low or moderate magnitude (Ohtsuji and Ikeda, 1972).

EXPOSURE TEST

The exposure test, as proposed by Piotrowski (1971), is based on total phenol determination in urine voided over the last two hours of exposure on any working day. The results are expressed in excretion rate, mg/h, from which the absorbed dose, through all routes, is calculated according to the formula:

$$y = 0.44 + 0.108\ x \qquad (1)$$

where y = the excretion rate of phenol in mg/hour, x = the absorbed dose in mg, and the constant 0.44 denotes the average excretion rate of phenol in physiological urine.

The precision of this test has been found to be extremely good (± 2.2 mg). Another version of the test is based on the concentration of the excreted phenol in urine; if results are normalized to the standard specific gravity of the latter (1.024) the regression equation assumes the form:

$$y = 0.5 + 1.6\ x \qquad (2)$$

where y = concentration of phenol in urine mg/l, standardized to specific gravity of 1.024, x = absorbed dose of phenol mg 0.5 — respective physiological concentration. The precision of this version of the test, however, is much worse (± 15 mg). Calculation of the absorbed dose according to equations (1) and (2) may apply equally well to 6 and 8 hours' exposures (Mogilnicka and Piotrowski, 1974). Evaluation of absorbed amounts of phenol from the standpoint of maximum allowable concentrations in the air (C) can be based on the following relationship:

$$D = CT(RV + \alpha) \qquad (3)$$

in which D = the absorbed dose as determined by means of the test, T = duration of daily exposure (6 or 8 hours, depending on the circumstances), R = fraction of the substance retained in the respiratory tract, V = lung ventillation (for high work it may be assumed as 0.8 m^3 per hour), α = skin absorption coefficient expressed in volume of air cleared of phenol per unit time (0.35 m^3/ hour). For the MAC of 5 mg/m^3 (USSR) and 19 mg/m^3 (USA) the value of D amounts to 35 and 135 mg, respectively. Expressed in the form of urinary excretion rate (U), the corresponding values would be 4.4 and 15.3 mg per hour.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

In bakelite plants, at phenol concentrations in the air reaching 12.5 mg/m^3, Ohtsuji and Ikeda (1972) found urinary concentrations of total phenol in the range of 200 - 350 mg/l. Mogilnicka and Piotrowski (1974) have studied the exposure of workers in plants manufacturing phenol from chlorobenzene and cumene, and also in factories in which caprolactam and phenolic resins were produced using phenol as a starting material. At an air concentration of phenol well below 5 mg/m^3, the absorbed doses did not usually exceed 35 mg, with sporadic cases reaching a level of 70 mg per working day. These authors found reasonable agreement between average amounts of absorbed phenol, evaluated either on a basis of the excretion rate or the urinary concentration, when the urinary concentration was normalized to urine of specific gravity = 1.024. This latter method, however, yielded sporadic results differing widely from those obtained using the excretion rate as the basic parameter of the test. From estimates presented by Mogilnicka and Piotrowski (1974), it may be concluded that, under prevailing industrial conditions studied, the contamination of skin and clothes does not seem to have a significant contributing role in the total exposure (absorption).

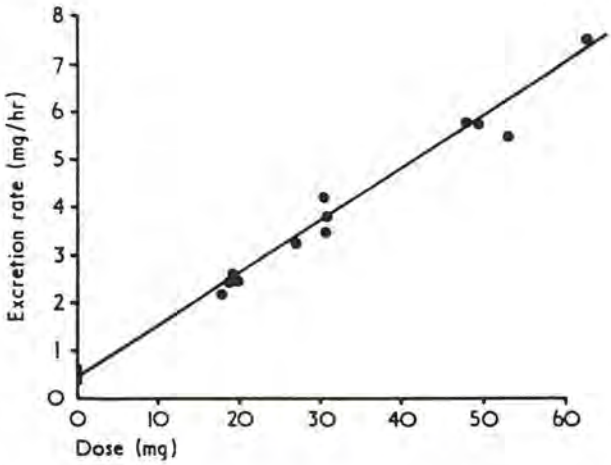

Fig. 11-1. Excretion rate of phenol in urine collected during the last 2 hr of exposure as a function of absorbed dose of phenol (Piotrowski, 1971).

Taken from: Piotrowski J.: *Evaluation of exposure to phenol.* **Brit. J. Industr. Med. 28,** 177-178, 1971. Page 176, Fig. 5.

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12. ANILINE

ABSORPTION

In industrial conditions absorption of aniline takes place in the respiratory tract and through the skin. The retention of aniline vapours in the respiratory tract is high (about 90 per cent), and does not vary with duration of the exposure. The vapours also penetrate the skin. The rate of systemic absorption via the skin at a given concentration of aniline in air is comparable with the rate of absorption in the respiratory tract; at low concentrations the skin absorption is even greater (Fig. 12-1). Elevated temperature of the environment enhances somewhat the cutaneous absorption rate (with temperature rise from 25 C to 30 C by about 20 per cent). The influence of increased air humidity manifests itself in a similar direction (increase of the humidity from 35 to 75 per cent leads to a rise of the absorption by 30 per cent). The above data relate to individuals exposed naked under experimental conditions. Normal working clothes reduce the cutaneous absorption rate by about 40 per cent (Dutkiewicz, 1961).

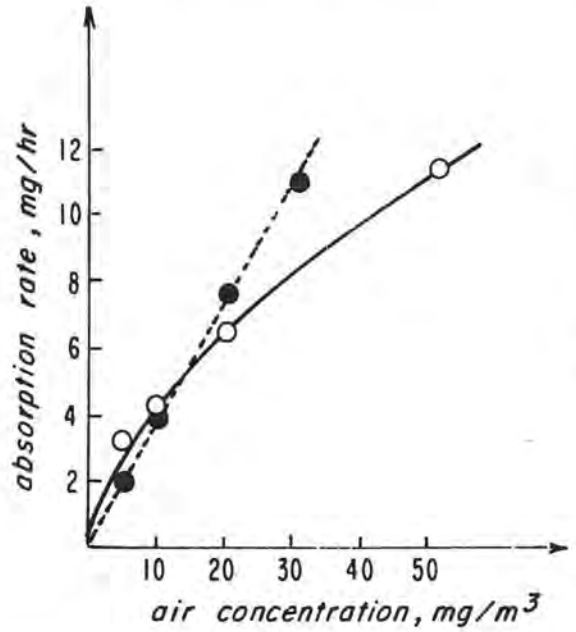

Fig. 12-1. Absorption rate of aniline vapours through the respiratory tract (dotted line) and skin (solid line) as dependent on the air concentration (Dutkiewicz, 1961).

In experimental conditions it was demonstrated that contact of the skin with liquid aniline (saturated layer) yields absorption rates of the order 0.2-0.7 mg cm^{-2} $hour^{-1}$. The rate does not seem to change with duration of the contact, but it shows a rising trend with increasing skin temperature and becomes considerably enhanced when the skin is moist (Piotrowski, 1957). In industry this absorption process may play a substantial role because, in some occupations (e.g., dye-stuff industry), a significant contamination of the skin (up to 2 mg/cm^2) and clothes (up to 25 mg/cm^2) has been found (Trojanowska, 1959). From estimates of the total systemic absorption by means of exposure tests and by taking the aniline air concentrations into account, Dutkiewicz (1961) demonstrated that in industrial conditions the skin formed the main route of absorption; the dominant role in the process was played by direct cutaneous contamination with aniline. Similar conclusions were reached in earlier studies in which aniline concentrations in the air in industry were compared with the frequency of subacute aniline poisoning in various seasons (Jamrog et al., 1954).

METABOLISM

The metabolism of aniline has been learned mainly from animal experiments; the human data are sketchy. Oxidation of aniline leads mainly to the formation of o- and p-aminophenols; the ratio of the two varies considerably from species to species (Parke and Williams, 1956). After the administering the ^{14}C-labelled compound to rabbits, it was possible to prove that m-aminophenol is formed, but with low efficiency. The excreted unchanged aniline accounted usually for less than 1 per cent of administered dose; however the percentage increased at high doses (Parke, 1960; Piotrowski, 1961). The aminophenols are excreted

in the urine, conjugated with glucuronic and sulphuric acids (Smith and Williams, 1949).

Biological hydroxylation of aniline is localized in the liver as a typical process that depends on the microsomal electron transport chain with $NADPH_2$ as hydrogen donor and atmospheric oxygen (Parke, 1968). From this it would be expected that the rate of aniline transformation in the body will increase under the influence of typical inducers of microsomal enzymes, such as phenobarbital and others. In rats, dietary iron deficiency may play the role of an inducing factor (Becking, 1972). Ability to induce slightly the microsomal enzymes in rats was shown by aniline itself if given repeatedly (Wisniewska-Knypl et al., 1975).

The metabolism of aniline has been less investigated in human tissues than in those of animal species. In humans the process also takes place in the microsomes of hepatocytes, but at a rate slower than in rats and not correlated with cytochrome P-450 level (Ackermann, 1972). However, it appears that the process discussed is not the only one possible. For instance, no metabolic activity of the microsomal fraction has been demonstrated in human placenta. Nonetheless, metabolism of aniline was localised in the cytoplasmic fraction (104,000 *g* supernatant), and proceeded in the presence of NADH or NADPH as the hydrogen donors, with molecular oxygen and hemoglobin or methemoglobin as catalyzers (Juchau and Symms, 1972).

It was demonstrated that, in man, p-aminophenol forms the principal metabolite of aniline. The metabolite is excreted in urine, conjugated with glucuronic and sulphuric acid. The efficiency with which the metabolite is formed seems to rise, within the range from 15-60 per cent, with increasing doses of aniline. The metabolism—including oxidation, conjugation, and excretion—is relatively rapid. With some approximation, outlined in the chapter "Kinetics", the process may be given a first order kinetic description with rate constant in the range of 0.1-0.3 $hour^{-1}$. Under conditions of repeated industrial exposure, therefore, there should be no cumulation of aniline in the body (Piotrowski, 1957; Dutkiewicz, 1961).

DETERMINATION OF THE METABOLITES

After hydrolysis, the total p-aminophenol may be assayed colorimetrically making use of the indophenol reaction. Complexity of the method depends on the range of concentrations to be determined and the required or assumed accuracy. Robinson et al. (1951) has made the reaction directly in a diluted hydrolyzate of animal urine, coupling phenol to p-aminophenol in sodium carbonate solution in the presence of potassium ferricyanide as the accelerating oxidant. Piotrowski (1954, 1957) determined urinary p-aminophenol in humans in a similar manner, but with different dilutions, and using phenol in ammonia without an oxidant. The method is not very accurate and the limit of detection is about 10 μg/ml urine. Because of its simplicity, the method has been recommended for practical use in the exposure test (Teisinger et al., 1956; Dutkiewicz et al., 1964). Gadaskina and Filov (1971) recommended the somewhat more accurate method of Dutkiewicz (1960). Developed for human urine, the indophenol reaction is carried out with 1-naphthol in diluted urine-hydrolyzate from which colouring substances were removed previously by extraction with n-butanol in acid medium. The reaction itself is made in ammonia solution, the indophenol for colorimetry is extracted subsequently with n-butanol. Dutkiewicz and Piotrowski (1961) have used both the methods in experiments on humans, and obtained consistent results.

The difficulties encountered while determining low concentrations of p-aminophenol in the urine are related, on the one hand, to the presence of substances inhibiting the indophenol reaction (ascorbic acid, uric acid, creatinine, colouring substances of urine), and, on the other hand, to the low stability of the compound to be measured in the neutral medium which is optimal for quantitative extraction. Greenberg and Lester, cited by Dutkiewicz (1960), proposed a radical procedure to circumvent the difficulties, namely: to extract p-aminophenol from a small volume of urine with dichloroethylene, followed by reextraction with hydrochloric acid. The physiological concentrations obtained using this method (mean = 3.2 μg/ml) were similar to those reported by Dutkiewicz (1960), who had used his own method (mean = 3.7 μg/ml).

OTHER METHODS

Aniline itself can be determined in blood after deproteinization with trichloroacetic adic. The diazo reaction is carried out and the product conjugated with 1-naphthol; the resulting coloured compound may be determined colorimetrically after extraction with n-butanol. P-aminophenol

can also be determined in blood. The blood is deproteinized using zinc- and barium sulphates; and the free, as well as the conjugated, p-aminophenol (after hydrolysis) is determined by means of indophenol reaction, using for this purpose 1-naphthol in an alkaline medium. These methods were used, so far, only in animal experiments (Salm, 1958).

For approximate evaluation of exposure to aniline and other aromatic amines, Marhold (1953), Hill (1953) and Dieteren (1965) have proposed determination of the total urine content of substances yielding a diazo product. The methods are non-specific, and there does not seem to exist a satisfactory basis for interpretation of the results.

EXPOSURE TEST

Piotrowski (1957) originally worked out the exposure test by exposing volunteers to aniline, applied directly to skin in a manner enabling assessment of the absorbed dose, followed by determination of urinary p-aminophenol. Later, subjects were studied by Dutkiewicz (1961), who exposed volunteers by the inhalatory route, while preventing at the same time contact of the vapours with the skin. Piotrowski demonstrated that the amount of absorbed aniline (in the range from 10 to 100 mg) may be assessed with reasonable accuracy, accepting as a measure the excretion rate of p-aminophenol in the last portion of urine voided in the exposure interval.

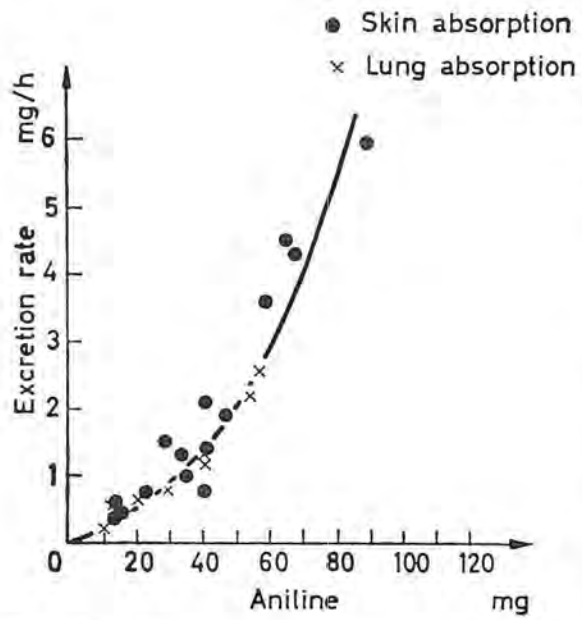

Fig. 12-2. Excretion rate of p-aminophenol in urine collected toward the end of exposure as dependent on the absorbed dose of aniline (Dutkiewicz and Piotrowski, 1961).

Taken from: Dutkiewicz T. and Piotrowski J.: **Pure Appl. Chem. 3,** 319-323, 1961. Page 320, Fig. 3.

Estimates based upon concentrations of p-aminophenol, even after normalization of the urine to constant specific gravity (1.024), are much less accurate. The relationship between the absorbed dose and p-aminophenol excretion rate is curvilinear (concave); this results from the increasing efficiency of the metabolic pathway with rising doses of aniline. Relations reported by both the authors are consistent, as shown in Figure 12-2. For the range of doses beyond those studied experimentally, Piotrowski proposed a formula permitting the extrapolation:

$$U = \frac{0.12D^2}{66 + D} \qquad (1)$$

where U = urinary excretion rate of p-aminophenol measured between 4 and 6 hours of exposure (mg/hours), D = absorbed dose of aniline (mg).

For practical use of the test, the following procedure is proposed: from the excretion rate of p-aminophenol, the absorbed dose is read from the curve in Figure 12-2 or calculated from formula (1). To relate the obtained result to MAC of aniline vapours, a general formula may be applied:

$$D = CT(RV + \alpha) \qquad (2)$$

where D = absorbed dose (mg), T = duration of daily exposure (hour), R = retention of the vapours in the respiratory tract = 0.9, V = lung ventilation rate (for light work 0.8 m^3/hour may be substituted), and α = proportionality coefficient for skin absorption of the vapours.

From Figure 12-1 it can be read that the value of α varies with aniline concentrations, decreasing from about 0.6 m^3/hour for low concentrations (5 mg/m^3) to about 0.2 m^3/hour at 50 mg/m^3. The author of this review proposed to use, for practical purposes, the values of α corresponding to MAC values in a given country (e.g. 0.6 and 0.3 m^3/hour for 5 mg/m^3 (Poland) and 19 mg/m^3 (USA), respectively.

The value of C, obtained from transformation of equation (2), may be directly compared with the

respective MAC values adopted in any given country. For the values given above for Poland and the USA, the derived permissible daily doses, at assumed 6 and 8 hours of daily exposure, amount, respectively, to 35 and 150 mg. The corresponding excretion rates would be 1.5 and 13 mg/hour.

The relationship between the absorbed dose of aniline and the concentration of p-aminophenol in urine sampled toward the end of exposure is burdened with much larger errors. Figure 12-3 may be used as a basis of calculations of data encountered in the literature. The sensitivity of the exposure test allows the estimation of daily doses of aniline above a threshold of 20-30 mg. It is useless, however, at exposure levels as low as the recent MAC level in the USSR (0.1 mg/m³ of air) (Cahiers de Notes Documentaires, 1974). The latter value has been proposed based on observations performed on humans exposed in industry to aniline concentrations not exceeding on the average 3 mg/m³, where subtle changes in the blood picture as well as in the nervous system were observed (Vasilenko et al., 1972 a). Other data indicate, in humans, a slight elevation of the methemoglobin level after oral doses exceeding 25 mg (corresponding to air concentrations of 2-3 mg/m³) (Jenkins et al., 1972). It seems, therefore, likely that the MAC level will be considerably reduced in the future, and the postulated low exposure level will not be measurable anymore using the described exposure test.

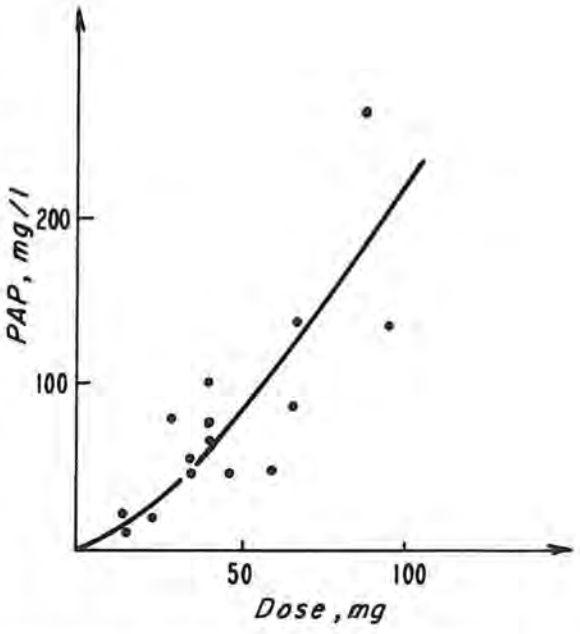

Fig. 12-3. Maximum urinary concentrations of p-aminophenol, as dependent on the dose of aniline absorbed through the skin (Piotrowski, 1960).

SPECIFICITY OF THE EXPOSURE TEST

The test, based upon the determination of urinary p-aminophenol, is nonspecific in situations where an individual has absorbed other aromatic compounds metabolised to the same end-product, i.e. p-aminophenol. From the practical standpoint, the most important role is played by the analgesic phenacetin, an ingredient of anti-headache pills. A few hours after intake of phenacetin the concentrations of p-aminophenol may reach values in the order of 200 mg/l and one day later oscillate still around 30 mg/l (Simko and Hassman, 1960). It is therefore necessary to ascertain whether a person whose urine is to be analyzed did take headache pills or other drugs over the preceding two days. As a control it is recommended that urine voided before onset of the exposure be analyzed; the result should be negative.

Nitrobenzene, absorbed in small amounts (up to 70 mg daily) does not interfere with the p-aminophenol exposure test for aniline (Salmowa et al., 1963). In workers exposed to dimethylaniline, a slight elevation of urinary p-aminophenol was observed (Vasilenko et al., 1972 b).

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

The bulk of the available data refer to earlier years, and should not be taken as reflecting the present hazard of exposure to aniline in industry. The data reported by Dutkiewicz (1961) and by Kodura and Sniady (1963) indicated that the daily absorption did not exceed 45 mg. Piotrowski (1968), who has studied more recently the exposure of workers engaged in the manufacture of aniline, found daily absorption between 30 and 80 mg. Exposure of the same order of magnitude has been reported from the Hungarian industry (Pacseri, 1959).

Most of the recent data from the USSR point to a very low exposure of workers manufacturing diphenylamine and other organic amino compounds. The average concentrations of p-aminophenol in urine did not exceed 20 μg/ml (Vasilenko et al., 1972 b). A slight rising trend in urinary p-aminophenol concentrations in subsequent days of the working week was reported.

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13. NITROBENZENE

ABSORPTION

In industry, nitrobenzene may be absorbed into the body by all possible routes, but mainly via the respiratory tract and through the skin. Retention of nitrobenzene vapours in the respiratory tract is high; on the average 80 per cent, from 87 per cent at the beginning down to 73 per cent after 6 hours of inhalation (Salmowa et al., 1963).

The vapours pass directly through the skin. The rate of absorption is roughly proportional to air concentration, from 1 mg/hr to about 9 mg/hr at 5 and 20 mg/m^3, respectively. The temperature of the air in the range from 25 C to 30 C did not influence the absorption rate; increasing the relative humidity from 35 to 67% increased the rate of absorption by about 40 per cent. Normal working denims reduced the cutaneous absorption rate of the vapours, relative to the values in persons exposed naked, by about 20 per cent (Piotrowski, 1967).

The cutaneous absorption of nitrobenzene also takes place after direct application to the skin (contamination). The maximal absorption rate resulting from creation of a saturated layer at the skin surface reaches values from 0.2 to 3 mg/cm^{2}h^{-1}, rising with skin temperature. The absorption rate, measured as a decrement of nitrobenzene from the contacting layer, decreased with duration of contact (Salmowa and Piotrowski, 1960). Absorption of nitrobenzene as a result of direct contamination of skin (and clothes) may be of practical importance in industry. As demonstrated by Trojanowska (1959), the contamination of the skin and working clothes of workers in the dye manufacturing industry may reach values of 2 and 25 mg/cm^2, respectively. Also, in a field study performed in a nitrobenzene manufacturing plant, Piotrowski (1966) has shown that the absorbed doses of the compound, estimated by means of exposure tests, exceeded considerably the amounts that could be attributed to absorption of the vapours.

METABOLISM AND ELIMINATION

Nitrobenzene is quite soluble in lipids; the coefficient of oil-water distribution is about 800 (Dutkiewicz et al., 1964). In rats, one hour after intravenous administration of a small dose of the substance, the ratio of concentrations of unaltered nitrobenzene, adipose tissue vs. blood, in internal organs and muscles was approximately 10 : 1 (Piotrowski, 1968).

Information on the metabolism of nitrobenzene is based mainly on animal experiments; the human data are fragmentary. The main pathways are directed in two ways: a) reduction to aniline with subsequent hydroxylation to aminophenols, and b) direct hydroxylation of nitrobenzene with formation of nitrophenols. Partial further reduction of nitrophenols to aminophenols may take place.

The reduction of nitrobenzene to aniline proceeds through the unstable intermediate products, nitrosobenzene and β-phenyl hydroxylamine, which are highly toxic and possess a pronounced methemoglobinemic activity (Uehleke, 1963). The process takes place mainly in the liver under the influence of "mammal nitro-reductase", localized partly in the microsomal fraction and partly in the cytoplasm. The process depends upon NADPH$_2$ and requires the presence of flavoprotein; oxygen exerts an inhibitory influence. The mechanism of further hydroxylation of aniline to aminophenols is probably the same as discussed in the chapter on "Aniline".

The hydroxylation of nitrobenzene to nitrophenols is not a microsomal process and is difficult to reproduce in animal tissues "in vitro". The process proceeds under the influence of peroxidase in the presence of oxygen (Buhler and Mason, 1961).

In rabbits, p-aminophenol is the main (31 per cent) metabolic product of biotransformation; other metabolites are p- and m-nitrophenols (9 per cent each), and o- and m-aminophenols (each 3-4 per cent). Only a minor fraction of nitrobenzene is excreted unaltered with the expired air; aromatic ring cleavage with resulting $^{14}CO_2$ expiration after administration of the labelled compound accounts only for about 1 per cent of the administered dose. The nitro- and aminophenols are excreted in urine conjugated with sulphuric and glucuronic acid (Robinson, et al., 1951).

Quantitative relations between metabolites vary with animal species and dose of nitrobenzene. In rabbits the share of p-aminophenol rises from 2-6 to 15-27 per cent with increase of the dose from 10 to 50-100 mg/kg, respectively; whereas the efficiency of the formed p-nitrophenol seems constant, of the order of 5-15 per cent. In contrast, in rats the efficiency of p-nitrophenol formation decreases with an increasing dose of nitrobenzene, the percentage of p-aminophenol formed remaining constant (Salmowa and Piotrowski, 1960; Salmowa, 1961).

Excretion of two metabolites in man—p-aminophenol and p-nitrophenol—have been studied. In experimental conditions of inhalatory exposure to nitrobenzene, the efficiency of p-nitrophenol formation was of the order of 6-21 per cent (in one individual up to 37 per cent); mean 13-16 per cent (Salmowa et al., 1963; Piotrowski, 1967). The respective efficiency for p-aminophenol may be estimated only indirectly from observation of acute poisoning cases, where the molar ratio of excreted p-nitrophenol to p-aminophenol was about 2:1 (Ikeda and Kita, 1964; Myslak et al., 1971). Combined excretion of these two metabolites in man accounts for not more than 20-30 per cent of the nitrobenzene dose. The fate of the rest remains obscure.

METABOLIC AND EXCRETION KINETICS

Experimental investigations of nitrobenzene metabolism in animals, in which large doses of compound were administered, pointed to a prolonged excretion of the metabolites. At moderate and low doses, however, the excretion of p-nitrophenol and p-aminophenol obeyed a first order kinetics with a rate constant of about 0.09 hour $^{-1}$. At a daily dosage of 10–20 mg/kg for a week, the cumulative effect was slight; and increase in the excretion rate did not exceed 40 per cent relative to the first day. In rats the excretion was somewhat slower, and the rate constants, obtained from following the urinary levels of p-nitrophenol and p-aminophenol, were about 0.08 and 0.06 hour $^{-1}$, respectively (Salmowa and Piotrowski, 1960; Salmowa, 1961).

From a study of nitrobenzene concentrations in the blood of an acutely poisoned individual, Teisinger et al. (1956) concluded that the compound persists in the human body for a prolonged time. Similar observations were made with respect to urinary excretion of the metabolites, p-nitrophenol and p-aminophenol, by patients treated for acute or subacute poisoning. The excretion coefficient for p-nitrophenol, followed in urine for 3 weeks, amounted to about 0.008 hour $^{-1}$ (Ikdea and Kita, 1964; Myslak et al., 1971). It may be concluded, therefore, that the metabolic transformation and excretion of nitrobenzene in man is slower by an order of magnitude than in rabbits or rats. A study of the metabolic kinetics of nitrobenzene in man, by means of the urinary excretion rate of p-nitrophenol after experimental exposure to low doses of nitrobenzene, demonstrated that it is a biphasic process that may be expressed by an equation:

$$U_t/U_o = 0.66\, e^{-0.14t} + 0.34\, e^{-0.0115t} \quad (1)$$

where t = time in hours.

This curve characterizes the, so-called, two compartment open model in which the role of the slowly turning over compartment can be attributed to adipose tissue. The prolonged systemic retention of nitrobenzene in man, and slow excretion of its metabolites, is predetermined by the low rate of the metabolic transformation of nitrobenzene itself, namely of reduction and hydroxylation; whereas the coupling and excretion of the metabolites (p-nitrophenol) is a rapid process. In accord with theoretical expectation in man, nitrobenzene cumulates in the course of repeated daily exposures, reaching an equilibrium after 3–4 days, at which time p-nitrophenol level in urine is 2–3 fold higher than on the first day of exposure (Piotrowski, 1967). This allows calculation of cumulative trends on the basis of the kinetic data derived from investigation of single exposures (Piotrowski, 1971).

METHODS FOR THE DETERMINATION OF THE METABOLITES

The exposure test is based upon determination in the urine of p-nitrophenol after hydrolysis. Basic methods for respective determination have been developed for assessment of exposure to parathion. All the methods include removal of interfering colour substances, hydrolysis, extraction of p-nitrophenol, re-extraction into the aqueous medium, reduction to p-aminophenol, and indophenol reaction yielding a blue product. The detection limit is similar for all methods (about 5 μg in a sample); and final detectability results

from the volume of urine taken for analysis (0.1 μ g/ml at a 50 ml, 0.5 μg/ml at a 10 ml sample). Various authors have recommended different methods or their modifications. The method of Salmowa has been applied in elaboration of the exposure test (Salmowa et al., 1963; Piotrowski, 1967). The method includes acid hydrolysis, oxidation of urine dyes with perhydrol in an alkaline medium, extraction of p-nitrophenol with a mixture of solvent naphtha and ethylether, re-extraction to the alkaline aqueous medium, reduction with zinc in an acid medium, and development of the colour with phenol in presence of ammonia. The limit of detection (for a 10 ml sample of urine) amounts to 0.5 μ /ml; the precision of the method is about ± 6 per cent.

The methods published recently for p-nitrophenol in urine make use of gas chromatography (see "Parathion").

OTHER METHODS

Determination of p-aminophenol—see: "Aniline".

For assessment of exposure to nitrobenzene and other aromatic mono-nitrocompounds, Ajtai and Csayi (1956) proposed polarographic determination of unaltered nitrocompounds after destillation of urine. No quantitative interpretation of this assay has been proposed.

EXPOSURE TEST

Originally, a test based upon determination of p-aminophenol in urine was recommended (Von Oettingen, 1941; Piotrowski, 1954; Teisinger et al., 1956). Later studies by Salmowa and Piotrowski (1960) and by Salmowa (1961) showed that the test could not be applied for the evaluation of low-level exposure due to poor sensitivity and lack of specificity of the method in the low concentration range.

Salmowa (1961) proposed to base the test upon the determination of p-nitrophenol; and experiments involving single (Salmowa et al., 1963) and repeated exposure (Piotrowski, 1967) demonstrated that the test may be applied for the assessment in man of an absorbed nitrobenzene dose (or average doses) above 10 mg. However, the test, as discussed here, should be treated as semiquantitative because the precision in the recommended version was not better than ± 10 mg nitrobenzene.

In the test, urine samples are collected in the last 2-3 hours of a working shift. Since in the first 3 days of the working week there is a systemic cumulation of nitrobenzene, it was suggested that the test be performed on the 4th, 5th, and possibly 6th day of the week and that the estimate of absorption be based on the mean values for individual workers.

The test is based on the measurement of the excretion rate of p-nitrophenol (μg/hour); for according to Salmowa et al. (1963), this parameter leads to better accuracy than the measurement of the concentration of the metabolite itself.

In the case of single exposures, interpretation of the test is provided by equation:

$$I = 3.6\ E \qquad (2)$$

where: I = excretion rate measured toward the end of daily exposure (μ g/hour); E = absorbed dose in mg.

In the case of daily repeated exposures, if the measurement is made at the end of week, the relationship should undergo a change, the expected magnitude of which may be derived from parameters of equation (1). The result would be:

$$I = 10.8\ E \qquad (3)$$

indicating a threefold increase of excretion relative to the first day of exposure. The experimental relationship obtained by Piotrowski (1967) was:

$$I = 7.9\ E \qquad (3a)$$

Equations 3 and 3a denote the range of uncertainty in the interpretation of the test in conditions of repeated exposure. Figure 13-1 presents the dynamics of increase of p-nitrophenol in urine, found experimentally, after repeated daily exposure of a constant magnitude.

The final interpretation of the test is derived by relating the absorbed dose to the permissible dose D_m; resulting from exposure to the maximal permissible concentration in air C_m. The relation between these two parameters is as follows:

$$Dm = CmT(VR + \alpha) \qquad (4)$$

Mean values of the parameters may be chosen as follows: lung ventilation V = 0.8 m³/hour (light work), lung retention R = 0.8; coefficient of va-

pour skin absorption α = 0.25 m³/hour. The permissible dose is determined, therefore, by two factors (daily exposure time T and maximum permissible concentration in air C_m), depending on the regulations, specific for a given country. In the USSR, at 6 hours daily work with aromatic nitro- and aminocompounds, and a MAC in the air of 3 mg/m³ (Sanitarnyje Normy, SN 245-71), the permissible dose D_m will be 15 mg; in the USA, at 8 hours of daily exposure and C_m = 5 mg/m³, D_m = 35 mg. According to equations 3 and 3a these values corresponded to the permissible urinary excretion rate of p-nitrophenol of 130-180 and 280–390 μg/hour, respectively.

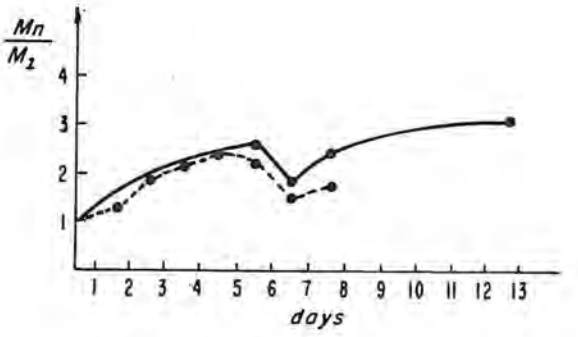

Fig. 13-1. Increasing trend of p-nitrophenol in urine, following daily exposure to nitrobenzene. Ratio of daily excreted amounts on the subsequent n-th day to the first day of exposure. Solid line — theoretical trend, dotted line — mean experimental data (Piotrowski, 1966).

SPECIFICITY OF THE TEST

Among the aromatic nitrocompounds tested thus far in rats, a positive reaction for p-nitrophenol in urine was given by o-chloronitrobenzene and 2,5-dichloronitrobenzene, for which relative to equimolar doses of nitrobenzene the readings were 34 and 10 per cent—respectively. Other aromatic nitrocompounds, such as m-nitrobenzene, o- and p-nitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, o- and p-ethylnitrobenzene, p-chloronitrobenzene, m- and p-nitroaniline, and 1-nitronaphthalene— have yielded either negative or equivocal results (Piotrowski, 1967). On the other hand, Kodura and Sniady (1963) reported from a field study positive results with the p-nitrophenol test in workers exposed to p-chloronitrobenzene. However, due to the character of production in the factory, concurrent exposure of the studied individuals to o-chloronitrobenzene could not be excluded.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

The early published data related to Polish industry. Serious systemic absorption (doses of the order of several hundred mg per day(had been described by Piotrowski in workers in a nitrobenzene producing plant as early as 1954. When the working conditions were improved, at similar production levels 10 years later, the absorbed daily doses were estimated at 20–65 mg, with sporadic values up to 130 mg. Exposure to nitrobenzene in course of aniline production was not appreciable, absorbed doses being about 7–9 mg (Piotrowski, 1966). A moderate exposure to nitrobenzene, apart from nitrobenzene manufacture, was found earlier in other studies, mostly in the Polish dye-stuff industry (Salmowa, 1961; Kodura and Sniady, 1963).

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14. BENZIDINE

ABSORPTION

Under industrial conditions, benzidine may pass into the system via all routes: the respiratory, gastrointestinal, and the skin (Dutkiewicz et al., 1964). Absorption through the skin seems to be dominant under normal conditions; this is borne out by experiments on volunteers (Meigs et al., 1951), as well as by observations made in industry. It has been demonstrated repeatedly that a positive correlation exists between benzidine concentrations on the skin, underwear, and clothes of workers and its concentration in the urine. Furthermore, when a daily change of underwear and working denims and an afterwork bath were introduced, the urinary level of benzidine was reduced by a factor of 2 to 3 (Meigs et al., 1951; Meigs et al., 1954; Ader et al., 1958).

METABOLISM AND ELIMINATION

In the body, benzidine undergoes conversion mainly into 3-hydroxybenzidine. In mice, this metabolite and its N-acetyl derivative is excreted predominantly estrified with sulphuric and glucuronic acids; these esters account for about 2/3 of all the metabolites. Of the others, mono- and diacetyl benzidine, as well as N-sulphuric and N-glucuronic derivatives, were identified in urine (Seiarini and Meigs, 1961). In this and an earlier paper (1958), these authors were unable to confirm the data on biotransformation of benzidine to the dihydroxy-derivative.

Part of the absorbed benzidine is excreted unaltered in the urine; the percentage varies among the species. In the first experiments on humans, Meigs et al. (1951) recovered 10 per cent of the compound unchanged in urine. According to Sciarini and Meigs (1961), who investigated urinary excretion in three workers exposed to considerable amounts of benzidine in industry, the free compound and mono- and diacetylbenzidine accounted for 3.6–5.6 and 6.7–15.9 per cent, respectively, of the total excretion; the rest was present in the form of 3-hydroxybenzidine (esterified).

The dynamics of benzidine metabolism may be evaluated on the basis of its rate of disappearance from urine. Ader (1957) estimated an elimination half-time at about 20, 5.8 and 5.4 hours in the rabbit, dog, and man, respectively. From these values it follows that after repeated industrial exposure, systemic cumulation of benzidine in man is unlikely.

DETERMINATION OF BENZIDINE AND ITS METABOLITES IN URINE

Glassman and Meigs (1951) developed a method for the determination of free benzidine in urine based on ether extraction from the alkaline medium, evaporation of the solvent, and reaction of the compound with chloramine T which yields a coloured product (merichinoid, with an absorption maximum at 440 nm). When fifty milliliters of urine were used, the limit of detection was at about 20 μg/l; however, quantitative determination was possible only above 100 μg/l. Modification of this method (Ader and Chrzaszczewska, 1957) simplified somewhat the analytical procedure, leaving however the basic parameters unchanged. Sciarini and Mahew (1955) developed a rapid method recommended also by Gadaskina and Filov (1971), in which a smaller volume of urine (15 ml) is extracted with ethyl acetate; the colorimetric determination, however, is based on the same principle as in the previously described procedures. Again, the quantitative range for this method lies above 150 μg/l. A method based on the same principle suitable for lower concentrations was described recently by Piotrowski and coworkers (1971); 150 -250 ml of urine is continuously extracted with ether, benzidine is reextracted from the latter into a small volume of hydrochloric acid, and the colour reaction is performed with chloramine T. The resulting dye is extracted again with a small amount of chloroform and read at 445 nm. The limit of detection is about 2 μg/l with quantitative determination possible above 6 μg benzidine per liter urine (precision ± 9%).

The methods listed above are nonspecific in the sense that, like benzidine, the derivatives—e.g. dichlorobenzidine, dianisidine, tolidine—will be determined if they are present. The limits of detection for all these compounds are similar.

IDENTIFICATION AND DETERMINATION OF INDIVIDUAL FREE DIAMINES USING PAPER CHROMATOGRAPHY

Meigs et al. (1954) and Ader and Chrzaszczewska (1957) separated benzidine from its derivatives (o-tolidine, o-dichlorobenzidine, o-dianisidine) by means of paper chromatography. Partly evaporated ether extract of urine was applied to the paper, developed with petroleum-ether, and the resulting patches stained using chloramine T.

Ghetti et al. (1968) developed the chromatogram with a mixture of iso-butanol, acetic acid and water (3:1:1). Half of the strip was used for the determination of positions of the compounds by staining with p-(dimethylamino)benzaldehyde. The remaining half was cut in pieces, extracted, and the substances determined by diazotization and coupling with N-1-naphthylethylenediamine (absorption measurement of 575 nm). According to the authors, the limit of detection lies at about 5 μg of diamine per liter of urine.

APPROXIMATE EVALUATION OF THE TOTAL CONTENT OF AROMATIC AMINES IN URINE

Marhold (1953) described a method*, based on diazotization of amines and coupling with phenyl-7-acid directly in a sample of urine (50 ml). The resulting dye is absorbed on a piece of cotton fabric and, after drying, the colour is compared with the standards; the sample may be preserved as a lasting evidence of the level of the exposure. The method, as described, is nonspecific and of low sensitivity. The sensitivity was considerably increased by Ghetti et al. (1968) who performed the reaction in a small volume of the extract, obtained from the original volume of urine = 1.5 l. According to these authors, the sensitivity of this version of the method is to 6 μg/l.

* FIAT, 1313, vol. 4, page 400.

DETECTION AND DETERMINATION OF BENZIDINE METABOLITES IN URINE

For detection of individual benzidine metabolites in urine, Laham et al. (1970) applied paper chromatography with subsequent staining with Ehrlich or Gibbs reagents. The method, as developed, enables the detection in urine of benzidine, mono- and diacetylbenzidine, as well as of 4,4-diaminodiphenyl-3-sulphate and its monoacetyl derivative. The acetyl derivatives of benzidine and 4,4-diaminodiphenyl-3-sulphate yield coloured products after acid hydrolysis.

Sciarini and Meigs (1958, 1961) proposed methods for the quantitative determination of these substances, based upon two reactions: a) diazotization and coupling with N-1-naphthylethylenediamine (Bratton and Marshall, 1939) (the reacting substances are: benzidine, 3-hydroxybenzidine, and its sulphuric ester), and b) reaction with nitrous acid (Burkhardt and Wood, 1929) (yellow colour) for the determination of 3-hydroxybenzidine. Both reactions are made directly in a small volume (1–4 ml) of urine; the sensitivity seems too low for evaluation of industrial exposure to benzidine. The same authors proposed (1961) analyzing the urine of people exposed to significant amounts of benzidine by the above methods—however, after extraction of the metabolites from urine with ethyl acetate. In this way the sensitivity was considerably improved, rendering determination of 3-hydroxybenzidine possible at concentrations of the order of 4–20 mg per liter of urine.

OTHER METHODS USED IN PROPHYLACTIC EXAMINATIONS

The basic hazard of benzidine is related to the increased incidence of urinary bladder cancer (see Case et al., 1954; Chwat, 1957). Thus, additionally, or irrespective of the chemical evaluation of the magnitude of the exposure, biochemical and morphological tests are being applied for early detection of precancerous stages. Beta-glucuronidase activity levels in the urine were elevated in the exposed people, particularly in those in whom urological changes were already found by cystoscopy (Popler et al., 1964; Kleinbauer et al., 1969). Urine may also be screened for the presence of cancerous cells, a sufficienty sensitive technique has been developed by Rofe (1957).

EXPOSURE TEST

As yet, for the evaluation of exposure to benzidine, only detection of the free compound in the urine has been developed. Apart from the preliminary observations of Meigs et al. (1951), no experiments have been performed on volunteers; and thus the interpretation of the benzidine concentrations in urine is mainly comparative. Attempts to assess the absolute amounts absorbed are based on more or less speculative assumptions. The latter may be summarized as follows:

(i) The maximum urinary excretion rate occurs about 2-3 hours after termination of the exposure, declining thereafter with a half time of 5 to 6 hours (Ader, 1957). This precludes significant systemic cumulation of benzidine, and the levels found in urine may be legitimately ascribed to the absorption on a given day.

(ii) The concentration of benzidine in urine, collected directly after termination of the exposure (C), is related in a simple way to the daily amount excreted in 24 hrs urine (m) by a formula (Ader, 1957): m = 2C.

(iii) From data of Meigs et al. (1951), as well as of Sciarini and Meigs (1961), it follows that in man 4–10 per cent of the absorbed benzidine is excreted unaltered in urine; by analogy to the canine excretory pattern, the Polish authors (Ader et al., 1958; Bolanowska et al., 1972) assumed an average value of 7 per cent.

Quantitative assessment of the absorbed amounts has found application for the evaluation of the relative importance of the role of the various absorption routes under industrial conditions. Comparison of the evaluated absorbed amount in benzidine, based on urinary excretion, with that calculated from concentrations of the compound in air, provides relevant information on the role of dermal absorption.

The maximum permissible absorption rate of benzidine and its maximum permissible concentration in the urine have not yet been defined. From the older literature it follows that in periods, when urinary concentrations of the order of 100 μg/l and above were common, the incidence of the cancer of urinary bladder was appreciable (see Ader et al., 1958; Chwat, 1957). A drastic reduction of exposure to benzidine in Poland has been accomplished only recently; and the follow-up period is too short to see the effect of this new exposure, lower by an order of magnitude, upon the incidence of this occupational cancer.

Tentative values of the maximum permissible urinary concentrations of benzidine resulted mainly from the sensitivity of the methods applied for its determination. In Poland the earlier and contemporary values applied were 20 and 2 μg/l, respectively.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

First reports on the magnitude of occupational exposure of workers to benzidine in manufacturing plants were published in the fifties in the USA (Meigs et al., 1951, 1954) and in Poland (Ader et al., 1958); later data come from the same centers (Sciarini and Meigs, 1961; Bolanowska et al., 1972).

The earlier data published in the fifties had been collected under conditions of relatively primitive production procedures with hygienic precautions not carefully observed. Urinary concentrations of benzidine (and its derivatives such as dichlorobenzidine, tolidine, dianisidine, that had been determined jointly at a similar sensitivity of method as then applied) were high, of the order of 100 μg/l or more. Current interpretation would indicate a daily absorption rate in the order of several milligrams. At the same time, air concentrations of benzidine in the industrial premises reached the level of 0.09 mg/m^3 air (Meigs et al., 1951). If a more efficient method of benzidine collection from the air were applied, the concentrations appeared even higher, and in a Polish plant, as investigated by Ader et al. (1958), the mean concentrations clustered in the range form 0.15 to 0.4 mg/m^3. Calculations made by all the authors pointed to the dominant role of dermal absorption. Ader et al. (1958) estimated the dermal route contribution to the total absorption at 80 to 90 per cent. The urinary levels were higher in summer; moreover, the levels found in consecutive days of the week displayed a steep rising trend. The latter resulted from increasing contamination of clothes and of the skin (Fig. 14-1). Massive exposure of workers to benzidine continued in the USA later. Thus Sciarini and Meigs (1961) reported mean urinary concentrations of 230 ± 158 and 674 ± 534 μg/l in the cool and warm season, respectively. The most recent data indicate an extremely high incidence of

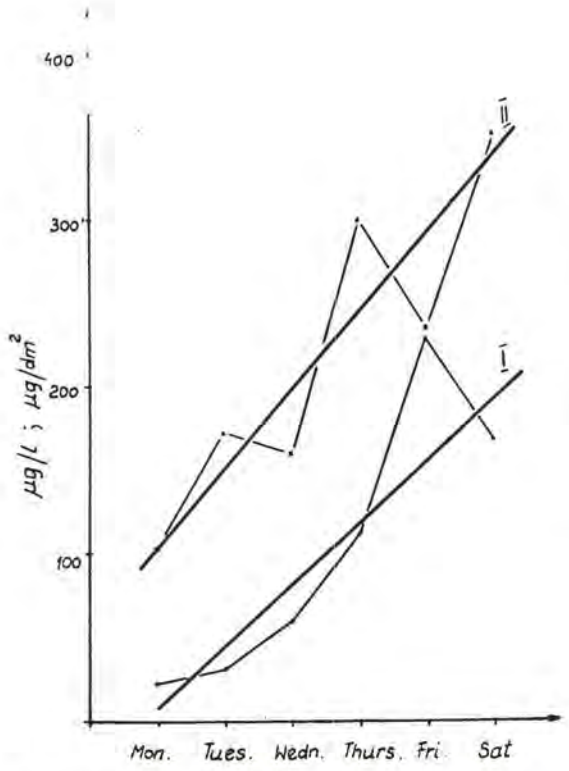

Fig. 14-1. The increase of benzidine concentrations in urine (II) compared with the rising contamination of the skin (I) in subsequent days of the working week (Ader et al., (1958).

Taken from: Ader D., Piotrowski J., Zaremba Z.: *Chemiczna ocena narazenia na benzydyne w przemysle.* **Medycyna Pracy 9,** 207-217, 1958. Page 211, Fig. 2.

bladder cancer in workers manufacturing benzidine; on the other hand, concentrations of benzidine in urine (below 160 μg/l) may indicate some drop in actual exposure as compared with earlier data (Zavon et al., 1973).

The necessary practical steps resulting from the picture presented above were taken in Poland in the late sixties. Improvements in manufacturing technology and the introduction of strictly controlled hygienic procedures led to a reduction in exposures estimated from all the indices by at least one order of magnitude: mean concentrations in the air were down to 7-11 μg/m³, amounts in the working clothes to about 200 mg/m², on the skin (chest) to 5-8 mg/m², and urinary levels to 9 μg/l (Bolanowska et al., 1972). It should be also noted that at present in Poland people below the age of 40 are not allowed occupational contact with benzidine. For those above that age, the permissible period of exposure is 3 years.

Considerable reduction of exposure to benzidine in a newly constructed manufacturing plant is born out in the reports of Czechoslovakian authors (Popler et al., 1964). These data, however, could not be easily compared with previously reviewed reports due to the lack of data on concentrations of benzidine in the urine of workers. In the laboratory of these authors (see also Kleinbauer et al., 1969) evaluation of exposure to benzidine was based upon concentrations of the compound in the air, on clothes and skin (these parameters are used by others mainly as supplementary data), and upon activity of beta-glucuronidase in urine.

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15. TRICHLOROETHYLENE

ABSORPTION

Under industrial conditions the respiratory tract forms the main route for trichloroethylene (TRI) absorption. The retention of the TRI vapours measured under similar circumstances amounted on the average to about 70 per cent (Grandjean et al., 1955). Studying the retention in controlled experiments, the Czech authors found the retained percentage varied between 58 and 70 (mean of 64, Soucek and Vlachova, 1960) or 51 to 64 (mean 58, Bartonicek, 1962). At variance with the above data are results obtained by the Japanese investigators (Nomiyama and Nomiyama, 1971) who found a rather low retention of about 36 per cent. Skin absorption of trichloroethylene is probably of minor importance (Frant and Westendorp, 1950; Smith, 1966), although it may take place when direct contamination of the skin occurs (Stewart and Dodd, 1964). It appears that the absorption of the vapours through the skin has not been studied in controlled conditions.

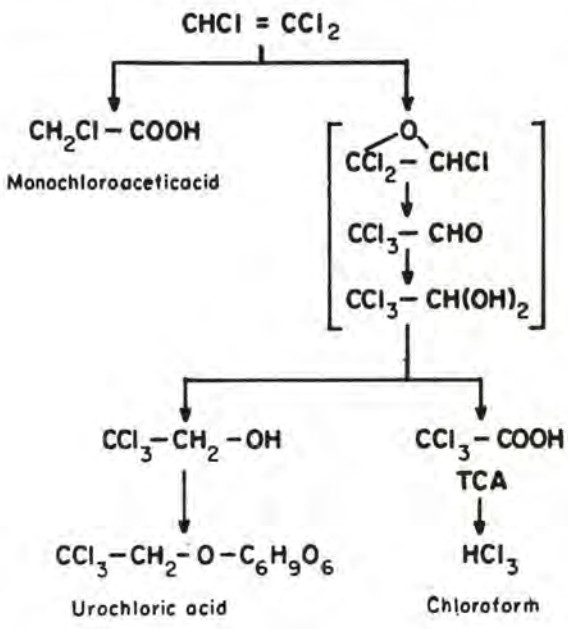

Fig. 15-1. Metabolic pathways of trichloroethylene (Williams, 1959).

METABOLISM

The absorbed trichloroethylene is rather uniformly distributed in various tissues. However, the earlier data reviewed by Gadaskina and Filov (1971) indicated that the lowest concentrations are encountered in muscle and the highest ones in adipose tissue.

According to Soucek and Vlachova (1960) and Bartonicek (1962) some 70-80 per cent of systemic trichloroethylene is metabolised in man. The principal metabolites are: trichloroacetic acid (TCA) and trichloroethanol (TCE). TCE is excreted in urine as glucuronide-urochloralic acid. Smaller amounts of monochloroacetic acid (MCA) (Soucek and Vlachova, 1954) and chloroform (the same authors, 1955) are also found. Based upon earlier studies, Gadaskina and Filov (1971) proposed a scheme of metabolic pathways for trichloroethylene as presented in Figure 15-1. The metabolites of the main pathway (trichloroethylene epoxide, trichloroacetic aldehyde, and chloral hydrate) are partially hypothetic. It was only in recent studies that Kimmerle and Eben (1973a) were able to detect chloral hydrate in the blood of rats exposed to trichloroethylene; this observation, however, could not be confirmed in a human experiment (Kimmerle and Eben, 1973). The presumption that two principal metabolites (TCA, TCE) are direct products of a common precursor (chloral hydrate) lacks direct experimental support.

In man the most abundant metabolic product is TCE (about 50%), then TCA (about 19%), and MCA (about 4%) (Soucek and Vlachova, 1960). The ratio of the two principal metabolites, TCE/TCA, disputed at length in the literature, varied in individual reports from 2.6 (Soucek and Vlachova, 1960) through 2.1 (Bardodej and Krivucova, 1958) to 1.4 (Barbonicek, 1962). It seems that the principal source of discrepancy lies in the fact that frequently the ratio has been based on the concentrations of the metabolites

found in spot urine samples. This procedure seems to be inadequate because of the pronounced differences in the kinetics of excretion of TCE and TCA, leading to considerable variations of the ratio with time after cessation of the exposure. The ratio seems to be higher in males than in females (Nomiyama and Nomiyama, 1971).

Relatively copious data have been accumulated on the systemic behaviour of trichloroacetic acid. In guinea pigs, tissue distribution of this metabolite is relatively uniform with highest concentrations in the adrenals, spleen, and testes. Blood has been most extensively studied; however, the concentrations of TCA are not high relative to other tissues (Fabre and Truhant, 1952). Most of the blood TCA is found in the plasma (Soucek, 1955). In human beings the concentration ratio of plasma/red cells amounted to 4.8 (Bartonicek, 1962). It is a commonly accepted view that in plasma the compound is protein bound. Whether administered directly into the blood stream in the form of a sodium salt or originating metabolically from TRI, TCA shows the same systemic effect (Smith, 1966).

The biochemistry of TRI metabolism has not been fully clarified. Smith, (1966), relying on earlier studies of Butler (1949), expressed the view that the formation of TCA is not limited to one organ only. Fabre and Truhant (1952) found in their study on rats that the highest metabolic activity is displayed by the lungs and spleen. In dogs and rats, the formation of TCA is considerably diminished by the administration of tetraethyl thiuram disulphide (TETD) (Forssman et al., 1955). In humans, TETD inhibits the formation of both TCA and TCE, with TCE being least affected (Bartonicek and Teisinger, 1963). Intraveous administration of glucose and insulin enhances the formation of all TRI metabolites (Soucek and Vlachova, 1960). These facts point to a similarity of TRI metabolism with that of ethanol. It was found that alcohol dehydrogenase catalyzes one of the postulated metabolic steps, the transformation of chloral hydrate into TCE (Friedman and Cooper, 1960). The transformation of TRI into the former and of chloral hydrate to TCE (in presence of NADH) or to TCA (in presence of NAD), could be accomplished in post mitochondrial supernatants of hamster and rat liver. The Michaelis constants Km are: 10^{-2}M, 7 x 10^{-3}M; and 6 x 10^{-4}M for the transformations of TRI $\rightarrow$ chloral hydrate, chloral hydrate $\rightarrow$ TCA; and chloral hydrate $\rightarrow$ TCE, respectively (Ikeda and Immamura, 1973). It was also reported that the transformation rate of TRI can be enhanced by administration of phenobarbital, a well known inductor of microsomal enzymes (Leibman and Mac Allister, 1967). The values of Km did not change; only the maximal rate of reaction, as measured in vitro, was increased. Repeated exposure of animals to low TRI concentrations did not stimulate the metabolism; however, the stimulatory effect was seen after massive exposure (Ikeda and Immamura, 1973). The biotransformation of trichloroethylene is partly inhibited by the presence of toluene (Ikeda, 1974).

ELIMINATION

Reviewing the early studies by Czech authors, Teisinger and coworkers (1956) concluded that, in human beings, after cessation of a single exposure to TRI vapours, its metabolites are excreted in the following fashion: unaltered TRI mainly in the expired air (19%) and only traces in urine (up to 0.6%); TCA, TCE and MCA leave the body in the urine in amounts of about 16, 35 and 3-6 per cent of the retained amount, respectively. With considerable delay chloroform appears in the exhaled air, in varying amounts, reaching in some cases 15 per cent of the TRI on a molar basis. This efficiency of elimination displays wide variation, and thus the numerical values reported from various laboratories differed considerably. Due to prolonged excretion of TRI metabolites and the varying time of observation, the reported values are not always directly comparable. Bartonicek (1962) concluded that other routes of elimination of TRI metabolites (e.g. faeces, sweat, saliva) are of secondary importance.

The kinetics of elimination of TRI on one hand, and of its metabolites on the other, show basic differences. Unchanged TRI is eliminated directly in exhaled air after discontinuation of exposure, and its concentrations drop fast with time. From the data of Stewart et al. (1962, 1970), it follows that the disappearance rate of TRI from the exhaled air varies with the duration of exposure, the rate being inversely correlated with the duration. The elimination curves are multiexponential, and therefore the half-time, as determined, depends upon the interval when the measurements are made: over the first two hours after cessation of an exposure of 1 to 4 hours, the half-time is of the order of 0.5 hour; after longer exposures and determined later, it increases up to 15 to 20 hours. Up to 2.5 to 5 hours after discontinuation of exposure, other authors reported curves with two exponential

terms. The half-time at the beginning of observation was of the order of 0.5 hour, increasing to 1 to 2 hours toward the end of the period (Klyin et al., 1967; Nomiyama and Nomiyama, 1971; Kimmerle and Eben, 1973 b).

TRI metabolites excreted in the urine reach their maxima at different times after discontinuation of the exposure. The kinetics of their disappearance vary considerably with the metabolite. The concentration of monochloroacetic acid reaches a maximum directly after cessation of TRI inhalation and decays relatively fast; the half-time amounts to about 15 hours. The maximal concentrations of trichloroethanol are observed several hours later and their decay is biphasic: at first a half-time of 24 hours predominates, later a half-time of 40 hours is observed. Trichloroacetic acid reaches a maximum only after 1 to 2 days, and the biphasic decay follows with half-times of 50 and 70 hours (Soucek and Vlachova, 1960). Similarly Ikeda et al. (1971) observed kinetics of elimination of the metabolites which may be characterized with the following two half-times of urinary concentrations: TCE = 6 and 40 hours; TCA = 25 and 85 hours. The data reported by other authors seem basically consistent with the above picture (Bartonicek, 1962; Stewart et al. 1970). The kinetics of elimination of TRI and its metabolites is schematically presented in Figure 15-2; a multi-compartment model has been also presented that could form a basis for a mathematical description of the metabolic kinetics of TRI. As a matter of fact, the complexity of the model may be advocated to explain why the elimination processes have never been described as a whole, and individual authors have limited their description only to the presentation of the half-times. Regardless of its basic relation to material truth (existence of two intermediate metabolic precursors of TCE and TCA is assumed), the model could be solved only with the use of a computer.

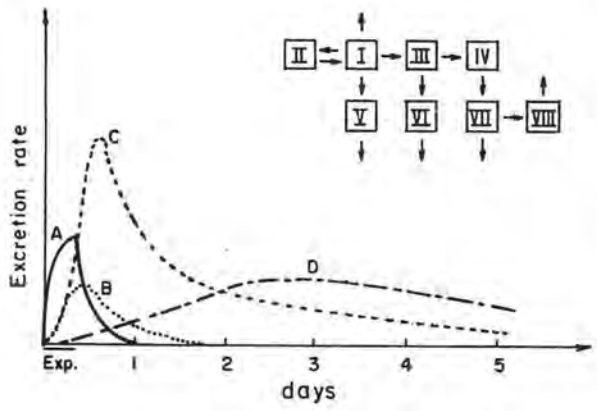

Fig. 15-2. Schematic presentation of the kinetics of excretion of trichloroethylene and its metabolites, following single exposure. Lower diagram: A — trichloroethylene in expired air, B — MCA, C — TCE, D — TCA, all in urine. Upper scheme — the corresponding model, leading to excretion of: trichloroethylene (I), MCA (V), TCE (VI), TCA (VII) and chloroform (VIII).

Approximate analysis of the kinetics of elimination of TRI metabolites, as postulated by the model, makes it possible to predict some phenomena. For instance, after a single exposure the ratio of excretion rates of the two principal metabolites, TCE/TCA, should be a steeply decreasing function of time. In fact, Nomiyama and Nomiyama (1971) found that the ratio declined from about 30, directly after exposure, to a value of 0.2 several days later.

Applying a simple principle of graphic summation (as proposed by Soucek and Pavelkova, 1953), it may be predicted that, after daily repeated exposures, the level of TRI metabolites in biological materials will be the greater 1) the longer the time required, after a single exposure, for the maximum concentration to appear in the material; and 2) the slower is the rate of decay of that concentration. Experimental data indicate that in man, trichloroethylene itself is not subject to significant cumulation (Stewart et al., 1970; Kimmerle and Eben, 1973). The theoretical considerations of Ikeda and Immamura (1973) postulated a 2 weeks' period of repetitive exposures for reaching a steady-state; this seems exaggerated due to assumed arbitrary values taken for the computation. A significant cumulation is seen for TCE and TCA. Of course, this must hold whether the predictions are based on urinary or blood concentrations. As might have been predicted, the concentration ratio TCE/TCA decreases gradually with time.

The experimentally determined growth of TCE urinary concentration over 5 consecutive exposure days was not appreciable: about 60 per cent relative to day 1, and a steady-state was reached after 3 days. TCA concentrations in the urine rose almost linearly with the duration of exposure, reaching a level 7–12 times higher than those on the first days; no steady-state was reached after 5 days. In the same period the TCE/TCA concentration ratio decreased from about 10 to 2 (Ertle et al., 1972; Fig. 15-3). A similar picture was seen by Muller and coworkers (1972),

who measured the concentrations in blood (Fig. 15-4). The difference in excretory kinetics of the two metabolites, TCE/TCA ratio may be used to evaluate the character of exposure and the assessment of the time elapsed between cessation of the exposure and sampling of the urine. Under conditions of repetitive exposure the steady-state is represented by a TCE/TCA ratio of 1.5–3.0. Values substantially higher represent early periods after single exposures; values of the ratio below 1 are obtained several days after discontinuation of the exposure. Apart from this potential usefulness, the differences in the elimination kinetics of TCE and TCA complicate the picture significantly. However, it is interesting to note that the problem may be considerably simplified if only a sum of all trichlorocompounds in the urine is determined. From the data of Nomiyama (1971, Fig. 15-5), it follows that after a single exposure the excretion rate of trichloroderivatives in the urine declines monoexponentially with a half-time of about 1 day. On the other hand, Ikeda et al. (1971) reported that, after a single inhalatory exposure, the maximum excretion rate of total

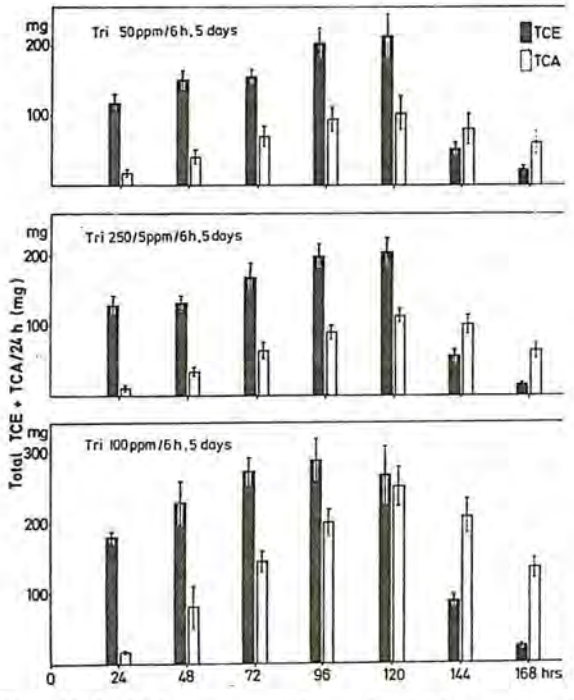

Fig. 15-3. Urinary excretion of trichloroethanol and trichloroacetic acid in daily intervals (mg/24 hr) following repeated exposure on 5 consecutive days (Ertle et al., 0972).

Taken from: Ertle T., Henschler D., Muller G., and Spassovski M.: *Metabolism of trichloroethylene in man.* **Arch. Toxikol. 29,** 171-188, 1972. Page. 179, Fig. 2.

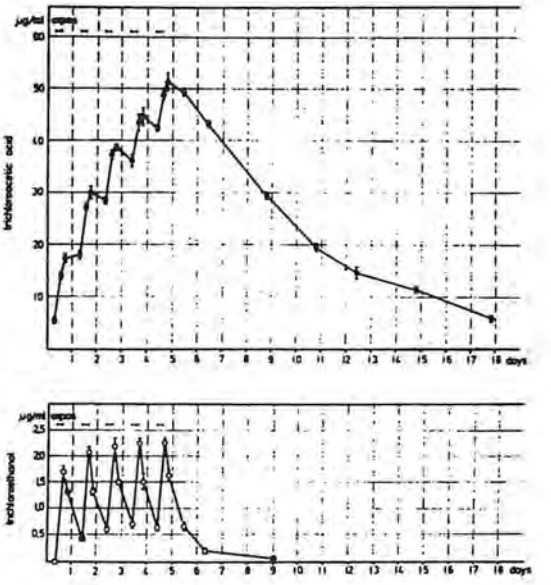

Fig. 15-4. The increasing trends of TCA in plasma (upper graph) and TCE in whole blood (lower graph) in volunteers exposed to trichloroethylene for 5 consecutive days (Muller et al., 1972).

Taken from: Muller G., Spassovski M., and Henschler D.: *Trichloroethylene exposure and trichloroethylene metabolites in urine and blood.* **Arch. Toxikol. 29,** 335-340, 1972, Page 337, Fig. 1.

trichlorocompounds appeared 2 to 4 hours after discontinuation of inhalation; over the first 24 hours the concentration declined with a half-time of about 8 hours. The same authors, however, observed a patient hospitalized several days after intoxication; the decline of excretion of total trichlorocompounds could be characterized by a half-time of 3 to 4 days.

Ikeda and Immamura (1973), summarizing the data of various authors, concluded that the half-time of total trichlorocompounds in urine, when studied on volunteers under controlled conditions, was bracketted by the values 31 to 50 hours. The same parameter, when studied on workers under industrial conditions, fell in the range of 26 to 50 hours. The mean for both groups was close to 40 hours.

METHODS FOR DETERMINATION OF TRI AND ITS METABOLITES

Trichloroethylene. In earlier studies TRI was determined in expired air and in biological materi-

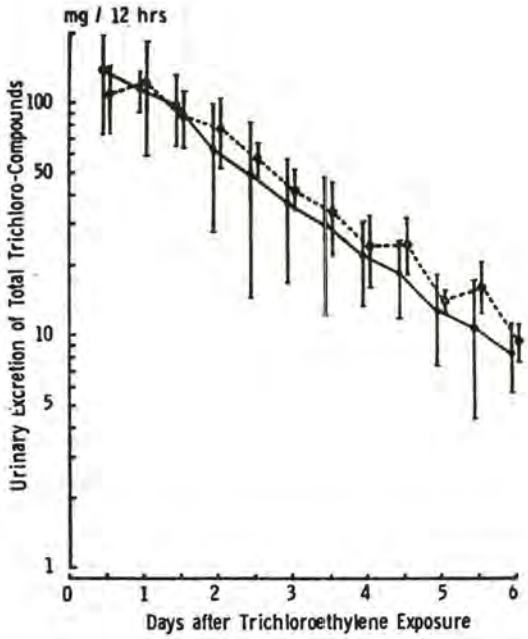

Fig. 15-5. Urinary excretion of total trichloro-compounds following single short exposure to trichloroethylene (Nomiyama, 1971).

Taken from: Nomiyama K.: *Estimation of trichloroethylene exposure by biological materials.* **Int. Arch. Arbetismed. 27,** 281-292, 1971, Page 285, Fig. 2.

als (blood, urine) after distillation or aeration, followed by absorption in pyridine, with subsequent color reaction of Fujiwara. The earlier methods have been reviewed by Smith (1966). Teisinger et al. (1956) recommended the method as presented by Soucek and Franklova (1952). Ehrner-Samuel (1963) proposed a determination in blood by extraction with isooctane, with subsequent gas chromatographic measurement (nitrogen, electron capture detector). Gas chromatography in different modifications has been used in more recent reports (Kylin et al., 1967; Ertle et al., 1972; Stewart et al., 1962, 1964, 1970). Stewart et al. also used infrared spectrophotometry (λ = 11.78 μ in gas-cuvettes of 10 m light-path length).

Trichloroacetic acid. The usually applied colorimetric method makes use of Fujiwara's colour reaction, performed by heating the alkaline pyridine extract. Teisinger et al. (1956) recommended the method in the version of Soucek and Franklova (1952). The same, or a very similar modification, has been used by Bardodej and Vyskocil (1956), Soucek and Vlachova (1960), Bartonicek (1962), Kundig and Hogger (1970), and Laham (1970). In the most recent studies the same method was used in the modification of Tanaka and Ikeda (1968) (Ikeda et al., 1971; Ertle et al., 1972; Kimmerle and Eben, 1973). Ehrner-Samuel et al. (1973) recently proposed a gas-chromatogrphic method for TCA determination in urine. A toluene extract of urine containing TCA is methylated using borontrifluoride-methanol reagent, and TCA methyl ester is then determined with a Varian gas chromatograph electron capture detector. The method is suitable for serial determinations, and its level of detection has been evaluated at about 3 μg/ml.

Trichloroethanol. In most studies a modified method has been used: TCE present in urine in the conjugated form with glucuronic acid is hydrolyzed by prolonged heating with a strong acid in a sealed glass ampoule. Trichloroacetic acid is then removed from the solution on an ionic exchanger and TCE is oxidized to TCA by means of dichromate and then determined. A version of this procedure as described by Vlachova (1957) has been recommended by Teisinger et al. (1956), Dutkiewicz et al. (1964), and by Gadaskina and Filov (1971).

Bardodej (1962) proposed a method according to which TCE is steam-distilled and determined colorimetrically (240 nm.), without oxidation, after reaction with pyridine and alkali. For tissue assays Cabana and Gessner (1967), and for urine Ogata et al. (1970), conducted the reaction with alkali and pyridine without separation of the metabolites. TCE and TCA concentrations are calculated after measurements at two wave-lengths (440 nm and 530 nm). Vlachova (1956), Seto and Schultz (1956), and Tanaka and Ikeda (1968) conducted two parallel determinations: TCA directly and a sum of trichlorocompounds after hydrolysis and oxidation. TCE was calculated from the difference.

Sedives and Flek (1969) proposed TCE determination in urine by means of gas chromatography with flame ionization detection. Eben developed a method which was applied later by Ertle et al. (1972) in which TCE is determined using gas chromatography with an electron capture detector. For acid hydrolysis of urochloralic acid several authors (Ogata et al., 1970; Ertle et al., 1972; Kimmerle and Eben, 1973) substituted enzymatic hydrolysis using -glucuronidase.

Total trichlorocompounds (TTC). The principle of this method for TTC consists of hydrolyzing the sample, with subsequent oxidation and utilization of the Fujiwara reaction as described above for trichloroethanol. Recently, Imamura and Ikeda (1973) proposed a modification of the method for rapid determination: a sample of urine is heated with CrO_3 in HNO_3, alkalised after cooling, and pyridine added. Short heating in a boiling water bath then follows, with extinction measurement at 530 nm.

EXPOSURE TESTS

Introductory remarks. It seems that a practical importance should be ascribed to the following determinations: 1) TRI in expired air, and also 2) TCE, 3) TCA and 4) TTC in urine. The concentration of TRI itself in exhaled air may provide information on the magnitude of exposure on the day of sampling; urinary TCE should give an evaluation of the mean exposure over the last 2-3 days; and finally, TCA and TTC in urine will provide information about the averaged exposure in the period of preceeding week or more. Although the data regarding the kinetics of TRI elimination in the exhaled air are not fully consistent, it seems that those obtained in controlled human experiments (single exposure) may serve as a basis for interpretation. When urinary TCE is considered, the data obtained by those authors who had carried out experimental inhalations studies on human volunteers, over several consecutive days until the steady-state was reached, may be utilized. For TCA and TTC in urine, it should be noted that the exposures have not been continued for a sufficiently long period in any of the experiments. In this case the urinary levels reflecting steady-state may be obtained from field studies (bearing in mind the lack of precision in assessing the real concentrations to which workers have been exposed over preceeding period of some 10-20 days), or by means of calculation from kinetic extrapolation. A basis for the latter was created for TCA in urine by Soucek and Pavelkova (1953).

Finally for TTC, due to relatively simple kinetics of its excretion, the sum of TCA + TCE data for the steady-state may be computed from kinetic equations, assuming an excretion half-time of about 40 hours.

TRI in expired air. For evaluation of exposure, determination of TRI in expired air sampled after inhalation has been recommended by Boettner and Muranko (1969), Stewart et al. (1970), Nomiyama (1971), and by Pfaffli and Backman (1972). All these authors have applied gas chromatography for analysis of the air sampled into Saran bags. The proposal regarding interpretation of the results put forward by Nomiyama (1971) and its rationale, as understood by the present author, may be presented as follows. In its simplest form, the concentration of TRI in expired air, as a function of time after discontinuation of exposure, may be presented by a curve as in Figure 15-6. Immediately after cessation of TRI inhalation, the concentration forms a given fraction of that in the inspired air during exposure and decays relatively fast. After a short time this rapid elimination ceases and then follows a period of slower elimination with concentrations following an exponential function.

If the intercept of this second exponential at t = 0 is denoted as "b", and the concentration of the inhaled TRI as "a", and assuming b:a = constant, we obtain:

$$C_{et} = \frac{b}{a} C_i \, e^{-kt} \qquad (1)$$

The purpose of the assay is to evaluate the concentration C_i from C_{et}. After transformation of equation (1) one obtains:

$$C_i = \frac{a}{b} C_{et} \, e^{kt} \qquad (2)$$

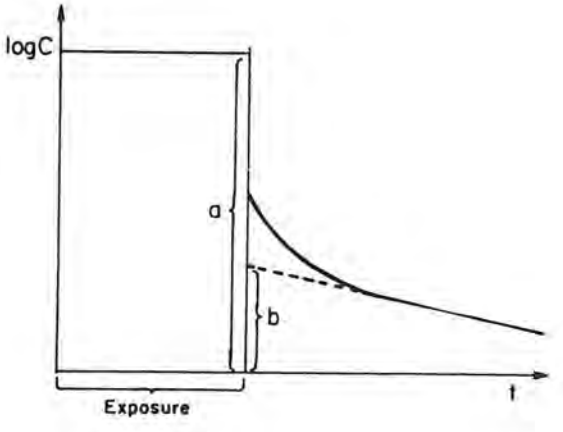

Fig. 15-6. The principle underlaying evaluation of exposure to trichloroethylene from its level in expired air, in the procedure of Nomiyama (1970).

According to recalculated data of Nomiyama (1971), a/b = 12.5 and k = 0.3 h^{-1}.

The information assembled by Nomiyama on the comparison of the calculated values of C_i (equation 2) with those directly determined are presented in logarithmic coordinates in Figure 15-7. From the graph it follows that: i) existing data cover only the range of high concentrations above 100 ppm; ii) the method yields calculated values of C_i with an uncertainly factor of 4 (i.e. the concentration as determined may actually lie in the range from 100 to 400 ppm).

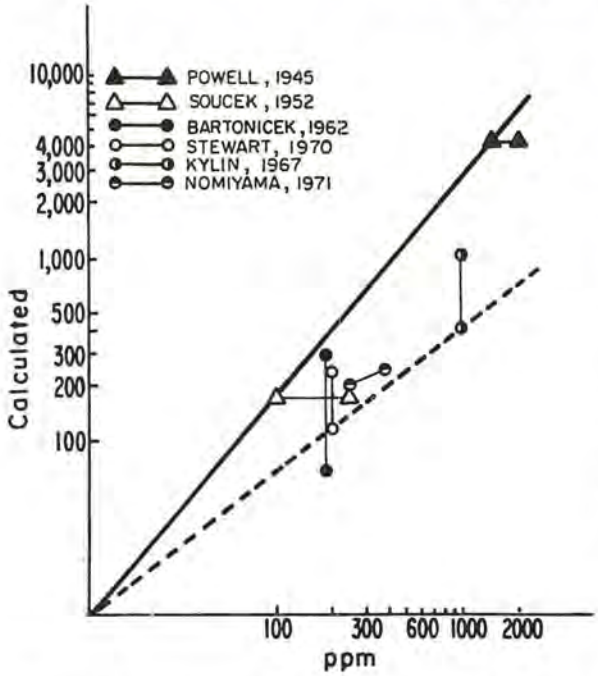

Fig. 15-7. Evaluation of the Nomiyama's method of exposure evaluation from expired air analysis by comparison of respective data of various authors.

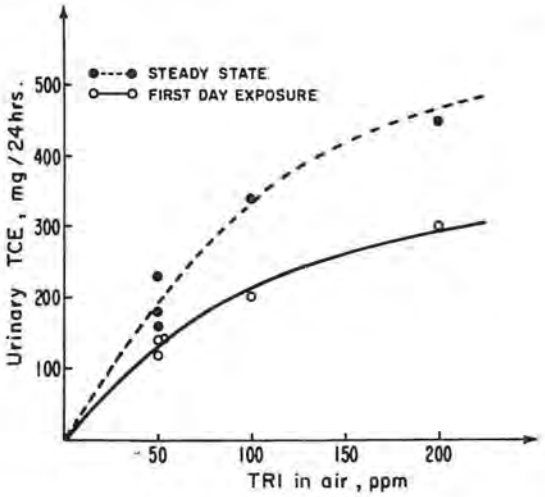

Fig. 15-8. Urinary excretion of trichloroethanol following one day and 3-5 days exposure, as dependent on the concentration of trichloroethylene in the air. Data of various authors standardized for 7-hrs exposure duration.

To improve the interpretation of this exposure test will require more experimental studies. It would be desirable to refrain from too much sophistication in designing th experiments, because exhalation of TRI must be a composite function of many variables. For practical purposes experiments will suffice in which a wide range of low TRI concentrations in the air are used, with constant duration of exposure (e.g., 7 hours corresponding to the effective duration of an industrial shift) and constant sampling time, sufficiently early (for instance 1 hour after the end of shift) to render the test feasible without retaining a worker for too long in the factory after work. Final interpretation could be worked out directly from empirical data.

Trichloroethanol in urine. Several authors have recently conducted experiments on TCE excretion in urine in subjects exposed daily until a steady-state in the excretion rate was reached (Stewart et al., 1970; Ertle et al., 1972; Muller et al., 1972; Kimmerle and Eben, 1973). TCE has been determined in 24-hours samples of urine, but this is not practical under industrial conditions. In the experiments reviewed here, the duration of daily exposure varied from 4 to 7 hours. The data were normalized for 7 hours of exposure assuming this is the effective duration of exposure during a normal 8 hours working shift. All the data reported in the papers cited above consistently indicate that TCE rises through the 5th day of exposure, tapering off thereafter. To calculate the steady-state values, the means of the excretion rate were determined on days 3 through 5. The results are presented in Figure 15-8. It appears that the relation between the magnitude of exposure and the daily urinary excretion of TCE shows a satisfactory precision. The mean of all individual relations may be recommended for practical purposes. It should be noted that the relationship (exposure — excretion) seems to be curvilinear; this may point to the fact that the proportion of TCE in all TRI metabolites diminishes with increasing absorbed dose.

The data of Muller et al. (1972) on the TCE levels in the blood (Fig. 15-4) suggest a rather fast

increase and decay of TCE in blood, related to the rhythm of exposure. It should be expected, therefore, that similar fluctuations might be seen in the urine. A given daily urinary excretion of TCE might be accompanied by considerable variation of the concentrations in individual fractions of urine, the maximum occuring directly after discontinuation of the exposure and the minimum in the morning of the following day. Ogata et al. (1971) found that at the end of exposure to concentrations of 100 ppm of TRI in air, the excretion concentration of TCE in urine was 530 mg/l.

Trichloroacetic acid in urine. Attempts to use TCA in urine as a measure of exposure to TRI were made in the late 1940's utilizing empirical correlations between TRI concentration in air on the one hand, and urinary TCA on the other. These early studies were reviewed by Bardodej and Vyskocil (1956), Teisinger et al. (1956), and Dutkiewicz et al. (1964). It is accepted that both variables are linked by an approximately linear correlation, represented by a line on a graph (Fig. 15-9) as proposed by Bardodej and Krivucova (1955). Assuming linear proportionality, a concentration of TRI in the air of 0.4 mg/l should be followed by mean concentrations of TCA in urine of: 140 mg/l (Bardodej and Krivucova, 1955), 150 mg/l (Frant and Westendorp, 1950), and 220 mg/l (Friberg et al., 1953; Grandjean et al., 1955). Interpretation of the test, as accepted in practice, is usually based upon the data of Bardodej and Krivucova, (1955).

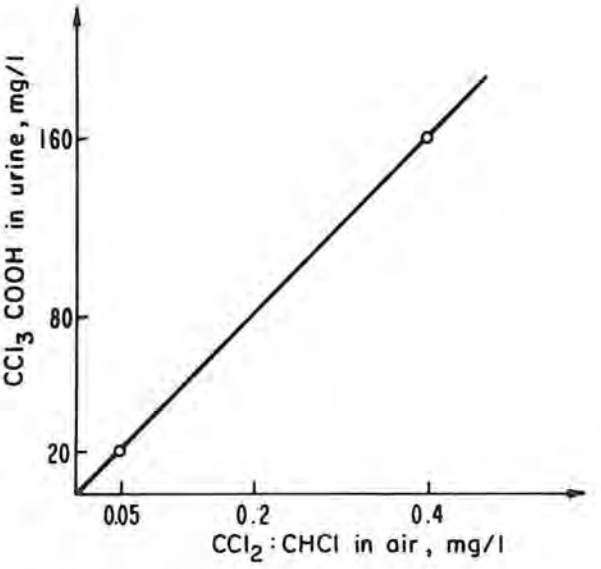

Fig. 15-9. TCA concentration in urine as dependent on the trichloroethylene concentration in air (Bardodej and Krivucova, 1955).

The above data, obtained from field studies, have not been verified in controlled experimental studies. Stewart et al. (1970), Ertle et al. (1972) and Kimmerle and Eben (1973) have studied TCA excretion in the urine of volunteers exposed for 5 consecutive days. The results (Fig. 15-3) indicated a linear progression of the concentrations with duration of the studies. It should be expected, therefore, that steady-state values must be appreciably higher. Nonetheless, by the 5th day of daily exposure, the authors found high urinary levels of TCA. Normalizing the data in such a way that they corresponded to a 7 hour exposure to TRI at 0.4 mg/l, and assuming a mean daily volume of urine of 1.2 l., the resulting TCA concentrations would be directly comparable with those obtained from the field studies. The data were: according to Stewart et al. 130 mg/l; according to Ertle et al., in a different series, from 160 to 190 mg/l. In general, the TCA concentrations exceeded those reported by Bardodej and Krivucova (1955) even if the steady-state had not been reached. The problem certainly calls for further experimental investigations. At the same time it should be mentioned that urinary concentrations, as seen in field studies, may be either higher than in a laboratory experiment due to greater lung ventilation, or lower as a result of shorter effective real exposure as compared with the product of official working time and average concentration at a given work site. The comparison as presented above might be interpreted as suggesting that these differences in actual exposure could have led to the discrepancy. On the other hand, too little is still known about possible changes in the metabolism of TRI in the course of prolonged exposure to exclude the possibility of lowered efficiency with which TCA is produced in the body, as was suggested by Bardodej and Krivucova in 1955 and supported recently by Ikeda et al. (1972). It should also be recollected that Grandjean et al. (1955) pointed to a phenomenon that the ratio U/A (U = TCA concentration in urine, mg/l; A = TRI in the air, ppm) is related to age, varying from the value of 6 in young subjects to only 2 in elderly people. A sex effect on TRI metabolization to TCA also appears to exist, as the ratio TCE/TCA, was higher by a factor of 2 in males (Nomiyama and Nomiyama, 1971). Assuming that reasons for the discussed discrepancy should be sought in variation of TRI metabolism in the body (variation of proportions of TCE and TCA in the total

pool of metabolites), a more universal approach to the problem would consist of a shift to determination of the sum of trichlorocompounds, as suggested by Nomiyama and Nomiyama (1971).

TOTAL TRICHLOROCOMPOUNDS (TTC)

The sum represents the total of TCA and TCE, and thus the bulk of TRI metabolites in urine. The metabolic yield of TTC does not depend on sex, and the kinetics of excretion may be expressed by a single exponential equation (Nomiyama and Nomiyama, 1971). Utilizing TTC for the evaluation of exposure to TRI has been suggested by numerous authors, namely: Bardodej and Krivucova (1958), Medek (1958), Tanaka and Ikeda (1968), and Nomiyama (1971). Interpretation of the test is based on the U/A ratio being equal to 6 (U = TTC in urine; mg/l; A = TRI in the air, ppm). Due to the simple characteristics of the excretion, the results may be easily interpreted even if the determinations are made several days after cessation of the exposure, assuming an average half-time of TTC excretion of 40 hours (see "Elimination").

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

There are voluminous data on industrial exposure, obtained utilizing the exposure tests, mainly of TCA excretion. Apart from the papers cited above there should be included the reports of: Ahlmark and Forssman (1961), Waelschova (1954), Teisinger et al. (1955), Hickish et al. (1956), Medek (1958), Sukhotina (1969), Kundig and Hogger (1970), Ikeda et al. (1972), Rosowski et al. (1974). The information relates to the exposure in the course of TRI production, and various applications of the substance, mainly in dry cleaning and degreasing of metals. Concentrations or daily excreted amounts of TCA in urine have encompassed a wide range of values, from those close to zero up to 300–400 mg/l; and in earlier studies even up to 700-1000 mg/l.

One of the problems very often discussed in earlier studies was the evaluation of TCA determinations in urine for the diagnosis of chronic TRI intoxications. From the onset opinions were divided. Frant and Westendorp (1950) were unable to find a significant correlation between TCA level and intensity of the intoxication symptoms. However, it has been claimed that in most workers exposed for a prolonged period to TRI and displaying urinary concentrations of TCA above 150 mg/l, symptoms of chronic poisoning were present. Frequency of the symptoms were significantly increased at TCA concentration above 20 mg/l, a value which has been recommended as safe. It is interesting to note that this value corresponds to the air concentration of TRI = 0.05 mg/l, which is accepted as the maximum acceptable one in the Soviet Union.

Although at present the use of urinary TCA as an individual diagnostic test is not being discussed, the accumulated data proved that TRI exposure, reflected by urinary TCA excretion at concentrations of more than 100 mg/l, may lead after prolonged exposure to symptoms accepted at present as signs of deleterious action of TRI on the human organism (Bardodej and Byskocil, 1956; Grandjean et al., 1955; Waelschowa, 1954; Ertle et al., 1972).

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16. TETRACHLOROETHYLENE

ABSORPTION

Pulmonary absorption constitutes the main route of entry for tetrachloroethylene into the body. Under experimental conditions in man, it was found that at the beginning the retention of tetrachloroethylene in the respiratory tract amounted to about 74 per cent; this decreased slightly in the course of the exposure and after 2 hours stabilized at 62 per cent (Bolanowska and Golacka, 1972). These values are close to those observed in mice given the ^{14}C-labelled compound by inhalation (70 per cent, Yllner, 1961). Tetrachlorethylene undergoes cutaneous absorption if applied as a liquid to the skin (Stewart and Dodd, 1964). It seems that systemic absorption of the vapours through the skin has not been studied.

METABOLISM AND ELIMINATION

From animal experiments it appears that tetrachloroethylene is metabolized only to a very limited degree, being mostly eliminated from the body unaltered in the breath. Daniel (1963) administered ^{36}Cl-tetrachloroethylene orally to rats and recovered 98 per cent of the radioactivity in the exhaled air. The data reported by Dmitriewa (1967) and Yllner (1961) indicated that elimination via the respiratory tract accounted for only about 70 per cent of the total. Among the metabolites observed in animals were: trichloroacetic acid and ionic chlorine (Daniel, 1963), and ethylene glycol and oxalic acid (Dmitriewa, 1967). Both authors maintained that chloral hydrate should not be listed among the intermediate metabolic products (see: "Trichloroethylene").

The metabolism of tetrachloroethylene in man is poorly understood. Judging from the 60 per cent steady-state respiratory retention, Bolanowska and Golacka (1972) presumed that this is the percentage that undergoes biotransformation in the body. On the other hand, excreted trichloroacetic acid, which has attracted most of the attention, accounted only for few per cent of the systemic retention. Two per cent of the inhaled tetrachlorethylene was excreted as TCA in the urine over a 67 hours collection period. There are no human data on other metabolites.

Elimination of tetrachloroethylene in the breath may be related to the blood level. The coefficient of distribution blood/air, studied *in vitro*, assumed a value of about 9 (Morgan et al., 1970). The disappearance rate of tetrachloroethylene from the blood is quite high; 30 minutes after discontinuation of inhalation the compound could not be detected. On the other hand, due to the sensitivity of the determination, elimination of the unchanged compound in the expired air could be followed for prolonged periods (Stewart et al., 1961). Stewart et al. (1970) followed the elimination of tetrachloroethylene for about 100 hours in human subjects after a single exposure, and for about 330 hours after repeated daily exposures for 5 days (about 100 ppm, 7 hours a day).

From the latter data it follows that, over the first few days, the compound disappears from the breath with a half-time of about 70 hours. Bolanowska and Golacka (1972), who have analyzed the elimination curve more closely for 40 hours after cessation of inhalation, found 4 contributing exponential terms in the equation for expressing disappearance in the expired air, of which the fastest one could have been neglected due to its quantitatively minor role.

The elimination equation, related to the intercept at time t = 0 (the first very fast term neglected), assumes the form:

$$E_t/E_o = 0.61\ e^{-1.2t} + 0.25\ e^{-0.14t} + 0.14\ e^{-0.02t} \quad (1)$$

where E_0 = concentration of tetrachloroethylene in breath at discontinuation of inhalation, E_t = the same concentration at time = t_0 (hours).

The corresponding half-times are 0.6, 4.8, and 34 hours. After repeated exposure, some increase of tetrachloroethylene concentration in the breath is observed (Fig. 16-1, Stewart et al., 1970). Bolanowska and Golacka (1972), who evaluated

the phenomenon from a kinetic viewpoint, concluded that the maximal increase above the concentration on day 1 should be in the order of 20 per cent.

However, the metabolites of tetrachloroethylene, excreted in the urine, should most likely display a considerable cumulation in the course of repeated exposures. Ikeda and Immamura (1973) observed that the half-time of urinary excretion of total trichlorocompounds in workers exposed to tetrachloroethylene is twice as long as that after inhalation of trichloroethylene, and amounts to 144 hours. Tada (1969), on the other hand, found maximum urinary levels of trichloroacetic acid between days 3 and 5 in human subjects exposed, under controlled experimental conditions, to tetrachloroethylene at concentrations of 50-160 ppm for 4-6 days. This picture did not differ from similar observations for trichloroethylene exposure. From the work reviewed, however, it seems rather unlikely that TCA levels in the urine can cumulate so as to reach the order of magnitude characteristic for exposure to trichloroethylene.

METHODS FOR DETERMINATION OF TETRACHLOROETHYLENE AND ITS METABOLITES

The methods based on the Fujiwara reaction are not suitable for tetrachloroethylene determination, because the sensitivity of the reaction is 20 times lower than for trichloroethylene (Lugge, 1966).

Recently, for the analysis of air in toxicological chambers and in human breath, either direct infrared absorptiometry in gasphase in 10 m long cuvettes (Stewart et al., 1961; Stewart et al., 1970) or, more often, gas chromatography has been applied. In the latter case, the selection of a flame ionization detector has been most usual (Stewart and Dodd, 1964; Stewart et al., 1970; Ogata et al., 1971); less frequently electron capture (Stewart and Dodd, 1964); and sometimes even argon ionization detectors (Golacka and Bolanowska, 1972) were in use. For breath sampling glass pipettes, Saran sacks (Stewart et al., 1970), or sacks made of aluminum foil coated with polyethylene (Golacka and Bolanowska, 1972) were employed.

DETERMINATION OF TRICHLOROACETIC ACID (TCA) AND OF TOTAL TRICHLORO-COMPOUNDS (TTC)

See section on "Trichloroethylene".

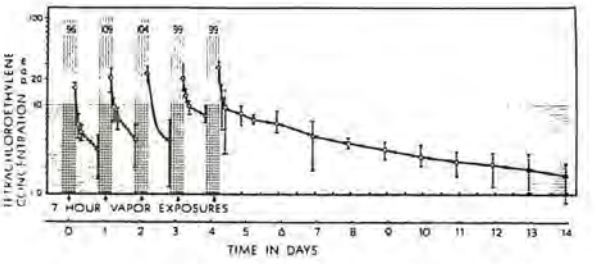

Fig. 16-1. Breath concentrations of tetrachloroethylene in the post-exposure period on successive days of repeated exposure (Stewart et al., 1970).

Taken from: Stewart R.D., Baretta E.D., Dodd H.C. and Torkelson T.R.: *Experimental human exposure to tetrachloroethylene.* **Arch. Environ. Health 20,** 224-229, 1970. Page 228, Fig. 2.

EXPOSURE TESTS

Attempts to use TCA or TTC in urine for the evaluation of systemic tetrachloroethylene absorption were made by several authors — however, with negative results (Friborska, 1969; Tada, 1969; Kundig and Hogger, 1970; Ogata et al., 1971; Bolanowska and Golacka, 1972). Ikeda and Ohtsuji (1972) demonstrated that relative to trichloroethylene, when calculated per total amount of excreted trichlorocompounds (TTC), tetrachloroethylene yields only 8 per cent of the amount of Fujiwara reaction positive compounds.

Therefore, at similar conditions of experimental exposure (200 ppm) to both the compounds, the TTC urinary concentration for tetrachloroethylene is lower by an order of magnitude. Moreover, at concentrations of the latter in air above 100 ppm, the TTC urinary level becomes stabilized at relatively low values of about 50 mg/l.

Under these conditions, growing importance should be attributed to the attempts to base the exposure test upon tetrachloroethylene determination in the breath. From the data of Stewart et al. (1961), it may be concluded that, at a given time after discontinuation of inhalation, the concentration of tetrachloroethylene in the breath may be related both to the concentration in the inhaled air and to the duration of the exposure. Directly after cessation of a TCE inhalation of 7 hours' duration at the concentration of 100 ppm, the concentration in the breath is of the order of 20 ppm; slightly less after the first, and mod-

erately above this value after exposure on subsequent days (Stewart et al., 1970). However, as may be judged from Figure 16-1, in time intervals that are suitable for performance of exposure tests, the magnitude of exposure could not be deciphered with reasonable precision from breath concentrations of tetrachloroethylene. From the data of Bolanowska and Golacka (1972), it could be calculated that 15 minutes after discontinuation of 6 hours' exposure to the vapours at a concentration of 380 mg/m^3, the exhalation rate is of the order of 15 mg/hour.

The data referred to above provide general answers to the basic questions and give guidance to further studies aimed at development of a test. However, they do not form as yet a basis for quantitative evaluation of exposure.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

Due to poorly advanced exposure tests for tetrachloroethylene, there are no data available from field studies that would contribute significant information to the subject.

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17. CARBON DISULPHIDE

ABSORPTION

Carbon disulphide is absorbed into the system by all routes; however, under conditions of industrial exposure the inhalatory route is of primary importance. Due to rapid saturation of the blood, the retention of carbon disulphide vapours in the respiratory tract decreases rapidly with the duration of inhalation, reaching a steady-state level after about 3 hours. According to the data of Teisinger, Soucek, and coworkers (Teisinger et al., 1956), the initial and steady-state values of retention amount to about 80 and 15-40 per cent, respectively. Later investigations of Demus (1964) and Jakubowski (1966) yielded the corresponding values of 30-60 and 35-50 per cent. The compound in fluid form, as well as its water solutions, are absorbed through the skin. This may be of practical importance in viscose rayon spinning mills (Baranowska, 1965). In the rabbit, dermal absorption of carbon disulphide vapours was demonstrated by Cohen et al. (1958) and Petrun (1967); in man this route seems of little, if any, significance (Baranowska, 1965).

METABOLISM AND EXCRETION

A large proportion of absorbed carbon disulphide is eliminated unchanged in the expired air. The data from animal experiments vary widely, depending on the species studied, the dose, and the route of administration. The extremes range from almost 100 per cent (rats, intragastric administration in doses of 80 and 200 mg per kg; Soucek, 1959) to 13-25 per cent (mice, studies using ^{35}S-labelled compound; Strittmatter et al. 1950). Data obtained from human inhalation experiments point to a considerable recovery of CS_2 in expired air in the period of desaturation. Quantitative assessment of the magnitude of this fraction is subject to discussion because of the difficulty in defining the absorbed dose in inhalation experiments which last for several hours, when the low "retention" may be partly accounted for by the parallel elimination of the CS_2 via the same route. Thus, in early Czechoslovakian publications, the amount of CS_2 exhaled, relative to net retention in the body, varied from 5 to 23 per cent of the dose (Teisinger and Soucek, 1949; Soucek and Pavelkova, 1953; Soucek, 1959). A less disputable assessment was obtained from studies with short inhalation periods (0.5-2 hours), in which the average exhalation was about 23 per cent (Harashima and Masuda, 1962). The process in itself is quite rapid; the half-time of disappearance from rat tissues is 0.5-1.25 hours (Freundt and Schnapp, 1970), that of exhalation in rats is about 1.4 hour (Soucek, 1959), and in humans less than 1 hour (Demus, 1964). These data relate to the main phase of elimination and disregard the very fast decline in concentration of CS_2 in expired air seen directly after discontinuation of the exposure. A later, very slow phase of elimination can be demonstrated which most likely should be linked with liberation of CS_2 from adipose tissue and from the so-called "bound carbon disulphide".

In urine, carbon disulphide is found in minor quantities (less than 1% of absorbed dose) in the free and bound form (subject to aeration from acidified urine) (Bartonicek, 1958); the unbound fraction amounts to about 30 per cent of the total urinary amount (Demus, 1964).

From these data it follows that about 25 per cent of the absorbed CS_2 is eliminated unaltered, and about 50 percent is metabolized. The intermediate products of the biotransformation consist most probably of compounds resulting from CS_2 reactions with proteins, peptides, and amino acids. The latter have been studied *in vitro* and *in vivo*, and were identified as dithiocarbaminocarbonic acids (Soucek and Madlo, 1956). From reactions with peptides, following cyclization, compounds of the thiazolidone type are produced. (Cohen et al., 1959).

The globulins display little reactivity with CS_2, whereas albumins form relatively stable compounds possessing free-SH groups, which in contrast to dithiocarbaminocarbonates do not liberate CS_2 in acid media (Bobsien, 1954; Madlo and Soucek, 1956).

The terminal metabolic products of CS_2 are sulphates, mainly the inorganic ones, which are ex-

creted in urine in amounts of about 30 per cent of $C^{35}S_2$ dose, as a well as small quantity (1–2% of the dose) as sulphur compounds, whose characteristic feature is a capacity to catalyze the iodine-azide reaction (Yoshida, 1955; Jakubowski, 1971). Jakubowski (1968) has demonstrated that the latter compounds are not identical with those found in normal urine. Pergal et al. (1972 a, b) identified two CS_2 metabolites in human urine: thiourea, and 2-mercapto-2-thiazolin-5-on, the former in greater abundance than the latter.

The urinary excretion of iodine-azide catalyzing compounds is a rapid process with a half-time in humans of about 1.4 hr. (Jakubowksi, 1966). However, Djuric et al. (1965) and Graovac-Leposavic et al. (1966) have observed that in some workers the compound catalyzing iodine-azide reaction can be found in high concentration in urine in the morning before the start of consecutive daily exposures. These authors proposed to use this phenomenon as an early diagnostic test of carbon disulphide intoxication.

DETERMINATION OF CARBON DISULPHIDE AND ITS METABOLITES

Free and bound CS_2 in blood and urine: A review of the older methods was published by Djuric (1966). The method recommended by Teisinger and coworkers (1956) consists of aerating CS_2, using nitrogen, transferring the latter to an absorbing solution containing diethylamine and copper acetate, and colorimetric or polarographic determination of the formed Cu-diethyldithiocarbamate. A similar procedure may be used for the determination of the bound urinary carbon disulphide after acid hydrolysis of the sample (Bartonicek, 1958).

The compounds catalyzing the iodine-azide reaction: The original method developed by Vasak (Vasak, 1963; Vasak et al., 1963) is based upon the measurement of the time necessary for the oxidation of sodium-azide by a given amount of iodine. The rate of this reaction depends on the type and concentration of the catalyzing compounds present in the sample of urine. The reading of the test is expressed as a coefficient of exposure E, calculated from the formula: $E = c \log t$—where t = reaction time in seconds, c = concentration of creatinine in mg/ml. The reaction is also seen in the urine of nonexposed people; but the time for the reaction is then long, and the values of E fall usually above 6.5. The correlation between the concentration of CS_2 metabolites and the values of exposure coefficient E is a negative one.

The chronometric method has proved useful for evaluating relatively high exposures to CS_2 (Jakubowski, 1965; Locati and Sassi, 1966; Salvadeo et al., 1967; Djuric et al., 1968). When the exposure is to low concentrations of CS_2, the sensitivity of the method is not satisfactory; moreover, difficulties are also encountered because of the long reaction time in samples of urine with a low concentration of creatinine (Tiller — see Djuric, 1966; Jakubowski and Piotrowski, 1966).

Jakubowski and Piotrowski (1966) have elaborated a modification of the method based on titrimetric determination of the iodine consumed in the reaction. The reaction is performed under conditions which discriminate in part the influence of the catalyzing compounds present in physiological urine. The results are expressed in mg iodine per 1 ml urine, corrected for a standard concentration of creatinine of 1.5 mg/ml. Physiological values are usually in the range from 8 to 12 mg I_2/ 1.5 mg creatinine. The readings are directly proportional to the concentration of the catalyzing compounds. The results obtained by this method correlate with those from the Vasak test, and approximate mutual translation of the data is possible. The method of Jakubowski and Piotrowski (1966) can be used at lower CS_2 exposure levels than the original Vaska-test, and it is the former upon which the exposure test of Jakubowksi (1966) was based.

The amount of iodine used in the iodine-azide reaction can also be determined colorimetrically, as described by Magos (1972), for the urine of rats. The latter method has not been applied so far for assessment of industrial exposure to CS_2.

For this purpose, at low levels of the exposure, the elaboration of a method for the determination of CS_2 metabolites catalyzing the iodine-azide reaction seems desirable. This method would eliminate the influence of compounds with similar properties encountered in normal urine.

EXPOSURE TESTS

A correlation between CS_2 concentration in blood and/or urine and the magnitude of exposure (CS_2 concentration in the air) has been sought for the past 20 years. The relevant studies have been reviewed by Djuric (1966). Bobsien (1954) and Weist (1959) have found a positive correlation between CS_2 in the blood and in the air. Demus (1964) investigated a similar urine/air relationship.

The data reported by Demus (1964) relate to a high level of exposure, in the range of 100-450 mg/m³. The concentration ratio urine/air (both expressed as μg/l) was about 4. Teisinger and coworkers (1956) concluded that analysis of blood, urine, and exhaled air for CS_2 may lead only to very approximate evaluation of the exposure.

The interest in the CS_2 exposure test was revived after the study by Vasak et al. (1963) of the test based on analysis of urine for its content of compounds catalyzing the iodine-azide reaction. The chronometric method, referred to above, was derived by the authors from experiments on humans exposed to CS_2 under controlled conditions; however, the range of exposures applied was limited to rather high values. The proposed interpretation of the test was approximate, based on classification of exposure coefficient (E) values into rather broad categories.

A modified version of the test was developed by Jakubowski (1966). The differences, relative to Vasak's test, consist of: a) application of iodometric titration, and b) reference of the results to the absorbed dose of carbon disulphide. The interpretation is based upon the increment of the amount of iodine used, relative to physiological urine. Due to considerable seasonal fluctuation of physiological values (6–11 and 8–18 mg I_2/1.5 mg creatinine in winter and spring (May), respectively), the author proposed to base the evaluation of the exposure on the difference between pre- and postwork readings for a given subject. The coefficient of correlation between the amount of CS_2 absorbed into the system over 6 or 8 hours of exposure and the reading of the test was r = 0.88, with a precision of dose estimation of ± 25 per cent. The relationship is presented in Figure 17-1.

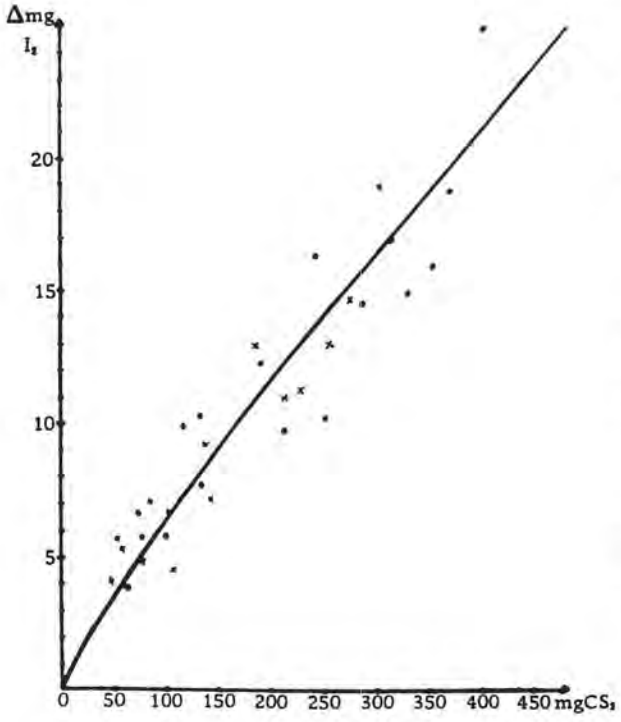

Fig. 17-1. Exposure test for carbon disulphide. Increase of iodine consumption (mg) per 1.5 mg of creatinine, against the absorbed dose of CS_2 (Jakubowski and Piotrowski, 1966).

Taken from: Jakubowski M. and Piotrowski J.: *Practical evaluation of the iodine-azide test for the estimation of exposure to carbon disulphide.* In: **Excerpta Medica Monograph "Toxicology of carbon disulphide", Proceedings of a Symposium,** Prague, 1966. Page 70, Fig. 1.

DATA ON INDUSTRIAL EXPOSURE

A large proportion of the information reviewed above was obtained from direct observations under conditions of industrial exposure, and repetition is not warranted. In the fifties and early sixties the concentrations of carbon disulphide in the air in numerous viscose rayon plants was very high. Jindrichova still reported in 1957 average concentrations in the order of 250 mg/m³. At such levels of exposure, positive results could have been obtained when exposure tests based on CS^2 determinations in urine or blood were used. Jindrichova found blood and urinary concentrations of 10-70 μg/100 mg and 50-600 μg/l, respectively. The ratio of the two concentrations (blood/urine) was close to unity. The data on CS_2 concentrations in air and urine are in basic accord with the relation found by Demus (1964). From investigations of both the authors it follows that urinary elimination is a rather slow process: the concentrations in the morning urine of the exposed workers amount to some 25 to 33 per cent of the maximal workday values.

In the early nineteen-sixties, in most viscose rayon plants, carbon disulphide concentrations in the air were lowered to a range of 50-150 mg/m³, with higher values seen only exceptionally. Under these conditions the Vasak-test in its original form was used with success (Roubal et al., 1963; Jakubowski, 1965; Djuric et al., 1965; Kaszper and Rogaczewsak, 1965). New technological improvements have lead to further reduction of air

CS_2 concentrations in many of the viscose rayon plants. Poland may serve here as an example — by 1964/65 average concentrations clustered around 20-30 mg/m³, with only a few departments in various plants having higher concentrations (Jakubowski and Piotrowski, 1966). At these levels of exposure Jakubowski's iodine-azide reaction may be applied — however, mostly as a collective test. None of the tests developed so far permit evaluation of exposures below the level of 20 mg/m³.

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18. PARATHION
(O,O-diethyl O-p-nitrophenyl phosphorothioate)

ABSORPTION

Parathion may be absorbed through all routes. The essential question of the relative importance of parathion absorption via the respiratory tract and through the skin prompted many controlled laboratory experiments and field studies. It seems beyond reasonable doubt now that both routes are important in conditions of occupational exposure; differences in opinions relate only to the biological consequences that may be attributed to absorption via each route. Thus Hartwell et al. (1965) and Hartwell et al. (1964), on the basis of their controlled studies on volunteers and field type observations, emphasize that cholinergic effects are particularly pronounced when inhalatory absorption is considered. These authors maintain that for prevention of cholinesterase inhibition, respiratory protection is of particular importance; cutaneous absorption, quantitatively comparable, is not followed by the expected biological effects (Hayes et al., 1964). The latter opinion however, should be received with some caution: in inhalatory experiments in which Hartwell et al, (1964) applied a thermal generation technique for attaining the desired concentrations, a more toxic paraoxan was formed in the gaseous phase. Moreover, other observations point to the occurrence of serious parathion intoxications in humans due to cutaneous absorption (Prinz, 1969). A high toxicity of parathion applied to the skin in experimental animals was also observed (Gainess, 1969). The available data do not support the contention that parathion undergoes hydrolysis (inactivation) in the skin (Fredriksson et al., 1961). Therefore, some authors maintain that the transformation rate of parathion is independent of the route by which the compound reaches the systemic circulation (Nabb et al., 1966). These authors applied the determination of cholinesterase activity in the serum for the assessment of parathion absorption rate through the skin in rabbits, and estimated the rate at about 3.5 mg/cm^2.hour. The technique, however, as pointed out by Arterberry et al. (1961), could withstand criticism only if there were a correlation between parathion absorption, p-nitrophenol excretion, and cholinesterase inhibition.

For cutaneous absorption of parathion to occur, it is not necessary to contaminate the skin directly with the compound; the gaseous phase penetrates the skin leading, nevertheless, to analytically measurable amounts on the skin surface (Hayes et al., 1964). The available data demonstrate unequivocally that under the conditions in which parathion is applied in agriculture, the potential hazard related to dermal absorption by far outweighs the risk of inhalatory intoxication (Batchelor and Walker, 1954; Durham et al., 1972).

METABOLISM AND ELIMINATION

The metabolism of parathion has been intensively studied due mainly to its toxic action, particularly the inhibition of acetylcholine esterase. The basic relevant reports have been reviewed in earlier monographs, e.g. O'Brien (1960) and Heath (1961). The present views on parathion metabolism in mammals, corresponding also to informations on the human metabolism, are presented in Figure 18-1. According to this scheme the compound is metabolised mainly via two parallel pathways: the first leads to hydrolysis with the formation of nontoxic diethyl phosphorothionate; the other one leads through activation to highly toxic paraoxon that in turn undergoes hydrolysis to diethylorthophosphate. The hydrolysis of parathion, as well as that of paraoxon, liberates p-nitrophenol which is excreted in the urine. From the metabolic quantitative point of view, the relevant underlying mechanism is that related to the mixed function oxidase which is localised in microsomes; the liver is the chief but not the only metabolising organ (Norman et al., 1973; Poore and Neal, 1972). Organs which accumulate parathion in significant amounts (e.g. kidneys, lungs, intestinal walls — Fredriksson and Bigalow, 1961), display metabolic activity independent of that present in liver. Hydrolysis of paraoxon proceeds to a substantial degree in the

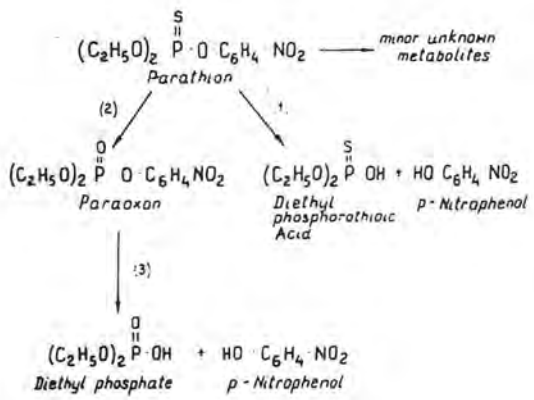

Fig. 18-1. The metabolism of parathion by mammalian hepatic microsomes (after Poore and Neal, 1972).

Taken from: Poore R.E. and Neal R.A.: *Evidence for extrahepatic metabolism of parathion.* **Toxicol. Appl. Pharmacol. 23,** 759-768, 1972, Page 760. Fig. 1.

blood under the influence of phosphatases, as well as a relatively specific hydrolase, called paraoxonase (Geldmacher et al., 1973). Dependence of parathion toxicity upon the route of administration, as discussed above, could result from the varying activity of the two main metabolic pathways (Fig. 18-1) in different tissues (organs), particularly those of the liver and lungs. The available data however, do not permit an unequivocal assessment of the extent the above explanation is likely to be true (for discussion see Neal, 1972). In any case, it appears certain that the final metabolite in both cases is p-nitrophenol, regardless of the share of the total taken by either of the metabolic pathways. Thus, at least theoretically, the determination of the total excreted p-nitrophenol may be a measure of the quantity of parathion absorbed into the system.

The metabolic rates vary considerably with animal species. For instance, one step of the transformation, hydrolysis of paraoxon under the influence of blood phosphatases, is 50 times faster in rabbit than in man (O'Brien, 1960). Urinary excretion of p-nitrophenol in the cynomolgus monkey lasts for a month after administration of parathion; for more than the first ten days, the decline of the excretion rate is marginal (Lieben, et al., 1952; Waldman et al., 1954). The same authors have observed prolonged excretion of p-nitrophenol in humans exposed to parathion (Lieben et al., 1953); however, the data reported by others indicate that the process is in fact much faster. From the data of Vlachova (1956), the maximal excretion rate of the metabolite occurs within the first day after exposure; thereafter the decline is so fast that within a day the rate declines to about 1/10 of the maximum values. The data of Hayes et al. (1964), obtained from repeated cutaneous exposures, indicate a fast decline of excretory levels between consecutive daily exposures also. Therefore, the excretion of p-nitrophenol becomes stabilised already after the second day of exposure, and does not indicate any further cumulation of parathion in the body.

DETERMINATION OF PARATHION AND ITS METABOLITES

The methods relevant for purposes of exposure evaluation are those for nitrophenol determination in urine, discussed in the section "Nitrobenzene". In recent years wide application of the sensitive method of Elliot et al. (1960) has occurred. At present the prevailing tendency is to introduce gas chromatography with electron capture detector by which a sensitivity in the order of 50 ppb from a small amount of urine may be reached. An increase in the volatility of p-nitrophenol silanization has been proposed by means of hexamethyldisilizane (HMDS) (Cranmer 1970) or transformation into ethyl ether using diazoethane (Bradway and Shafik, 1973).

Until recently determination of the unaltered parathion itself has not been considered for exposure evaluation. The issue may be of intrinsic interest due to possibility of gas-chromatographic determination of the compound in the serum. It has been suggested that this procedure may form a basis for an exposure test of still greater sensitivity than determination of p-nitrophenol in urine (Roan et al., 1969; Watanabe, 1972).

INTERPRETATION OF URINARY P-NITROPHENOL DETERMINATIONS

It appears that experimental evidence is lacking which would allow a direct estimate of parathion absorption from the urinary excretion of p-nitrophenol in man. Approximate assessment of the absorbed amounts seems possible if the following assumptions are accepted:

a) At repeated exposure the excretion of p-nitrophenol becomes stabilized by the second day on a level related to the magnitude of exposure (absorbed dose).

b) Maximum excretion rate is usually reached several hours after discontinuation of the exposure, and therefore representative samples of urine cannot be obtained by sampling directly after the end of a work shift. Bearing this in mind it appears reasonable to base quantitative assessment on the determination of p-nitrophenol in 24 hours urine.

c) The assumption seems rather well founded that in man virtually all p-nitrophenol is eliminated in the urine. Therefore a daily urinary amount of p-nitrophenol should directly correspond, on a molar basis, to the amount of parathion absorbed on the day of assay. This assumption is not inconsistent with the data of Vlachova (1958, 1962) who found in rats a urinary excretion of p-nitrophenol corresponding to 40-60 per cent of the administered parathion (depending on the sex); the same percentage is excreted by these animals after administration of p-nitrophenol itself.

The parathion exposure test used most commonly is based on the degree of blood cholinesterase inhibition. Total agreement as to the existence of a correlation between both tests is lacking, and it is beyond doubt that determination of p-nitrophenol is a more sensitive assay. Nonetheless, in animal experiments (rats) a relationship was found between the dose of parathion and the degree of cholinesterase inhibition (Fiserova-Bergerova, 1962). Arterberry et al. (1961) also found a correlation between the two discussed indices. A similar conclusion seems to follow from the study of Hartwell et al. (1964), with the reservation that in this case inhalatory exposure was involved. On the other hand, lack of the relationship was found by Roan et al. (1969).

In discussing the mutual relationship of both tests the following phenomena, which make unequivocal and simple conclusions less likely, should be borne in mind:

a) At a constant daily exposure to parathion the excretion of p-nitrophenol reaches a plateau already on the second day; whereas the inhibition of cholinesterase proceeds gradually for 2-3 weeks, and becomes stabilized only afterwards at a level reflecting the magnitude of the daily exposure (Glome and Swensson, 1958).

b) In experiments on human volunteers, low doses of parathion do not lead to a depression but to an elevation of the cholinesterase activity in both erythrocytes and serum; inhibitory effects may be expected only after daily doses via the alimentary tract in excess of 0.05 mg per kg body weight (Williams et al., 1958).

c) There seems no definitive answer to the question whether blood cholinesterase inhibition is, for a given dose of the compound, independent of the route of absorption.

From a practical viewpoint, information is needed on the concentrations (amounts) of p-nitrophenol in urine at which no inhibition of blood cholinesterase occurs. In human beings these concentrations seem to be in the order of 70-150 μg/l (Lieben et al., 1953) or 200 μg/l in 24 hour urine (Vlachova, 1956). A similar order of values follows from the investigation of Hartwell et al. (1964), and from computation of the doses that had been applied by Williams et al. (1958) at which no decline of cholinesterase activity was found.

The concentrations of p-nitrophenol in 24 hour urine, corresponding to maximum permissible concentrations in the work environment (0.05 mg/m^3 — USSR; 0.1 mg/m^3 — USA), may be calculated assuming full efficiency of absorption in the respiratory tract, ventilation of 0.8 m^3/h for 8 hours, and similar cutaneous absorption from the gaseous phase. At 100 per cent transformation into p-nitrophenol the concentrations of the latter in daily urine should be of the order of 250-500 μg/l.

AVAILABLE DATA ON INDUSTRIAL EXPOSURE

The data on the magnitude of exposure to parathion in the course of its manufacture are not voluminous. The reviewed literature relates mainly to the issues resulting from application of insecticides in agriculture. In addition to the papers cited above, others include: Wolfe, 1972; Watanabe, 1972; and Guthrie et al., 1972.

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19. FENITROTHION
(Metathion, Sumithion)

Fenitrothion, O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate, is an analogue of parathion, of pronounced toxicity for insects and relatively low toxicity for mammals.

ABSORPTION

The compound can be absorbed into the system by all routes: pulmonary, cutaneous, and through the alimentary tract (Matyushina, 1966). A negligible difference in the efficiency and dynamics of excretion of the main metabolite (p-nitro-m-cresol) in rats after intraperitoneal and intragastric administration of fenitrothion points to a rapid and efficient absorption from the gastro-intestinal tract (Hladka and Nosal, 1967). Absorption through the skin is also easy. DL_{50} after dermal application is higher by a factor of two than after alimentary administration (Matyushina, 1966). Observations exist that suggest initial retention of the compound in the skin with somewhat delayed resorption into the bloodstream (Chruscielska, 1968).

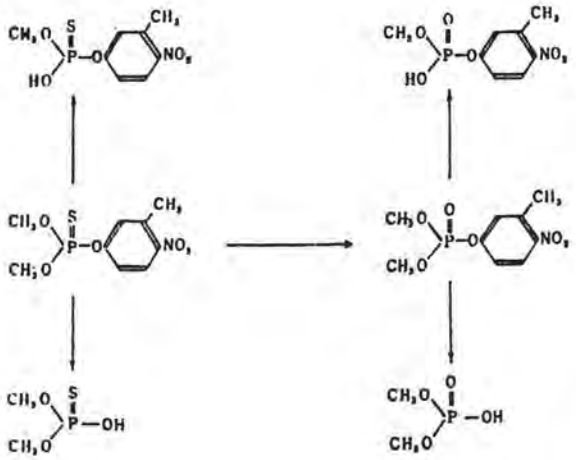

Fig. 19-1. Initial metabolic pathways of fenitrothion (Miyamoto et al., 1963).

Taken from: Miyamoto J., Sato Y., Kadota T., Fujinami A., Endo M.: **Agricultural and Biological Chemistry 27,** 381-389, 1963. Page 389, Fig. 7.

METABOLISM AND ELIMINATION

Miyamoto et al. (1963) studied the fate of the compound, labelled with ^{32}P, in rats and guinea-pigs, and proposed a scheme of metabolic pathways as presented in Figure 19-1. According to these authors, fenitrothion and also its desulphuration product, fenitrooxon, are subject primarily to hydrolysis yielding either methanol or p-nitro-m-cresol.

The latter is the chief metabolite in rats (Hladka and Nosal, 1967) and in man (Nosal and Hladka, 1968), accounting respectively for 62-66 and 48-59 per cent of the administered dose (in man after oral administration). At increasing doses in man the efficiency of this metabolic pathway declines.

A small percentage of the dose (3%) was found in rats' urine in the form of a reduction product, p-amino-m-cresol (Hladka, 1969). The biotransformation mechanism of fenitrothion is probably analogous to that found for parathion. Chronic exposure to organohalogen insecticides (e.g. heptachlor) increases the toxicity of fenitrothion by elevating the activation rate to fenitrooxon, with a concomitant increased excretion of p-nitro-m-cresol; these facts point to induction of microsomal enzymes (Mestitzova et al., 1970).

The metabolism of fenitrothion is relatively rapid. In rats, excretion of p-nitro-m-cresol is biphasic, and over the first 24 hours after intraperitoneal administration about 80 per cent of total originating p-nitro-m-cresol is eliminated (Hladka and Nosal, 1967). Its metabolism and elimination in man appear to be still faster. Almost all the p-nitro-m-cresol is eliminated within 24 hours after absorption, and maximal excretion rate follows directly the oral administration of

fenitrothion (Melichar and Franz, 1966; Nosal and Hladka, 1968). These observations clearly show that the cumulative effects of fenitrothion found by Matyushina (1966) could not have resulted from material cumulation of the compound in the course of repeated exposure.

METHODS FOR DETERMINATION OF P-NITRO-M-CRESOL IN URINE

The methods proposed for the evaluation of exposure in humans are based upon colorimetric determination of yellow nitro-cresolate in an alkaline medium. The introductory stage of the procedure includes hydrolysis and extraction with acetonitrile. To partly eliminate the influence of urinary pigments, Melichar and Franz (1966) introduced (H_2O_2) oxidation. To improve separation, Hladka and Hladky (1966) proposed thin-layer chromatography. In the latter version the method includes acid hydrolysis, extraction with acetonitrile from the alkaline medium, repeated extraction with a mixture of solvent naphtha and ethyl ether from the acidified medium, and thin-layer chromatographic separation on silica-gel. The colour is developed with ammonia vapour, and spectrophotometric determination is carried out at 402 nm in pyridine eluate. The method enables detection of 2 μg of p-nitro-m-cresol in 20 ml of urine (Hladka, 1969).

As with p-nitrophenol (see: "Nitrobenzene", and "Parathion"), it seems possible to determine p-nitro-m-cresol in urine by means of gas chromatography, as proposed for analysis of other media (Bowman and Beroza, 1969).

OTHER METHODS

For diagnosis of intoxication with phosphoroorganic compounds, including parathion and fenitrothion, determination of unlayered compounds in blood-serum by means of gas chromatography has been proposed (Watanabe, 1972 a, b).

EXPOSURE TEST

With respect to man, preliminary experimental data by Melichar and Franz (1966) and results of more systematic studies by Nosal and Hladka (1968), in which p-nitro-m-cresol has been determined, have been reported by Hladka (1969). In the latter studies fenitrothion was administered orally to volunteers in gelatin capsules in doses

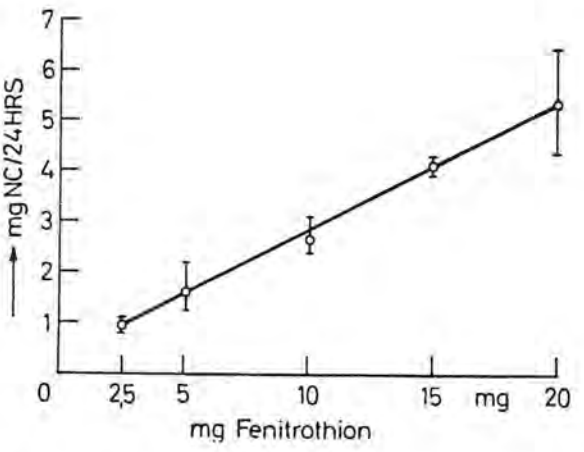

Fig. 19-2. Dependence of the quantity of nitrocresol dishcarged in 25 hours' urine of persons tested in relation to the dose of fenitrothion (Nosal and Hladka, 1968).

Taken from: Nosal M. and Hladka A.: **Int. Arch. Gewerbepath. Gewerbehyg. 25,** 28-38, 1968. Page 31, Fig. 1.

from 2.5 to 20 mg. The relationship between the dose and daily urinary excretion of p-nitro-m-cresol is preented in Figure 19-2. This relationship should also be valid for repeated exposures, because the authors experimentally demonstrated in human subjects that no essential cumulation of the metabolite occurred.

From the point of view of the route of administration of fenitrothion, reservations are pertinent that relate to the efficiency of absorption from the gastrointestinal tract and the degradation of the compound in the intestinal content (with possible participation of bacterial flora, etc.). The interpretation of the test with regard to dermal exposure does not seem possible. The same applies to the interpretation of results obtained from analysis of spot urine samples.

The above test may not be replaced by an assay of cholinesterase activity in blood, because, as shown by Nosal and Hladka (1968), changes in the latter (as well as in erythrocytes in the plasma) are not detectable after one- or four-days' exposure at the levels above the sensitivity limits of the test.

DATA ON INDUSTRIAL EXPOSURE

No data have been published so far on application of the test in industrial conditions.

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Watanabe S.: *Detection of organophosphate pesticides in blood serum from the patients suspected of acute and chronic pesticides poisonings and its clinical significance.* **Tohoku J. Exp. Med. 107,** 301-302, 1972(b). Abstract supplied by NLM MEDLINE.

20. DDT
(pp-dichlorodiphenyl-trichloroethane)

ABSORPTION

The absorption routes of DDT under conditions of occupational exposure are not well understood. It is obvious that absorption of DDT aerosols and vapours via the respiratory tract is possible, but there are no specific data as to the efficiency of the process. A part of the aerosol is most probably deposited in the upper respiratory airways and is subsequently swallowed. This part would be absorbed as if the compound were given orally. Most model experiments on the metabolism and systemic cumulation of DDT have been performed with oral administration of the compound. Generalization from these experiments involves the implication that intestinal absorption is highly efficient. Some doubt is cast on the validity of this presumption by the experimental data of Jensen et al. (1957), who demonstrated that considerable amounts of DDT were present in feces representing the unabsorbed fraction of the compound. It appears that cutaneous absorption of DDT has not been thoroughly studied.

METABOLISM AND ELIMINATION

Gadaskina and Filov (1971) claimed that after alimentary administration of DDT the compound appears rapidly in the blood, and the concentrations reach maximum after 6-8 hours, declining relatively rapidly thereafter. Organ distribution of DDT is rather uniform with the exception of adipose tissue, where the concentrations are much higher than the average. The same refers to organs rich in lipids, such as bone marrow, adrenal glands, etc. In the course of repeated exposures, it is possible to accumulate in animal adipose tissue total amounts of DDT much in excess of the lethal dose.

DDT, as well as its metabolites DDD and DDE are present in liver, mainly in the soluble fraction (cytoplasm) and partly in the mitochondria. The nuclei contain relatively small amounts (Kuzminskaja and Girenko, 1973). It is far from clear whether DDT in blood and tissues is present in a free form, or as biocomplexes (Hatanaka et al., 1967; Dale et al., 1967; Schoor, 1973).

In the body DDT is subject to complex metabolic transformations that follow two directions: the first pathway goes through DDD, DDMU, and a chain of intermediate metabolites to DDA, which is excreted as the final metabolic product. The alternative pathway leads to DDE. This compound displays little reactivity; and, if metabolized at all (to DDMU), the process must be slow. Therefore, because DDE is lipophilic (as in DDT itself), in course of prolonged DDT exposure the metabolite undergoes substantial cumulation in adipose tissue.

On the diagram presented in Figure 20-1 the metabolic pathways are given according to Peterson and Robinson (1964). The dotted arrows depict the somewhat different results of later studies by Datta (1970). Datta postulated further transformation of DDE with a common metabolic pathway starting with DDMU. The data of Roan et al. (1971), however, argue against further metabolic transformation of DDE to DDA in man.

The mechanisms of biotransformation of DDT to DDD on one hand, and to DDE on the other, are probably different. The data of various authors, reviewed by Alary et al. (1971), indicate

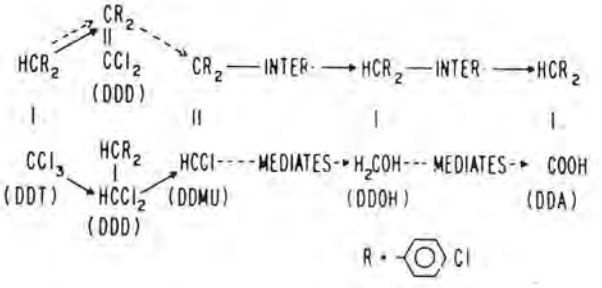

Fig. 20-1. Metabolic pathways of DDT according to: Peterson and Robinson (1964); Datta (1970).

that the transformation of DDT to DDD occurs predominantly in the liver, and is catalysed by microsomal enzymes that take part in the metabolism of foreign compounds. To some extent the transformation can be accelerated by induction of these enzymes with typical inductors, e.g. phenobarbital. The transformation of DDT to DDE may be, at least partly, a non-enzymatic process catalysed by iron porphyrins.

DDT itself, and to smaller degree its metabolites, have inductive properties with regard to the activity of microsomal enzymes (Street et al., 1966; Gillett et al., 1966). It may be presumed, therefore, that autoinduction occurs — however, only after high doses of DDT (Gillett, 1968).

Elimination from the body takes place via urine and faeces; the main eliminated product in both cases is DDA. The DDA reaches the intestinal content mainly with bile where complexes of DDA are formed, most likely with cholic acids. Considerable amounts of DDT or DDE in bile have not been found. Pronounced elimination of DDT with faeces represents most likely the unabsorbed fraction of the compound (Jensen et al., 1957). The so called "neutral metabolites" of DDT are also absent, at least in considerable amounts in the urine of humans exposed occupationally to DDT (Cueto and Biros, 1967).

DETERMINATION OF DDT AND ITS METABOLITES

For a long time DDT and its metabolites had been determined by use of a colorimetric method, known as Schechter-Haller method. In this procedure DDT and its metabolites are converted into tetranitro derivatives that yield colour products upon reaction with potassium ethylate (Schechter et al., 1945). Initially DDT had been isolated from the adipose tissue by extraction with carbon tetrachloride or chloroform preceded by sulfonation of the fat. Later Mattson et al. (1953) developed a method for separation of DDE from DDT, using a modified Davidov's column and applying alumina as the sorbent and acetone as the eluent. The Schechter-Haller method is still in use in numerous laboratories; attempts are being made to adjust the procedure for the determination of the individual metabolites by introducing separation of coloured products by means of thin-layer chromatography (Krechniak and Dubrawski, 1971). However, a contemporary approach to the determination of DDT and its metabolites is based totally on application of gas chromatography.

From the viewpoint of exposure evaluation, use may be made of DDT (and possibly DDE) determination in the blood, in the adipose tissue (obtained by means of biopsy), and of DDA determination in urine.

DETERMINATION OF DDT AND ITS METABOLITES IN BLOOD AND ADIPOSE TISSUE BY MEANS OF GAS CHROMATOGRAPHY

In principle, the gas-chromatographic methods applied here consist of extraction of the sample with hexane, introduction of an adequate amount of the dessicated extract into a chromatographic column, and measurement at adequately selected conditions (type of column and detector; temperature of separation technique). Among the most widely known procedures are methods developed by Hayes et al. (1965) for adipose tissue and by Dale et al. (1966) for blood. In the latter, a first attempt was made to leave out introductory clean-up of the extract before the chromatographic step; this tendency has been dominant in further methodologic studies. As stressed by Radomski and Fiserova (1965, 1967), cleaning-up of the extracts may be omitted while analyzing fat-tissue provided that a top class gas-chromatograph is used, equipped with a good electron-capture detector and able to work in the range from 2 to 50 picograms. It should be mentioned here, that when compared with the tritium detector the ^{63}Ni EC detector is of an advantage because the latter is less susceptible to contamination and may be used at higher temperatures than the former. The simplicity of this procedure may be essential for determination in biopsy-samples because a small amount of the material (200 mg) is sufficient for the analysis. Sensitive and precise methods of tissue analysis, in which a complete purification of the extract is applied before chromatography, require in general a substantially greater amount of the material to start with (for example, see Saschenbrecher and Ecobichon, 1967). However, there are methods available, developed more recently in which clean-up is performed on a column filled with activated magnesium silicate (fluorisil) that enable the analysis to be completed on a small amount of the adipose tissue, between 50 and 200 mg (Morgan and Roan, 1971). Thus, procedures including the clean-up step are still being developed (McLeod and Wales, 1972).

When analyzing blood for its content of DDT and metabolites, doubt has been expressed

whether simple extraction with hexane is satisfactorily efficient due to a possibility that DDT may be present in complexes which are not extracted. An alternative technique, proposed by Dale et al. (1967), is based on the principle of volatilizations. A small amount of plasma is put in a tube stoppered with siliconized glasswool for capture of sublimed pesticides. Hexane extraction is performed of both the residual content of the tube as well as of the glass wool. Using this technique higher results have been obtained in blood analysis relative to simple hexane extraction, but the results for standards were poor. Therefore the method has not been put into common use. Another, more recent attempt to improve the method is based on the modification of the extraction conditions. The extraction is made in the presence of anhydrous sodium phosphate and formic acid; the extract, after washing with potassium carbonate solution, is applied directly to the column. The role of formic acid is to improve considerably the separation (Palmer and Kolmodin-Hedman, 1972). Apart from the method of Dale et al. (1966) for analysis of blood, other chromatographic techniques are sporadically used. Morgan and Roan (1971) have used preliminary extraction with acetone and isooctane, purification on a column with n-pentane extraction, followed by evaporation and analysis of the extract.

DETERMINATION OF URINARY DDA BY MEANS OF GAS CHROMATOGRAPHY

As demonstrated by Cueto and Biros (1967), occupational exposure to DDT is not followed by an increased elimination of DDT and, so-called, neutral metabolites (mainly DDE) in urine. Earlier, the determination of urinary DDA was performed colorimetrically after separation of the metabolite from urine by means of ion-exchange chromatography (Cueto et al., 1956). Recently, gas-chromatographic methods are widely used. Laws et al. (1967) extract DDA from urine with chloroform after hydrolysis. After evaporation, methylation is performed by heating with anhydrous methanol in the presence of sulphuric acid; n-hexane extract is applied to the gas chromatographic column. Cranmer et al. (1969) used hexane extraction from an acidified medium followed (after evaporation) by esterification with borontrifluoride in methanol, purification on the fluorosil micro-column, and gas-chromatographic determination. To suppress the influence of "background", particularly that caused by minute amounts of DDT and its neutral metabolites against which the electron-capture detector is very sensitive, the authors proposed to use instead a Coulson conductivity detector. Roan et al. (1971) apply preliminary heating of urine with sulphuric acid followed by ether extraction of DDA. Esterification is then made with dimethylsulphate and the extract is analysed using a gas chromatograph with a microcolorimetric detector. The sensitivity of the method in this version reached 1 ng/ml.

BACKGROUND LEVELS OF DDT AND ITS METABOLITES

Background levels of DDT and its metabolites in human biological material depend on the scale at which DDT has been used. Thus the levels vary with time and geography. In general the suppression of the use of DDT has lead in recent years to a reduction of levels of DDT and its metabolites in human tissues.

In adipose tissue, obtained usually at autopsy and less frequently by biopsy, DDT or DDE, or a sum of both, have been determined. In Great Britain the average concentrations (sum of both substances) amounted to 2.2 ppm (Hunter et al., 1963) whereas at the same time in the USA the level reached 10-12 ppm (with individual values up to 22 ppm; Hofman et al., 1964; Quinby et al., 1965). Hunter (1968) reported that the lowest concentrations (1.8 ppm) were found in Australia and the highest (about 19 ppm) in Israel. In Poland, in one agricultrual area, the maximal levels noted in 1968 reached 30 ppm and were still high in 1970 — 16 ppm (Ochynski and Bronisz, 1972). Of the above values obtained for people not exposed occupationally to DDT, DDT comprised 28 to 34 per cent, the rest was due to DDE. The approximate ratio DDE/DDT in the adipose tissue is therefore close to 2.

At steady-state, the relation of concentration of DDT and its metabolites in blood to those in adipose tissue is relatively constant: at absolute concentrations in blood of about 20 ppb the concentration ratio fat/blood equalled about 140 (Brown 1972). Thus, similar to the DDT content in fat, the total blood DDT in occupationally nonexposed individuals varied from report to report. Perron and Barrentine (1970) reported values in the range of 5-25 ppb, of which 80-100 per cent was DDE. Apple et al. (1970) estimated the relation of DDT to DDE in serum; DDE con-

centrations of 5-25 ppb were accompanied by DDT at the levels of 2 to 5 ppb.

Relatively less information is available with regard to background levels of DDA in urine. In the experiments of Hayes et al. (1956), the levels in control groups were in the range of 0-90 ppb; Ortelee (1958) reported, for a group of individuals with a lowest daily intake of DDT of about 0.2 mg, urinary DDA concentrations in the range 40-150 ppb. Durham et al. (1965) found, in a general population, levels from below 20 up to 350 ppb. Hayes et al. (1971) reported the most frequent values in the range of 3-150 . μg/hr, which would correspond to 60-3000 ppb; the authors, however, were unable to exclude erroneous sporadic inclusion into the control group of individuals exposed experimentally to DDT. Cranmer et al. (1969) reported values in the range from 8 to 19 ppb for occupationally non-exposed, whereas Edmundsen et al, (1970) have found concentrations on the order of 7-22 ppb in people exposed to a mild degree. The last two sets of data, obtained by gas-chromatographic methods inspire the most confidence.

EXPOSURE TESTS

Studies on volunteers given known amounts of DDT orally will be reviewed and discussed. This is the only type of controlled experiments on human volunteers that involved significantly increased absorption of DDT. All reservations that were raised previously are pertinent here because of: a) non-complete absorption of the substance from G.I. tract, and b) a possibility of biotransformation of DDT in intestines under, for instance, the influence of bacterial flora.

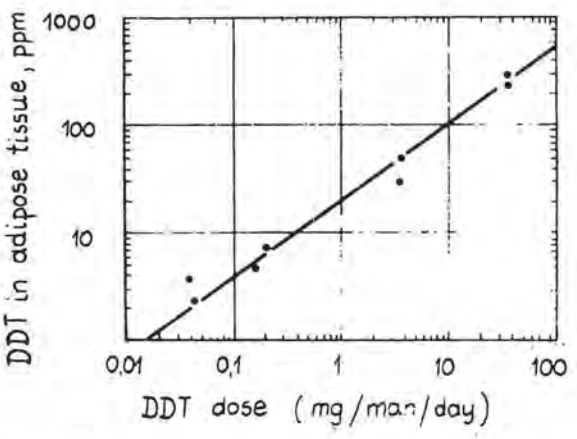

Fig. 20-2. Storage of DDT in the body fat (ppm) as dependent on the daily dosage (mg per man per day) in humans.

Taken from: Durham W.F., Armstrong J.F., Quinby G.E.: **Arch. Environ. Health 11,** 641-647, 1965.

DDT in adipose tissue. Hayes et al. (1956) conducted basic experiments on prisoner-volunteers and obtained the data from adipose tissue biopsy. They found that after a year of continuous administration of a technical preparation of DDT its cumulation in the tissue was completed and the steady-state values attained vere: 25-40 and 210 ppm for the daily intakes of 3.5 mg and 35 mg, respectively (the values for pure p,p'-DDT were higher). Recently the repeated systematic studies by Hayes et al. (1971) have pointed to a longer period necessary for attaining equilibrium (2 years) and steady-state values somewhat higher than the earlier ones; at the daily intakes of 3.5 mg and 35 mg, these were 30-50 and 200-300 ppm, respectively. Morgan and Roan (1971), in a shorter study (1/2 year) when the rising trends were still apparent, obtained toward the end of exposure adipose tissue levels of 50 and 120 ppm for respective intake rates of 7.7 and 15.4 mg per day. From these studies it seems to follow that: a) the tissue levels approach equilibrium late, after 1-2 years; and b) the concentrations of DDT in the fat are approximately proportional to the daily dose. Durham et al. (1965), on the basis of the studies by Hayes et al. (1956) and their own investigation in which a low controlled dietary intake of DDT was used, have assumed for interpretation a simple proportionality in a log-log coordinate system (Fig. 20-2). This could be modified only slightly in view of later studies.

For assessment of DDT absorption, the levels of DDE in adipose tissue seem to be of little use. Hayes et al. (1956) and Morgan and Roan (1971) have seen some increase of DDE concentrations after DDT, given in doses of 7-35 mg/day; however, the increase was much smaller than that of DDT itself, and displayed a much larger scatter of individual values.

DDT in serum. Apple et al. (1970) performed experiments on volunteers to whom DDT was administered orally for about 1/2 year in daily doses of 10 and 20 mg. The serum levels rose over the whole interval of exposure, reaching the respective values of about 150 and 300 ppb. In a later experiment of the same group, Morgan and Roan (1971) confirmed the rising trend of serum DDT over the whole 1/2 year period of ex-

posure, and obtained final concentrations of about 200 and 400-600 ppb for the daily intakes of 7.7 and 15.4 mg DDT, respectively.

From these data one could conclude that, similar to DDT in the fat, serum DDT may be used as a measure of chronic absorption of the substance. The data of all the cited authors, however, in particular of Apple et al. (1970), argue against the possibility of assessing the absorption rate from DDE levels in serum.

DDA in urine. Hayes et al. (1956) maintain that about 20 per cent of a DDT dose is excreted as urinary DDA. For daily oral DDT doses of 3.5 and 35 mg they found urinary concentrations of DDA (toward the end of exposure period) of 50-300 and 900-6000 ppb, respectively. Ortelee (1958) presented a relationship between daily DDT dose and DDA concentrations in urine in the form of a curve in log-log coordinates (Fig. 20-3). Roan et al. (1971) found in volunteers who were given 15 mg of DDT daily that the DDA levels in urine rose over the whole period of experiment lasting for 1/2 year, and reached at the end a daily excretion rate of 2 mg/24 hours, i.e. about 2000 ppb. This concentration was higher than predicted by Ortelle's curve. Finally, unexpectedly high DDA urinary levels were obtained by Hayes et al. (1971) in individuals administered a daily DDT dose of 35 mg. The excretion rate reached 2-3 mg/hour, and this value exceeded by an order of magnitude those expected from the data reported by others.

Discrepancies in the reviewed data warrant further well-controlled human studies.

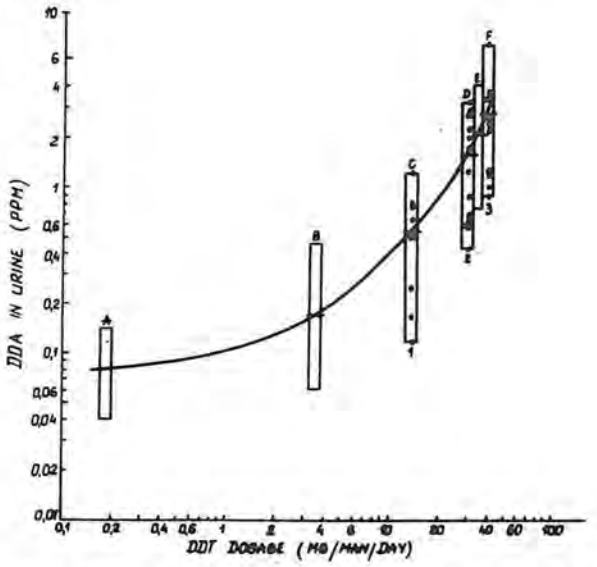

Fig. 20-3. Concentration of DDA in the urine of men with known daily oral intake of DDT (Ortelee, 1958).

Taken from: Ortelee, M.F.: **Arch. Ind. Health 18,** 433-440, 1958. Page 435.

AVAILABLE DATA ON OCCUPATIONAL EXPOSURE

Hayes et al, (1958) claimed that people exposed occupationally to DDT have greater deposits of the substance in their adipose tissue than nonexposed individuals. Also the contribution of DDE to the so-called "total DDT" should be lower in the former. Ortelee (1958) studied workers engaged in DDT manufacture and evaluated their exposure via analysis of urine for DDA content. The author concluded that the workers in the plants studied were absorbing daily 10-50 mg of DDT. Durham et al. (1965) found that at massive occupational exposure the urinary DDA exceeded by far (120-7500 ppb) the values observed in the control group, and reached values close to those observed after daily ingestion of 35 mg (volunteers). Laws et al. (1967) assessed DDT absorption in workers by two independent methods: a) determining DDT in the adipose tissue and interpreting the data according to Durham et al. (1965); b) measuring DDA urinary concentrations and applying Ortelee's diagram (1958). The mean values obtained for three groups of workers by either way were consistent with each other: 18, 6.3, and 3.6 against 17.5, 8.4, and 6.3 mg DDT/day, respectively. Edmundson et al. (1969a) studied changes in blood DDT and DDE, and urinary DDA, in workers after a single massive exposure to DDT; the steepest rise was found for urinary DDA. The same authors (1969b) recommended DDE determination in blood for the purpose of exposure evaluation. In a still later report, the same group (Edmundson et al., 1970) expressed the opinion that in occupationally exposed individuals there is an increase in the role of the detoxication mechanism that leads to the formation of DDA.

These authors were unable to find a direct relation between actual exposure, on one hand, and the DDT and DDE levels in blood and DDA in urine, on the other. This conclusion is strengthened by existing data on the kinetics of DDT turnover in the body. Perron and Barrentine (1970) found only minor differences in serum DDT concentrations between controls and oc-

cupationally exposed individuals; on the other hand a pronounced seasonal variation has been observed in both groups. The authors suggested that the variation resulted from seasonal application of DDT in the region in which the study had been performed; the rise in all studied groups seemed to have been induced by environmental exposure. Mean DDT and DDE levels of the order of 650 ppb (DDT) and 600 ppb (DDE) were found in the serum of workers engaged directly in the manufacture of DDT.

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21. OTHER SUBSTANCES

The author did not make a systematic literature survey with regard to substances that have not been discussed in detail in this book. Some information was obtained, however, in a preliminary literature search. For the period of 1969-1971 the literature was searched more systematically using MEDLINE as the information source. Earlier reports were included in older monographs on the subject. Thus, even if the information for the substances mentioned below may not be regarded as exhaustive, it should permit basic orientation as to the scope and soundness of the existing data.

ALIPHATIC ALCOHOLS

For ethyl alcohol a voluminous literature exists, mainly on methods of determination in blood. Essential information is contained in older monographs (Teisinger et al., 0956; Dutkiewicz et al., 1964; Gadaskina and Filov, 1971). The gas-chromatographic methods, not reviewed there, recently attracted the attention of two authors: Jain (1971) and Laplace and his coworkers (1971). The blood level may be also estimated indirectly by means of breath analysis (Yamamoto and Ueda, 1972). Interpretation of the results, however, may be regarded more as a practical task in forensic medicine, than as an exposure test in the sense of this book (Dutkiewicz et al., 1964). A contrasting view was however, expressed by Spassovski and Benchev (1969), who suggested that already at concentrations of ethanol in air of the order of 1000 mg/m^3 an elevated level in blood occurs both in volunteers and in workers exposed chronically in occupational conditions.

Methanol was extensively covered earlier in the older monographs referred to in this book. Both the methods of determination and the interpretation of the results relate mainly to evaluation of acute intoxications. Nevertheless, an approximate interpretation of urinary levels of methanol has been proposed for purposes of exposure evaluation in industrial conditions. The basic experimental data on humans were reported by Leaf and Zatman (1952), who found that a dose of 100 mg/kg was accompanied by a urinary concentration of about 190 mg/l. The results of field studies, made by Dutkiewicz and Blochowicz (1967), when recalculated, yield a similar relationship. More recent data, obtained from experiments on volunteers and from field studies in industrial conditions (Spassovski and Benchev, 1969) suggest that, numerically, the effective air concentrations of methanol (mg/m^3) to which a worker had been exposed may be obtained by multiplying urinary concentrations (mg/l) by a factor of 3.

For higher aliphatic alcohols, it seems, even preliminary human data are absent. There are, however, limited pharmacokinetic data for some of them, e.g. 2–propanol, obtained in animal studies (Abshagen and Rietbrock, 1969).

FORMALDEHYDE

An evaluation of exposure based on the determination of the substance itself in urine has been proposed (Spassovski, 1965, 1966), and later blood assays were suggested (Volkova and Sidorova, 1971). The latter authors, who studied the problem in field conditions, reported that at the air concentration of 7 mg/m^3 the blood levels were in the range from 0.06 to 0.4 mg%. This information still should be regarded as preliminary.

ACETONE

Studies on volunteers were conducted by Spassovski et al. (1967) and Di Vincenzo et al (1973). The first author recommended urinary determinations of the substance for exposure evaluation; at air concentrations of 200 and 2400 mg/m^3, 40 and 100 mg/l had been found in urine, respectively. Di Vincenzo et al. (1973), applying gas-chromatographic methods, were also unable to find a direct proportionality between concentrations in air and in urine; however, direct proportionality was found between concentrations in the air and those in the blood and the breath. The half-life of acetone in blood in humans is close to 3 hours, and this seems to preclude any possibility of cumulation. It seems these two

studies exhaust the available human data with regard to acetone.

ALIPHATIC ALCOHOL ESTERS

It appears that no attempts have been made so far to undertake elaboration of exposure tests for esters of aliphatic alcohols. An exception seems to exist here with regard to glycol dinitrate and diethylene glycol dinitrate. Both compounds have been studied in rats. Moreover, for the latter of two compounds some preliminary data for human subjects exposed in industry have been obtained. From the results, it seems to follow that evaluation of the exposure could be based, perhaps, upon urinary determination of nitrates that form the final metabolites of both compounds.

LOWER CHLORINATED HYDROCARBONS

There exist data for some chloroderivatives of methane, ethane, ethylene and acetylene. Of this group, tri- and tetrachloroethylene have been discussed in separate chapters in this book. Attempts to evaluate exposure to other compounds of this group have been based almost exclusively upon breath analysis. The general problems of this methodology have been discussed by Boettner and Muranko (1969) and by Backman and Pfaffli (1972). A general picture of the fate of methane chloroderivatives such as methylchloride, chloroform and carbon tetrachloride had been obtained earlier from animal experiments (Sperling et al., 1950; Soucek, 1962; Soucek, 1961a, b). Recently, elimination of methylene-chloride in the expired air has been studied in volunteers (Riley et al., 1966), as well as blood and urinary concentrations of the compound (Di Vincenzo et al., 1971); the results seem to be encouraging.

Metabolism of 1,1,1-trichloroethane has been studied in rats (Hake et al., 1960) and the dominant role of elimination of the unaltered compound with the expired air was demonstrated. Stewart (1968) recommended breath analysis for the evaluation of exposure in man, and both in this work and in the report of Gazzaniga et al. (1969) there exists a basis for approximate interpretation of the results. Similar analysis was made for vinyl-chloride (Baretta et al., 1969). An exception in this group is represented by 1,1,1,2-tetrachloroethane, which like trichloroethylene undergoes metabolisation predominantly to trichloroethanol and trichloroacetic acid (Phu-Lich et al., 1971).

AROMATIC HYDROCARBONS

Apart from benzene, toluene, xylene, ethylbenzene, and styrene, on which the available information has been reviewed extensively in other chapters of this monograph, there are isolated pieces of information on mesitylene, i-propylbenzene, and alpha-methylstyrene. For the first of these compounds methodical data are available on urinary determination of the main metabolite, mesitylenic acid (3,5-dimethylbenzoic acid) in exposed individuals. The interpretation is, however, of a preliminary character (Laham and Matutina, 1973). For i-propylbenzene there are available relatively exhaustive data obtained in a study on volunteers. The results, yet unpublished, suggest that the exposure evaluation might be based upon determination of dimethylphenylcarbinol in urine (Litewka, 1974). Alpha-methylstyrene was shown to be metabolised in the body similarly to styrene; the main metabolite in man is atrolactic acid (Bardodej and Bardodejova, 1966).

AROMATIC NITRO- AND AMINOCOMPOUNDS

Apart from nitrobenzene discussed extensively early in this book, others of importance are the following nitroderivatives of benzene: trinitrotoluene, dinitrophenol, and dinitro-o-cresol. Among aromatic amines, besides aniline and benzidine, of significance are their derivatives, as well as, the carcinogenic beta-naphthylamine. It seems that recently exposure tests for this group of compounds have not been attracting much interest; and the basic amount of information known from earlier reviews, and in particular from the monograph by Gadaskina and Filov, has not been expanded significantly.

Of other aromatic compounds, some interest has centered on pentachlorophenol. The metabolic data available are those obtained from animal experiments only (Pleskova and Bencze, 1959). With the evaluation of human exposure in mind, methods have been developed and refined only for the determination of the compound in urine (Bencze and Pleskova, 1959; Cranmer and Freal, 1970). Similar attention has been devoted to p-dichlorobenzene (McKinney et al., 1970): a gas-chromatographic method was developed for the determination after hydrolysis of its metabolite, 2,5-dichlorophenol in urine.

Among heterocyclic compounds, preliminary data are available for furfural, which finds application in the refining of mineral oils. As shown

in volunteers, and confirmed in industrial conditions, the exposure evaluation can be based on the determination in urine of the metabolite, furoylglycine (Kemka and Klucik, 1962).

Among other compounds not discussed in detail in this book, relatively exhaustive information exists for an organohalogen pesticide: aldrin and its epoxide, dieldrine; dieldrine is also a metabolite of aldrin. The situation in general is very similar to that for DDT (see chapter: DDT). Both aldrin and dieldrin are lipophilic and, therefore, their levels in adipose tissue and in blood increase with the duration of the exposure. Both are also found in individuals from the general population, and this circumstance has a bearing on the evaluation of occupational exposure (Robinson and Roberts, 1969). Methods for the determination of dieldrin in the blood have been discussed by Robinson et al. (1967) and Richardson et al. (1967). Based on this determination, a "diagnostic" version of the test was developed (Brown et al., 1964); however, the determination of the metabolites in urine has also aroused some interest (Cueto and Hayes, 1962). As the half-life of dieldrin in the human body is about 3 months, the problem of steady-state levels in tissue as a function of dose and duration of the exposure was studied in individuals exposed for a long period via the alimentary route, similar to that described for DDT (Hunter and Robinson, 1967). There are some data available on blood levels in people exposed occupationally, and on the biological effects (Mick et al., 1972; Avar and Czegledi-Janko, 1970; Versteeg and Jager, 1973).

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