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VOID

American Standard

Maximal Acceptable Concentration of Benzene

Sponsor

American Industrial Hygiene Association

Approved October 28, 1960
AMERICAN STANDARDS ASSOCIATION
INCORPORATED

American Standard

Registered United States Patent Office

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Foreword

(This Foreword is not a part of American Standard Maximal Acceptable Concentration of Benzene, Z37.4-1960.)

American Standard Maximal Acceptable Concentration of Benzene, Z37.4-1960, has been developed by a committee, national in scope, functioning under the procedure of the American Standards Association.

This committee was organized to co-ordinate all available information on the various air contaminants and to establish acceptable concentrations which could be used in the development of means for controlling such contamination. This standard on the acceptable concentration of benzene is one of a series of standards which have been prepared by the committee and approved by the American Standards Association.

The initial draft of this standard was prepared co-operatively with the United States Public Health Service. The first standard on this subject was approved January 15, 1941. The subcommittee which developed the present revision of this standard was under the chairmanship of Hervey B. Elkins.

For many years the need for standard acceptable concentrations of toxic dusts, gases, mists, vapors, and fumes in the air of work places has been recognized. A great deal of information on such concentrations has been published, but it frequently differs, due largely to the varying conditions under which observations and tests were made.

While research on the toxic effects of many dusts, fumes, mists, vapors, and gases is continuing in industry, governmental institutions, universities, and elsewhere, the concentration set forth in this standard reflects information obtained from all authoritative published data and the experience of the members of the committee.

The ASA Sectional Committee on Acceptable Concentration of Toxic Dusts and Gases, Z37, which developed this standard, had the following personnel at the time of approval:

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Contents

The Meaning of Maximal Acceptable Concentration

It is generally recognized that there are levels of concentration of atmospheric contaminants to which a person may be exposed without known ill effects or discomfort. Since it is not practical to maintain completely uncontaminated air in most industrial situations, it is necessary to establish maximal acceptable concentrations of atmospheric contaminants encountered in industrial operations. These concentrations in themselves do not represent a scale of relative toxicity; they represent the concentrations of contaminants below which ill effects are unlikely to be experienced by any but hypersusceptible individuals.

These concentrations are usually based upon data obtained by one or more of the following procedures:

(1) Laboratory tests on animals

(2) Laboratory tests using human subjects

(3) Environmental and medical investigations in plants

A common feature of these procedures is that the experimental exposure shall correspond to the normal work pattern. This should be taken into consideration also in the application of maximal acceptable concentration values.

Three criteria have governed the establishment of these concentrations:

(1) Organic or other tissue changes

(2) Functional reactions that have no discernible untoward effects on health but cause impairments, such as in-co-ordinations and increased proneness to accidents

(3) Discomfort or adverse sensory effects

In those cases in which the concentrations have been established on the basis of organic changes, it is logical to assume that repeated exposure to concentrations significantly in excess of the acceptable concentration probably would produce injury. When the level has been established on the basis of a functional change or discomfort, it is logical to assume that exposure to concentrations not greatly exceeding the acceptable concentration would not produce materially injurious effects. Hence it is important to understand the criteria upon which any such maximal acceptable concentration has been established.

Though the purpose of the acceptable concentration is to minimize health hazards, its immediate use is for guidance in establishing engineering procedures to prevent objectionable concentrations of toxic or noxious materials from being present in the air of work places. Comparison of the results of air analyses with maximal acceptable concentrations indicates acceptable conditions or otherwise the need, extent, and urgency of control measures. Sampling and analysis should be performed by competent personnel using an acceptable method that will provide a reliable measure of actual exposure.

Air analyses in conjunction with acceptable concentrations of such atmospheric contaminants as are encountered in industrial operations serve as a measure of exposure, but not as a means of diagnosis of occupational disease. Diagnoses should be based on a consideration of all factors, including results from clinical and physical examination, as well as from air analyses, from means of assessing amounts of materials in the body, and a knowledge of toxicological effects of the materials under consideration.

In the application of the standards, it should be kept in mind that:

(1) The acceptable concentrations serve as standards for good industrial hygiene practice.

(2) They serve as engineering guides. Design and operation should aim at maintaining all concentrations below the acceptable maximum.

(3) They should be applied and interpreted by competent individuals with a full understanding of the basis and limitations of the information from which the standard has been developed.

(4) It is advised that these standards are considered to be guides toward good industrial hygiene and are not intended as legal requirements.

(5) The levels are applicable only for exposure to a single substance. In the case of exposure to mixtures, the effect may be increased or decreased, and controls should be based on the specific situation.

American Standard
Maximal Acceptable Concentration of Benzene

1. Scope and Purpose

1.1 The maximal acceptable concentration of benzene applies to all places of employment.

1.2 The purpose of this standard is to prescribe the maximal acceptable concentration of benzene in the atmosphere of work places for guidance in design and operation, so as to protect the health of the workers.

2. Properties of Benzene [1] *

2.1 General Properties. Benzene (benzol) is the lowest homologue of the aromatic hydrocarbons. It should not be confused with benzine (benzin), a mixture mainly of aliphatic hydrocarbons. Benzene is a colorless liquid. Commercial benzene is seldom pure and may be contaminated with xylene, toluene, phenol, thiophene, carbon disulfide, acetonitrile, and also by pyridine and other substances.

2.2 Physical-Chemical Properties

Chemical formula:	C_6H_6	
Molecular weight:	78.11	[2]
Specific gravity:	0.879 at $\frac{20}{4}$ C	
Vapor density (air = 1.00):	2.7	
Boiling point:	80.1 C	
Melting point:	5.5 C	
Vapor pressure at 26.1 C:	100 mm Hg (millimeters of mercury)	
Flash point (closed cup):	—11 C (12 F)	
Explosive limits (percent by volume in air):	1.4-7.1	[3]
Autoignition temperature:	562 C (1044 F)	

2.3 Toxic Properties. Benzene absorption occurs mainly through inhalation of the vapor. Upon acute exposure, benzene acts predominantly as a narcotic, causing depression of the central nervous system.

With chronic exposure, benzene may cause bone-marrow damage, resulting in a change in the circulating white blood cells, platelets, and red blood cells. Individual susceptibility varies greatly.

Absorbed benzene is excreted partly as phenol conjugated with sulfate in the urine. Determination of the ratio of inorganic to total sulfates in the urine is a useful method of estimating benzene exposure [4].

Medical control procedures should emphasize periodic blood examinations.

3. Maximal Acceptable Concentration

3.1 The maximal acceptable concentration of benzene (benzol) shall be 25 parts per 1,000,000 parts of air by volume, corresponding to 0.08 mg (milligram) per liter at 25 C and 760 mm pressure, for exposures not exceeding a total of 8 hours per day, with the understanding that variations in concentration should fluctuate below this level.

3.2 The maximal acceptable concentration of benzene is based on field studies in plants where benzene poisoning has occurred. Serious cases of benzene poisoning have been reported where concentrations averaged 150 ppm (parts per million) [5], 105 ppm, [6], 100 ppm (3 cases) [7], and 60 ppm [8]. Blood changes were found in workers exposed to average concentrations of 100 ppm and 90 ppm [9], 80 ppm [5], 105 ppm [6], and 60 ppm [8].

The concentration of 25 ppm is intended to provide the average individual with a considerable margin of safety against serious benzene poisoning. A medical control program is recommended whenever workers are exposed to concentrations of benzene vapor approaching the maximal acceptable concentration.

4. Sampling Procedure and Analytical Methods

4.1 Sampling Procedure

4.1.1 The sampling procedure shall be in accordance with what experience shows is necessary to secure a sample which is *truly representative of the actual working conditions at the location tested and for the period of the employees' daily exposure.* This implies that the volume of the sample and the rate at which it is drawn into the sampling apparatus shall

* Numbers in brackets throughout the text refer to corresponding numbers in References on page 9.

be such that the taking of the sample does not itself change appreciably the atmospheric conditions at the location tested. The volume of the sample shall be consistent with the sensitivity of the analytical method employed.

4.1.2 Samples of air should be taken wherever exposure to benzene in the atmosphere is known or suspected to be of hygienic significance.

4.1.3 Samples should be taken at the breathing level, close to the mouth or nose of the worker, with special attention to the locations near the source of the benzene and in the path of air currents carrying it towards the worker, if such condition exists.

4.1.4 Samples should be taken in sufficient numbers to indicate whatever differences in concentration of benzene exist during the working period.

4.2 Analytical Methods. The analytical methods recommended for use are based on the formation of dinitrobenzene by the action of fuming nitric acid and concentrated sulfuric acid. The sample may be taken originally by passing the air through such a nitrating mixture, in a suitable bubbler, at a rate of 0.02 to 0.25 liters per minute [10]; or grab samples may be taken in suitable receptacles and then shaken with nitrating acid [11]; or the air may be passed over silica gel, and the latter heated in the laboratory to drive off the benzene, which is passed through nitrating acid.

The dinitrobenzene may be determined by the color produced by methyl ethyl ketone and alkali.

Any other analytical method may be used, provided it is equivalent in applicability and reliability to those cited.

NOTE: It is understood that the sampling and analysis of contaminated air as specified above, and the interpretation of the data in relation to the stated permissible concentration shall be done only by technically qualified and competent persons.

References to the Text

[1] Physical constants, unless otherwise referenced, are taken from or calculated from *International Critical Tables of Numerical Data, Physics, Chemistry, and Technology*. Published under the direction of National Research Council—National Academy of Sciences. New York: McGraw-Hill Book Company, Inc, 1926-1929.

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American Standards

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The ASA provides the machinery for creating voluntary standards. It serves to eliminate duplication of standards activities and to weld conflicting standards into single, nationally accepted standards under the designation "American Standard."

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On the job benzene vapor exposures of less than 5 ppm were measured concurrent with the 24 hour collection of urine from 52 employees. Urinary phenol levels of the collective study group, measured as either concentration or weight, showed a positive statistically significant correlation with benzene exposure. However, due to variances in individual baseline phenol levels, determination of benzene exposure at these low concentrations is relatively weak. Since baseline phenol concentrations exist over a range of values, adjustment for a single value of each individual's baseline did not improve correlation for the collective study group.

A study of benzene exposure versus urinary phenol levels

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introduction

The 1974 NIOSH proposed standard for benzene[1] requires the monitoring of urinary phenol levels of employees with time-weighted average (TWA) exposures exceeding 5 parts per million (v/v air). The correlation between benzene exposure and increased urinary phenol levels has been reported previously.[2] A level of 75 mg phenol/liter of urine (mg/l) is proposed as a signal of unacceptable benzene absorption requiring close medical surveillance. This study was designed to evaluate the proposed technique of "biological monitoring" in an industrial environment. It was also intended to determine if concentrations in the area of 75 mg phenol/liter of urine are indicative of *unacceptable benzene exposure.*

benzene vapor sampling procedure

Benzene is widely used as a raw material and solvent by The Dow Chemical Company. Five benzene-consuming production facilities were selected to represent industrial environments where benzene is handled along with other chemicals. Over a six month period, benzene exposures were studied for 52 employees from 25 job classifications, including laboratory technician, production operator, plant mechanic, and material transfer operator. Usually, samples were obtained from two employees in each job. Participation was voluntary since the collection of urine depended on each person's willingness to cooperate. Urine samples and breathing zone air samples were collected on the same day. Increases in urinary phenol levels were expected to correlate directly with inhalation of benzene vapors. Skin adsorption was assumed to be negligible since most jobs required the wearing of rubber gloves when handling benzene. Potential exposure to other chemicals should not have affected urinary phenol levels since none were known to interfere with, or contribute to the metabolism of benzene to phenol.

Actual TWA exposure to benzene was determined via personal samplers worn on each employee's collar throughout the course of his workday. Workdays varied from 8 to 12 hours in length. A small battery powered vacuum pump provided a low air flow rate, 100 to 250 milliliters per minute (ml/min), through charcoal packed stainless steel tubes. A few "extra large" commercially available sample tubes were used, but most of the sample collection tubes were custom-packed with Pittsburgh coconut base

For more information about authors, see "this issue's authors . . ." on page A-3

activated charcoal, 12-30 mesh. Charcoal was used because of its suitability for collection of a wide range of aromatic compounds which were likely to be encountered. The custom packed tubes were approximately .47 cm (3/16 in.) in diameter by 12.7cm (5 in.) in length and contained approximately one gram of charcoal. Efficiency of the total sampling system, including error of analysis, was determined by spiking sample tubes and submitting them for analysis with tubes used in monitoring. "Spiking" of the sample tubes was accomplished by preparing in a 100 liter Saran[R] bag a known concentration of benzene, both by itself and with a mixture of other chemicals found in the work place. Air from the bag was drawn through a sample tube at the same flow rate used in the plant studies.

Collection of urine began when the employee started work, and continued for 24 hours. The "day" sample was collected in a hospital specimen container while the employee was at work. A second container for the "night" sample was taken home and used until he returned to work the following day. Thus, the two "combined" urine samples represented approximately 24 hours of urine. A baseline urine sample was collected by each employee after being away from work for a minimum of 48 hours. No other restrictions were placed on the collection of the baseline urine sample. Information concerning consumption, within the last 24 hours, of any medication, tobacco, or alcohol was recorded by each employee for each urine sample. Any known chemical exposure was also noted. Information concerning previous or present health problems, particularly of the liver, kidney, or heart, was requested for each volunteer.

analysis of samples

air samples

The charcoal absorbent used in air monitoring was desorbed with cold carbon disulfide and analyzed by gas chromatography using a flame ionization detector. Specific chromatographic conditions varied over the several months of personnel sampling. Column packings included:

20-30% Carbowax 20,000
10% SP 1000
5% FAPP
5% QF 1/8% SE 30
10% UCW 98
10-20% Carbowax 20,000
5% SP 1200/1.75% Bentone 34
10% LAC 2R446
15% Carbowax 4000
10% DC 200
Durapak OPN/Poracil C 80/100

The columns ranged in length from 1.83m (6 ft.) to 4.57m (15 ft.) and were prepared from .95cm (1/8 in.) inside diameter stainless steel tubing. Column temperatures ranged from 18.3 to 68.3°C (65° to 155°F). Selection of a column depends on the combination of chemicals absorbed on the charcoal. A column packed with SP 1000, Durapak or SP 1200/Bentone would be suitable for benzene alone.

urine samples

Urine phenol analysis was based on the NIOSH proposed standard for benzene.[1] The urine samples were hydrolyzed with perchloric acid at 95°C for two hours, saturated with sodium chloride, and extracted with isopropyl ether. The ether extract was analyzed for total phenol using a flame ionization gas chromatograph. The only modification of the NIOSH method was the saturation of the hydrolyzed urine with sodium chloride. This step increased the extraction efficiency of the isopropyl ether. The chromatograph column consisted of a tube .95 cm (1/8 in.) in diameter, 1.83m (6 ft.) long and filled with 2% EGA CG-AW-DMCS (980-100 mesh) packing. The following temperatures were utilized: column at 160°C, injection port at 230-250°C, and detector temperature at 290-300°C. Maximum sensitivity under these conditions was about 2 mg phenol/liter urine.

results

data

Table I lists data collected from air and urine sample analysis. TWA benzene vapor exposure concentrations are given as ppm (v/v) and they are grouped according to the length of the workday. Concentrations of phenol in urine are

[R]Registered trademark of The Dow Chemical Company abroad.

TABLE I
Results of Air Sampling (ppm) and Urine Analysis (mg/l)

Benzene TWA Range	Number of People	Hours Worked	Concentration Range of Phenol in Urine, mg/l						
				Unadjusted			NIOSH Adjusted		
			Baseline	Day	Night	Combined	Day	Night	Combined
4.0	1	12	7	51	26	39	51	23	37
2.5	1	12	3	28	14	23	37	18	30
2.0	1	12	5	14	6	11	26	13	21
1.5	1	12	8	17	2	6	14	13	14
1.3	1	12	9	16	17	17	18	26	22
1.2	1	12	58	67	30	38	73	60	65
1.1	2	12	1-5	4-6	2-20	3-10	7-11	4-18	6-15
1.0-0.6	7	12	2-36	2-65	2-83	2-73	4-65	5-90	5-77
≤0.5	15	12	1-32	2-39	2-18	2-20	2-36	3-16	3-16
4.4	1	8	6	27	51	35	27	51	35
3.9	1	8	2	41	22	25	55	29	34
3.8	1	8	4	49	22	32	51	22	33
3.6	1	8	2	3	9	6	7	17	13
3.5	1	8	37	46	45	45	61	64	63
3.0	1	8	4	24	21	22	36	28	32
2.6	1	8	3	14	12	13	22	17	19
2.5	1	8	6	10	5	7	10	5	6
2.1	2	8	4-5	11-23	9-10	9-15	18-20	11-15	15-16
2.0	1	8	75	40	64	57	34	55	49
1.3	1	8	2	6	2	5	7	4	6
1.2	1	8	11	46	25	30	37	18	21
1.0-0.6	4	8	2-6	3-21	4-24	4-23	4-18	4-19	4-18
≤0.5	5	8	3-80	2-16	4-12	4-13	6-15	6-11	6-12

expressed as milligrams of phenol per liter of urine (mg/l). The concentrations are listed as measured, and as adjusted according to the NIOSH proposed benzene criteria. The following equation was used to calculate the adjusted values.

$$\text{Adjusted Concentration} = \frac{\text{Measured Concentration} \times \text{Last 2 digits of NIOSH average specific gravity}}{\text{Last 2 digits of measured specific gravity}}$$

TABLE II
Results of Air Sampling (ppm) and Urine Analysis (mg)

Benzene TWA Range	Number of People	Hours Worked	Weight Renge of Phenol in Urine, mg			
			Actual		Adjusted for Baseline	
			Day	Night	Day	Night
4.0	1	12	32	16	28	12
2.5	1	12	21	7	19	5
2.0	1	12	22	7	14	1
1.5	1	12	8	3	4	-7
1.3	1	12	12	18	5	8
1.2	1	12	16	25	2	-23
1.1	2	12	6	2-11	1 to 4	1 to 8
1.0-0.6	7	12	3-65	4-77	-51 to 39	-20 to 52
≤0.5	15	12	2-28	2-9	-10 to 23	-20 to 5
4.4	1	8	21	20	17	18
3.9	1	8	13	31	12	28
3.8	1	8	7	6	7	4
3.6	1	8	5	17	2	13
3.5	1	8	32	82	6	15
3.0	1	8	17	17	14	14
2.6	1	8	12	18	9	13
2.5	1	8	4	3	2	-1
2.1	2	8	5-12	10-11	3 to 10	5 to 6
2.0	1	8	15	52	-13	-9
1.3	1	8	5	1	4	1
1.2	1	8	8	16	6	8
1.0-0.6	4	8	2-6	2-8	0 to 5	1 to 7
≤0.5	5	8	2-6	5-10	-24 to 2	-56 to 3

TABLE III
Coefficients from Correlation of Benzene TWA Exposures with Weight of Urinary Phenol and Urinary Phenol Concentrations

	Urine Segment	Phenol Weight Coefficients	Phenol Concentration Coefficients	
			Measured	NIOSH Adjusted
TWA of 8 hour workday (22 men)	Day	0.66	0.55	0.66
	Night	0.46	0.50	0.63
	Combined	0.53	0.52	0.64
TWA of 12 hour workday (30 men)	Day	0.44	0.42	0.51
	Night	0.18	0.18	0.23
	Combined	0.32	0.34	0.41
TWA of combination of 8 and 12 hour workdays	Day	0.38	0.45	0.56
	Night	0.40	0.37	0.42
	Combined	0.42	0.43	0.50
TWA of combination of 8 and 12 hour workdays, 5 cases of "unacceptable" phenol concentrations deleted	Day	0.57	0.56	0.69
	Night	0.69	0.55	0.69
	Combined	0.72	0.61	0.77
TWA of combination of 8 and 12 hour workdays, 5 cases of "unacceptable" phenol concentrations deleted, 12 hour workday data adjusted to an 8 hour workday	Day	0.70	--	--

Note: A perfect correlation is ±1.0, no correlation is 0.0. Each product moment correlation coeffecient above was calculated as follows:

$$\text{Coeff} = \frac{X_1 Y_1 + X_2 Y_2 + \ldots + XnYn \quad \_\_(\text{mean value of x})\ (\text{mean value of y})}{(n\text{-}1)\ (\text{Std. deviation of X})\ (\text{Std. deviation of Y})}$$

Where n = number of observations
X, Y = parameters being correlated

The average specific gravity of urine used by NIOSH is 1.024. As a comparison, the respective average specific gravities of urine samples included in this report were 1.021, 1.021, and 1.022, for the day samples, night samples and baseline samples. These values are similar to those recently reported[7] for a large work population.

The weight of phenol measured in the urine samples is shown in Table II. Phenol weight was of interest as an alternative to concentration as a means of measuring benzene exposure. Measuring weight instead of concentration would eliminate the problem of low phenol concentration due to the intake of large volumes of liquid. This dilution of urinary phenol levels is handled in the NIOSH criteria by measuring specific gravity, comparing it with a traditionally used average, and adjusting the measured concentration accordingly.

correlation of data parameters

Two main parameters, urinary phenol concentration and phenol weight, were correlated with measured employee TWA benzene exposures. The correlation coefficients and the equation used in calculating them are shown in Table III. A correlation coefficient is a measure of the strength of relationship between two variables. In Table III, most of the coefficients exceed 0.3, a value representing the highest correlation which would be expected if random numbers were substituted for the reported sample values. This value assumes a sample size of 52 observations and a confidence level of 95% or $\rho < 0.05$. Since most of the coefficients exceed 0.3, in most instances a positive significant correlation exists between TWA benzene exposure and the two different measures of urinary phenol levels. Figures 1 and 2 are plots of the strongest relationships for phenol concentration and phenol weight respectively.

Besides comparing two methods of reporting urinary phenol levels, Table III indicates the different ways in which the experimental data was analyzed. Obvious subgroups exist in the total of 52 observations reported. Examination

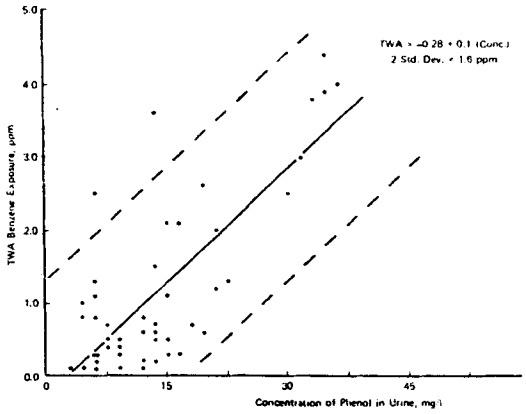

Figure 1 - TWA benzene expsoure versus adjusted concentration of phenol in urine (.5 cases of high baseline concentrations deleted).

of the data included analysis of these subgroups as well as the total group. Two of the sub-groups in Table III resulted from the sampling of employees with two different length, 8 and 12 hour, workdays. A third sub-group with the high baseline urinary phenol levels became evident after the data had been collected. High baseline levels are apparently normal for some individuals and these individuals may exceed the concentration of 75 mg/l, proposed in the NIOSH document as an unacceptable level of "absorption." The five individuals with high background phenol levels will be discussed in more detail later. They were deleted in the last two correlation groups in Table III in order to determine the degree of influence they exerted on the collective group.

The last correlation in Table III was an effort to further examine the relationship between excreted phenol weight and benzene exposure. Since the data had been collected using two different length workdays, it was logical to expect a higher weight of phenol to be excreted in the "day" samples by persons working the longer day. This was assuming vapor exposures were fairly equal throughout the workday. The individual phenol weights collected during the 12 hour workdays were reduced by 1/3 to equalize them with the 8 hour workday. The correlation of this adjusted "day" weight of phenol is reported as the last section of Table III.

This study agrees with previous studies showing a good correlation between benzene exposure and urinary phenol levels. The highest correlations in Table III occurred when the five persons with high background phenol levels were deleted from the data. These correlations are statistically significant at the critical level, $\rho \leq 0.005$. If persons with high baselines are included in the data field, the best correlations are significant at $0.0005 < \rho \leq 0.005$. However Figures 1 and 2 show the unpredictability of this association at relatively low exposure levels. These figures are plots of data, excluding high baseline phenol levels, which generated the highest correlation between phenol concentration/weight and TWA benzene exposure. Plots of data which included persons with high baseline phenol levels showed an even wider and more unpredictable scattering of points. The solid line in Figures 1 and 2 is a least-squares plot, or regression line. The broken lines represent plus or minus two residual standard deviations and should contain between them approximately 95% of the data points. Efforts to use the regression line to predict benzene exposure resulted in the equations shown in Figures 1 and 2. The ppm value for two standard deviations is given below each equation. Two standard deviations for these, the best two correlations, represent an unacceptable 35-40% of the experimental (0-5 ppm benzene) data range. For example, assume an employee's urine was monitored and it contained 25 mg/l of phenol. Based on Figure 1, the best conclusion that can be drawn is that the employee is 95% sure his TWA benzene exposure was between 0.4 and 3.9 ppm. Or, if 100 employees were monitored for one day, and all of the urine

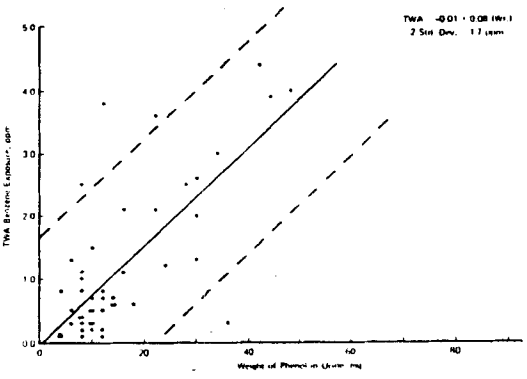

Figure 2 - TWA benzene exposure versus weight of phenol in urine (5 cases of high baseline concentrations deleted).

TABLE IV
Correlation Coefficients for TWA Benzene Versus Urinary Phenol Weight, Adjusted and Unadjusted for Background Phenol Levels

	Urine Segment	Unadjusted for base	Adjusted for base
TWA of 8 hour workday (2 men)	Day	0.66	0.59
	Night	0.46	0.59
	Combined	0.53	0.60
TWA of 12 hour workday (30 men)	Day	0.44	0.38
	Night	0.18	0.15
	Combined	0.32	0.29
TWA of combination of 8 and 12 hour workday	Day	0.38	0.36
	Night	0.40	0.40
	Combined	0.42	0.41
TWA of combined 8 and 12 hour workdays, 5 cases of "unacceptable" phenol concentrations deleted	Day	0.57	0.41
	Night	0.54	0.58
	Combined	0.65	0.53
TWA of combination of 8 and 12 hour workdays, 5 cases of "unacceptable" phenol concentrations deleted, 12 hour workday data adjusted to an 8 hour workday	Day	0.70	0.45

Note: Perfect correlation is ±1.0, no correlation is 0.0.

samples contained 25 mg/l of phenol, one could assume that approximately 95 out of the 100 had had a TWA benzene exposure between 0.4 and 3.9 ppm. Less than 10% of the time will an employee's actual exposure fall on (within 0.1 ppm of) the regression line. Urinary phenol levels are not an accurate method of predicting TWA benzene exposures in the range of 5 ppm or less.

Another observation exists. It is that NIOSH's recommended procedure for adjusting "spot" urine samples to a standard specific gravity slightly improved the correlation of the 24 hour urine samples. NIOSH recommends this

TABLE V
Summary of Employees* with High Baseline Urinary Phenol Levels

Benzene, ppm TWA	Age	Medication used at time of survey	History of medical problems	Work hours per day	Urinary Phenol Levels, mg/l Measured Day	Measured Night	Measured Base	NIOSH Adjusted Day	NIOSH Adjusted Night	NIOSH Adjusted Base
0.2	57	Esidrex	Hi blood	8	16	12	80	15	12	68
0.8			pressure	-	-	-	12	-	-	9
0.8	48	2 Aspirin day of 1st base	No Problems	12	65	83	26	65	91	33
				-	-	-	99	-	-	108
				-	-	-	62	-	-	64
1.2	23	Diet medication during workday sample, Rolaids on 3rd base	No Problems	12	67	30	58	73	60	87
				-	-	-	44	-	-	51
				-	-	-	63	-	-	58
2.0	33	None	No Problems	8	40	64	75	34	55	64
				-	-	-	59	-	-	64
				-	-	-	36	-	-	32
3.5	27	2 Aspirin day of 2nd base	No Problems	8	46	45	37	61	64	47
				-	-	-	6	-	-	7
				-	-	-	84	-	-	77

*All male employees

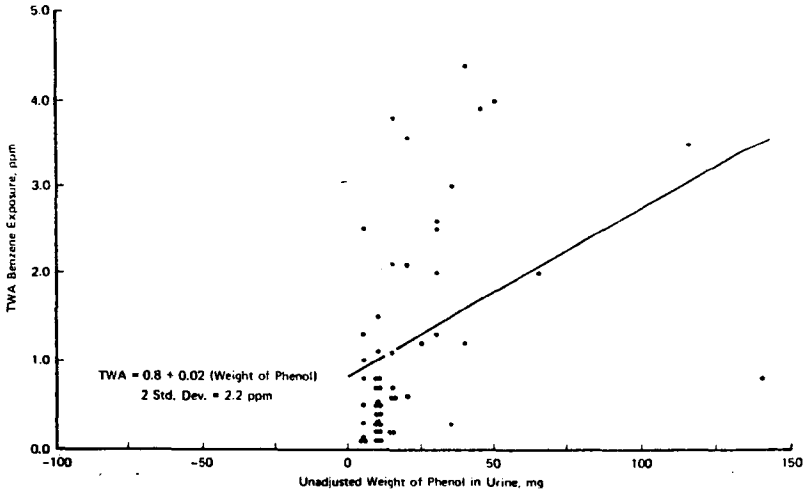

Figure 3–TWA benzene exposure versus unadjusted weight of phenol in urine.

procedure when a single urination is sampled, to allow for dilution of phenol concentrations due to large volumes of liquid intake. When this adjustment was applied to the various urine segments, Table III, the resultant concentration correlated better with TWA benzene exposure.

background phenol levels

Table IV, and Figures 3 and 4 present the result of adjusting measured urinary phenol levels according to each individual's normal baseline level. A baseline urine sample was collected after each volunteer had been away from work or any known chemical vapor exposure for 48 hours. Each baseline phenol weight was calculated and subtracted from the total weight of phenol in their "day" and "night" urine segments. Theoretically, this adjustment should have compensated for persons with normally high urinary phenol levels, leaving the adjusted weights of phenol entirely related to benzene absorption. Figure 3 is a plot of unadjusted urinary phenol weight for all 52 observations. Figure 4 illustrates the effect of subtracting the baseline ground weights of these 52 individuals. Table IV compares group correlation coefficients for TWA benzene exposures versus both unadjusted and adjusted weights of urinary phenol. In most cases, the unadjusted measured weights have a higher group correlation than the adjusted weights. This finding indicates the difficulty in using a single value for baseline urinary phenol levels. For any individual, a baseline range should be determined to accurately assess what phenol level is "above" normal. The negative phenol weights in Figure 4 also indicate that unknown factors are causing greater fluctuations in baseline levels than those resulting from low benzene exposures. A study of non-exposed controls is underway and will be reported in a future publication.

Volume of Urine			Specific gravity		
Day	Night	Base	Day	Night	Base
375	850	44	1.026	1.025	1.028
-	-	25	-	-	1.031
1000	925	78	1.024	1.022	1.019
-	-	140	-	-	1.022
-	-	225	-	-	1.023
235	-	1450	1.022	1.012	1.016
-	-	235	-	-	1.021
-	-	255	-	-	1.026
365	820	260	1.028	1.028	1.028
-	-	55	-	-	1.022
-	-	365	-	-	1.027
690	1825	695	1.018	1.017	1.019
-	-	13	-	-	1.021
-	-	445	-	-	1.026

The benzene ring will not react with water or hydroxyl ions unless it is substituted with a number of electronegative groups (Ayers and Nuder, 1964). Without this substitution, the hydrolysis of benzene will occur only under specialized conditions of temperature and pressure. Oxidation will occur only in the presence of catalysts and/or elevated temperature (Howard and Durkin, 1974). Benzene can be photolyzed at wave lengths less than 290 nm (Howard and Durkin, 1974); Kaplan et al. (1971) demonstrated one of the photoproducts of aqueous benzene to be 1,3-cyclopentadiene-1-carboxaldehyde. Benzene can be photolyzed in smog or heavily polluted air. The long-term stability of benzene under atmosphere conditions is unknown, but it is not completely inert (Howard and Durkin, 1974).

Toxicology Data

Plants

Dunstan et al. (1975) exposed Skeltonema costalom (diatom), Amphidinium carterae (dinoflagellate), Cricospaera carterae (coccolithophorid) and Dunaliella terticlecta (green flagellate) cultures to various concentrations of spectro-grade benzene. In Amphidinium, growth was inhibited at concentrations from .001 to 100 mg/l, while the growth of Cricosphaera carterae was inhibited at benzene concentrations of 10 mg/l to 100 mg/l; Dunaliella tertiolecta growth increased slightly at these concentrations.

Invertebrates

Tigriopus californicus, a tide copepod, was exposed to four different concentrations of benzene (0.87, 0.44, 0.22 and 0.087 mg/l) by Barnett and Kontogiannis (1975). The two highest concentrations proved lethal to 40 percent of the copepods within the first two days of exposure. Of the copepods exposed to the lower benzene concentrations (0.22 and 0.087 mg/l), 70 percent survived the 7 day observation period.

Vertebrates

In a acute toxicity study performed on striped bass (Morone saxatilis) in a continuous flow bioassay, the 72-hour and 96-hour LC50 values for benzene were identical - 10.9 ± 0.2 ul/l, 9.6 mg/l (Meyerhoff, 1975).

Korn et al., (1976b) examined the effects of 3.5 ul/l (3.07 mg/l) and 6.0 ul/l (5.27 mg/l) benzene on the behavior of striped bass, Morone saxatilis, in a flow-thorugh bioassay. Fish were examined at 7 day intervals for 28 days. Benzene caused decreases in dry weight, wet weight and percent fat, but no differences was observed in kilocalories per gram weight between the two groups. Hyperactivity and changes in feeding habits were observed in fish exposed to 5.27 mg/l benzene; fish at the lower concentration were only moderately affected. By one week after initial exposure, behavioral and feeding habits began to return to normal in both groups.

Pickering and Henderson (1966) studied the acute toxicity of benzene * 143
to fresh water fish under static bioassay conditions. In soft water * 145
(20 mg/l; EDTA) 96-hour TLm values for fathead minnows (Pimephales * 145
promelas), bluegill (Lepomis macrochirus), goldfish, (Carassius * 146
auratus) and guppies (Lepistes reticulatus) were 33.47, 22.49, 34.42, * 147
and 36.60, mg/l, respectively. In hardwater (360 mg/l hardness; EDTA) * 149
the 96 hour TLm was 32.00 mg/l for fatheads. * 150

Struhsaker et al. (1974) examined the effects of benzene * 152
concentrations ranging from 5 to 55 ppm-vol/vol on egg and larval * 153
forms of Pacific herring, Clupea harengus pallasi (exposed for up to * 154
96 hours) and on the northern anchovy, Engraulis mordax (exposed for * 155
up to 48 hours) in static bioassays. Herring eggs exposed to an * 156
initial mean concentration of 35.2 to 39.6 mg/l of benzene exhibited * 156
50 percent mortality after 96 hours, as well as delayed or abnormal * 157
development of the larval forms hatched from the exposed eggs. All of * 159
the larvae that survived longer than 33 days appeared normal. Initial * 160
mean concentrations of 17.6 to 22 mg/l caused 50 percent mortality in * 161
herring larvae exposed for 48 hours and observed for 7 days. * 161
Mortality was due to closure of the fore- and hindgut, resulting from * 162
changes in feeding habits. All concentrations greater than 8.8 mg/l * 164
caused a delay in larval development, retarded growth, an increase in * 165
respiration and an interruption of normal behavior. The authors * 167
stated that the benzene concentration in the assay decreased over each * 168
24 hour exposure period (e.g., 39.6 mg/l decreased to 10.6 mg/l or * 169

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less after 24 hours), due to volatilization. Anchovy larvae * 170
experienced 50 percent mortality at 17.6 to 22.0 mg/l benzene after 48 * 171
hour exposure. At a concentration of 4.1 mg/l for a single 24 hour * 172
exposure, benzene caused a slight acceleration of larval development. * 173
Yolk utilization and effects on egg development were directly related * 174
to the length of exposure to benzene. A 24 hour exposure to 4.1 and * 175
9.2 mg/l increased yolk utilization, while 40 hours of exposure to the * 176
same concentrations delayed utilization; developmental rates declined * 177
with increasing concentrations and length of exposure. Anchovy and * 179
herring eggs were more resistant to benzene toxicosis than the larval * 180
forms of both species (Struhsaker, et al., 1974). * 180

Non-Human Mammals * 183

Sprague-Dawley rats of different ages received oral doses of undiluted * 186
benzene to determine the oral LD 50 (Kimura et al., 1970). Acute LD * 188
50's are: Immature rats; 3.4 mg/kg; young adult rats, 3.8 mg/kg; old * 189
adult rats, 5.6 mg/kg. A concentration of 0.87 mg/kg body weight * 191
proved fatal to newborn rats. Deichman et al., (1963) exposed * 193
Sprague-Dawley rats to 15 to 831 ppm benzene vapor for 20 hours/week * 194
for 6 to 31 weeks. Rats exposed to a mean concentration of 65 ppm for * 195
26 out of 39 days showed a decrease in white blood cell count after * 196
two weeks in males and after four weeks in female rats. Animals * 198
exposed to 47 ppm and 31 ppm exhibited abnormalities of the spleen and * 199
lungs. Rats exposed to 831 ppm for 32 or 46 days showed a decrease in * 200

white blood cell count that remained constant throughout the period of exposure.

Hiraki et al. (1963) injected 5 female and 5 male mice with 0.1 ml of a 1 percent solution (0.87 mg) each week. Two mice died in 8 weeks; the remaining 8 mice were treated for 10 weeks. Of these, 2 males and 3 females developed subcutaneous sarcomas. Three of these tumors were transplantable into syngeneic mice. No controls were reported.

Humans

Dobashi (1974) measured the cell renewal rate as well as the rate of DNA synthesis in cultured human leukocytes and HeLa cells exposed to benzene. Both cell types exhibited 50 percent inhibition of growth at 2.2x10-3M (171.6 mg/l). The rate of DNA synthesis was 50 percent inhibited in leukocytes at 2.2x10-3M (171.6 mg/l), while this phenomenon occurred at 1.1x10-3M (85.8 mg/l) benzene in HeLa cells. In man, acute benzene poisoning is characterized by nausea, vomiting, ataxia and excitement, followed by depression and coma. Death is usually the result of respiratory or cardiac failure (Holvey, 1972).

Aksoy et al. (1976) reported that, of 34 patients, six patients having been exposed to 150 to 210 ppm benzene vapor for up to 28 years (mean exposure: 11 years) were diagnosed as having Hodgkin's disease. Twenty other patients, also exposed to benzene, were later diagnosed

as leukemics. Based on case studies, the authors stated that benzene, * 231
because of its toxicity to both the hematopoietic and * 232
reticuloendothelial system, is etiologically related to the onset of * 233
Hodgkin's disease. Alternatively, the authors proposed that benzene * 234
may act with other unknown factors contributing to the onset of this * 235
proliferative disorder. * 236

Aksoy et al. (1974) determined the distribution of types of leukemia * 237
in 34 patients (shoe workers exposed on the job) with chronic benzene * 238
poisoning. The incidence of leukemia was determined to be 13.5 in * 240
100,000, which is greater than the incidence of leukemia in the * 241
general population (6 in 100,000). Patients were chronically exposed * 243
to 210 to 640 ppm benzene vapor for up to 20 years and most frequently * 244
exhibited acute myeloblastic leukemia. Proleukemia, erythroleukemia * 245
and acute lymphoblastic leukemia were also diagnosed. The authors * 247
noted that the number of cases of leukemia among shoe workers declined * 248
in 1974; benzene usage was prohibited in the factory in 1969. The * 250
authors also stated that, in 3 of the workers, genetic predisposition * 250
to leukemia by virtue of a past family medical history of leukemia was * 251
contributory to the onset of the disease. * 252

Thorpe (1974) reported the results of a ten year study of workers * 254
chronically exposed to benzene vapors. In a population of 38,000 * 256
workers exposed to low levels of benzene, the numbers of leukemics was * 257
not abnormal when compared to the general population. * 258

Environmental Fate and Effects * 260

Benzene is susceptible to breakdown by microorganisms. While this has * 263
been proven under laboratory conditions, the extrapolations to * 264
environmental situations are difficult to make. Studies indicating * 265
that benzene can be degraded, with degradation rates depending on * 266
acclimation and incubation of the organisms, have been performed * 267
(Bogan and Sawyer, 1955; Malaney, 1960; Marion and Malaney, 1963; * 267
Chambers et al., 1963), but these studies with mixed cultures indicate * 268
only the possibility of degradation, and at a very slow rate. * 269

Atlas and Bartha (1973) indicated that the biodegradability of benzene * 272
in the marine environment is severely hampered by the limiting * 272
concentrations of nitrogen and phosphorus. It must also be considered * 274
that the possibility of microbes using benzene as the sole or * 275
predominant carbon soruce is rare. * 275

Korn et al. (1976a) investigated the uptake, distribution and * 277
depuration of 14C-benzene in the northern anchovy, Engraulis mordax, * 278
and the striped bass, Morone saxatilis. Anchovies were exposed to * 280
initial concentrations of .00069 ul/l (.00061 mg/l) to 3.7 ul/l (3.3 * 281
mg/l). Bass were exposed to an initial concentration of .088 ul/l * 283
(.08 mg/l). Exposure to 14C-benzene was static for 48 hours, followed * 284
by resumption of water flow for the duration of the experiment (to 9 * 285

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days). Benzene concentrations in the water decreased exponentially throughout the experiment.

The authors reported no deaths during the tests. Accumulation in the bass was greatest in the gall bladder, followed by the colon, liver, intestine, brain, gill, heart, stomach, and muscle. The gallbladder accumulated up to 53.4 and 8,450 times the initial water concentration and the muscle accumulated up to 1.11 and 135 times the water concentration in the bass and anchovy respectively. Maximum concentrations were attained from 0.25 to 4 days after exposure. Gall bladder, fat, and gill tissue retained labeled residue the longest. Depuration in the bass began on day 2 up to day 4 or 5 after cessation of exposure. Residues were undetectable in muscle tissue within 24 hours after exposure ended.

Anchovies showed greater uptake than did the bass, with maximum levels maintained the longest in brain, muscle and intestine. The liver accumulated up to 309 times the initial water concentration.

Benzene appears to accumulate in tissues that exhibit a high lipid content (Korn et al., 1976a) or represent major metabolic sites. The major route of depuration in the two species of fish appears to be liver, gall bladder, intestines and through the colon. In bass, most residues were not detectable beyond 7 days.

Deichman et al. (1963) exposed rats to an average of 47 ppm benzene vapor. Blood benzene levels ranged between 1.624 to 8.70 mg/l (mean = 4.2 mg/l) after 180 days of exposure to the vapor. Rats exposed to 44 ppm for up to 54, 7-hour periods maintained a blood benzene level range of 3.2 to 6.7 mg/l (mean = 4.31 mg/l).

Summary

Benzene has been demonstrated to:

1. Bicaccumulate up to 9450 times in the gall bladder of the northern anchovy.

2. to be a significant environmental pollutant because of its presence in gasolines.

3. to cause changes in feeding habits in fish at 5.27 mg/l.

4. to cause 40 percent lethality in copepods at a concentration as low as .44 mg/l in 48 hours.

5. to be fatal to new born rats at an oral dose of .87 mg/kg body weight.

6. have a 96 hour LC50 for striped bass of 9.6 mg/l.

7. to be implicated as a causitive agent for leukemia in humans.

Criterion Formulation * 341

Based on the bioaccumulation properties of benzene an application * 343
factor of 0.01 is used. When the 0.01 application factor is * 344
multiplied by the 96-hour LC50 of 9600 ug/l for the striped bass, * 345
Morone saxatilis, an economically important food organism, a criterion * 345
of 100 ug/l is derived. Since benzene is considered to be a suspected * 347
carcinogen, all human exposure should be avoided. * 347

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CRITERION DOCUMENT

POLYNUCLEAR AROMATIC HYDROCARBONS

Criterion An interim value of 0.2 ug/l total polycyclic aromatic hydrocarbons is recommended for ambient waters. Because many polynuclear aromatic hydrocarbons are carcinogenic in animals, human exposure should be minimized.

Introduction

Polynuclear aromatic hydrocarbons (PAH's) represent a large class of compounds, many of which are tumorigenic in animals. PAH's are formed as a result of incomplete combustion of organic compounds without sufficient oxygen. This leads to the formation of C-H free radicals which can polymerize to form various PAH's (Groll, 1933; Ellis, 1937). Domestic and industrial soots are the products of incomplete combustion of carbonaceous materials such as wood, coal, and oil. The correlation of exposure to soots and disease was first reported by the British surgeon, Percival Pott (1775), who observed a high incidence of scrotal cancer in chimney sweeps. Three years later, as a result of Pott's finding, the Danish Chimney Sweeps Guild suggested that its members remove soot from their clothing and bathe daily. A century later, Butlin (1892) revealed that, as a result of these

precautions, scrotal skin cancer became relatively rare. Thus, the first example of disease prevention by reduction in direct exposure to the carcinogenic agent(s) was revealed.

Butlin (1892) observed greater incidences of skin cancer among workers in contact with coal-tar and pitch, which are residual products of coal distillation. Naturally formed oil in shale and petroleum, formed by heat and pressure in the earth's crust from decomposed animal and vegetable matter, contain PAH (Berenblum and Schoental, 1943; Catchpole et al., 1971).

The first case of skin carcinoma as a result of exposure to shale oil was reported by Bell (1876) and the carcinogenicity of shale oil was later shown by Leitch (1922).

The following list shows PAHs that have been identified from various sources (IARC, 1973).

Table 1

Some Sources of Polycyclic Aromatic Hydrocarbons

MPOUND	COAL-TAR PITCH	SOOT	SHALE OIL	CRUDE OIL	CRACKED OIL	G E E
nzo(a) pyrene	+	+	+	+	+	
z(a) anthracene						
nzo(b) fluoranthene	+	+				
benz(a,h) anthracene	+	+				
enzo(a,e) pyrene						
enzo(a,h) pyrene	+					
enzo(a,i) pyrene	+					
rysene	+	+				
nzo(j) fluoranthene	+	+				
leno(1,2,3-cd) pyrene						

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57
69
70.

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Concentrations of PAH in carbon-black soot have been reported as high as 20680 mg/kg for benzo(e)pyrene and benzo(a)pyrene (Falk et al., 1958). All compounds listed in Table 2 have been shown to be carcinogenic in laboratory animal tests. These components alone or in combination are believed to be responsible for the carcinogenic effects of these pyrolytic products. Pyrene and fluorene, which are inactive carcinogens, have been found in coal tars (Windholz, 1976).

Polynuclear aromatic hydrocarbons find their way to waterways adsorbed onto aerosols or bacteria (Andelman and Suess, 1970). Although their solubility in water is essentially zero, they may exist in water in association with organic matter or colloids (micelles) as formed by synthetic detergents. Andelman and Suess (1970) reported that carcinogenic PAH concentrations in groundwater were, 0.001 to 0.01 ug/l; treated river and lake water as, 0.01 to <0.025 ug/l; and surface water as, 0.025 to 0.100 ug/l. In highly contaminated surface water levels greater than 0.1 ug/l were found. Borneff and Kunte (1969) have examined ground water concentrations of six easily detected polycyclic aromatic hydrocarbons, fluoranthene, 11,12-benzfluoranthene, and 1,12-benzperylene, which have not been shown to be carcinogenic and 3,4-benzfluoranthene, 3,8-benzpyrene and indeno(1,2,3-cd)pyrene, which have been shown to be carcinogenic.

> .136 ug/l, from 48 samplings. Since these levels would be difficult to remove for drinking water purposes, the authors recommended that a maximum concentration of total polycyclic aromatic hydrocarbons be limited to a level not to exceed 0.2 ug/l for human consumption. The World Health Organization (1970) has also recommended 0.2 ug/l in drinking water as a maximum level for the safety of consumers.

Contaminated waters can have seriously large PAH concentrations. Andelman and Suess (1970) have also quoted benz(a)anthracene concentrations of 0.025 to 10 ug/l and benzo(a)pyrene concentrations of 0.001 to 1.84 ug/l in industrial and bitumen contaminated effluents. Waste water from households, trades, roads, and industrial sources had up to 31.4 ug/l benzo(a)anthracene and 34.5 ug/l benzo(a)pyrene.

Physical and Chemical Properties

Polynuclear aromatic hydrocarbons are a diverse class of compounds consisting of substituted and unsubstituted polycyclic and heterocyclic aromatic rings. They vary in color from white to yellow crystals with diverse melting points (e.g., benzo(c)acridine 108 degrees (Beilstein's handbook); dibenzo(h,rst)pentaphene 320-321 degrees (Beilstein's handbook)). PAHs are not very soluble in water. A few compounds in which

most produced epithelial proliferation but no tumors. Of the three, dibenzanthracene was the most potent.

Application of 1,2,5,6-dibenzanthracene or 3,4-benzopyrene to the amputated tail of the newt, Triturus viridescens, failed to produce tumors; however, the rate of tail regeneration was markedly reduced by these compounds (Finnarello, and Wolsky, 1966). Lecamp and Delsol (1947) reported also that 3,4-benzopyrene did not induce tumors in regenerating limbs of the toad but inhibited the rate of regeneration and appearance of the formed limb. Similar results were observed by Prada (1946) in Triton vulgaris.

Matoltsky (1947) reported no tumor formation in the amphibians, Rana esculenta and Triton cristatus injected with a 0.3 percent solution of 3,4-benzopyrene. However, he observed hemorrhaging in the kidney and liver, parenchymal degeneration, fatty degeneration, and necrosis. In the frog (R. esculenta) he observed pulmonary edema, cellular infiltration, and edematous swelling of the alveolar walls as well as edema of the skin and abdomen. Bonte (1950) also failed to induce tumors in frogs with 3,4-benzopyrene, implanted or painted on the skin; however, the compound produced atrophy and regression of the mucous glands of the skin and increased the permeability of the skin to water.

Pontenwill (1953) observed inhibition of cleavage and disturbances in the formation of blastomeres and neurolation in Triton and Axototl eggs exposed to unspecified concentrations of 3,4-benzopyrene. Colombo (1948), however, found that benzo(a)pyrene concentrations of 1:2,000 to 1:20,000 had no effect on the development of the ova, morula, or gastrula of the frog, Rana esculenta. Ruhland and Weiss (1954) observed that 1:1,000 to 1:10,000 solutions of benzo(a)pyrene reduced the motility of the sperm of Rana fusca; however, eggs fertilized by these sperm developed normally.

Birds

Intratracheal administration of 3-methylcholanthrene (dose not specified) to ducks produces acute and chronic inflammation, and prolonged administration of the compound produced a variety of pulmonary tumors. Administration of 3,4-benzopyrene also produces chronic pulmonary inflammation, but no tumors (Rigdon and Neal, 1965). Benzopyrene does not appear to be acutely toxic to ducks or chickens given a single oral dose of 250 mg (Rigdon, and Neal, 1963).

Administration of up to 2.5 mg benzopyrene/g of food for 24 days does not affect the growth or survival of chicks, nor does a diet of 0.1 mg/g of food have any effect on the sperm, ova, egg

fertility, or chicks from eggs obtained from treated hens (Pigdon, and Neal 1963). Hatchability of fertilized chicken eggs was determined after injection of 0.1 ug/egg of different PAH (Peno, 1968). The hatchability was reduced to 51 percent of the controls for benzo(a)pyrene, 73 percent for benzo(k)fluoranthene, 70 percent dibenzo(a,h)anthracene, 55 percent dibenzo(a,j)acridine and 76 percent benzo(r,s,t)pentaphene. Different combinations of these PAH led to a greater than additive effect.

Mammals

In an in vitro study on the effect of 5 PAH compounds on the activity of selected enzymes, Gemant (1967) observed that the activity of catalase, an enzyme that acts on hydrogen peroxide and thus regulates the amount of this compound in tissues, was reduced by up to 50 percent by the PAH compounds. In order of decreasing inhibition potency, the compounds were 3-methylcholanthrene, 3,4-benzopyrene, 1,2-benzanthracene, 9,10-dimethyl-1,2-benzanthracene, and anthracene, which suggests that the catalase-inhibiting potency of the PAH compounds is related to their carcinogenic potency. None of these compounds had any effect on the activity of peroxidase.

activity of lipoxygenase, another oxidoreductase, was only 352
44 percent of the control level in the presence of 352
3,4-benzopyrene, the most potent inhibitor, and only 46 and 353
61 percent of the control value in the presence of 354
methylcholanthrene and dimethylbenzanthracene. Of the three 355
carcinogens, dimethylbenzanthracene inhibited that activity of 356
ribonuclease most and benzopyrene was least effective. None of 357
the carcinogens had any effect on the activity of trypsin. 357

In mice injected with 1.25 mg of 3,4-benzopyrene or 2.5 mg of 360
3-methylcholanthrene (Draganov, 1966), a significant increase 360
occurred in succinic dehydrogenase activity in lung tissue 60 362
days after injection but not at 30 days after the injections. He 363
reported that enhanced succinic dehydrogenase activity occurred 363
at the same time that pathological changes in the lung tissue 364
were observed. Zinnari (1964) observed that same effect in liver 365
tissue, but enhanced succinic dehydrogenase activity occurred 366
much more rapidly. In mice injected with a 2 percent solution of 367
3,4-benzo(a)pyrene, the activity of succinic dehydrogenase 367
increased about 3-fold above the control level on the second day 368
and gradually decreased to near control levels by the 30th day. 369
However, the degree of morphological changes in the liver 370
mitrochondria increased with time, rather than with enhancement 371
of succinic dehydrogenase activity. 371

The effect of PAH compounds on the activity of cathepsin from subcellular fractions of rat liver homogenates was investigated by Lomsadze and coworkers (1969). They found that dimethylbenzanthracene, 3-methylcholanthrene, 1,2-benzo(a)pyrene, and anthracene lowered the activity of the enzyme at a concentration of 0.005M. Anthracene was lease effective. These investigators also found a reduction in cathepsin activity in the rat liver 2 to 5 months after a single injection of 5 mg of dimethylbenzanthracene to rats.

DeLuca (1969) reported no changes in liver glutamic-oxalacetic transaminase (GOT) or glucose-6-phosphate activity in rats injected with 0.1 mg 3,4-benzo(a)pyrene; however, at a dose of 0.5 mg, the activity of GOT was enhanced.

Intravenous injection of 0.5 mg of anthracene, pyrene, perylene, 3,4-benzopyrene, or 1,2,5,6-dibenzanthracene caused an increase in liver SH levels in mice within 15 minutes to 1.5 hours. Subsequently, the SH levels dropped to below normal.

A list of several PAHs are given in Table I with carcinogenic data for different animal species. This table represents only a few of the PAHs tested and are included in the list of PAHs selected by the International Agency for Research on Cancer Working Group (1973) based on their carcinogenicity in animals.

e PAHs were administered orally, in the diet or applied on the 397
skin. 397

Table 2

Compound	Animal	Administered	Effect
enzo(a) anthracene	mice	oral	papillomas hepatomas
enzo(b) fluoranthene	mice	cutaneous	papillomas, carcinom
enzo(j) fluoranthene	mice	cutaneous	carcinomas
enzo(a) pyrene	mice	diet	stomach tumors
	rats	oral	mammary tumors
	hamsters	oral	papillomas, carcinom
	rabbits	cutaneous	carcinomas
enzo(a) pyrene	mice	cutaneous	papillomas, carcinom
hrysene	mice	cutaneous	papillomas, carcinom
ibenz(a,h) anthracene	mice	diet	carcinomas
ibenzo(a,e) pyrene	mice	cutaneous	papillomas, carcinom
ibenzo(a,h) pyrene	mice	cutaneous	papillomas, epitheli
ibenao(a,i) pyrene	mice	cutaneous	papillomas, epitheli
ndeno,(1,2,3,cd) pyrene	mice	cutaneous	papillomas, carcinom

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