INTRODUCTION

The carcinogenic potential of certain polycyclic aromatic hydrocarbons (PAH's), e.g., benz(a)pyrene and benz(a)anthracene, has been well documented (IARC Monograph, Volume 3, 1973; Dipple, 1976). However, comparatively little information exists upon which to base an evaluation of the carcinogenic potential of chrysene. Further, isolated exposure to chrysene occurs only in specific operations, i.e., chrysene manufacture, laboratory experimentation, and possibly in the synthesis of anthraquinone vat dyes. The number of workers thus exposed is small relative to the number of workers exposed to chrysene ad-mixed with other PAH's in industrial processes which involve the pyrolysis of organic matter. This notwithstanding, sufficient quantities of chrysene may now be in use to elicit concern over any biological effects exerted by this chemical. Thus, an integration and evaluation of all experimental data pertinent to the carcinogenic potential of chrysene appears warranted.

There is now no federal occupational health standard for chrysene, or for any other individual PAH. Nor has the American Conference of Governmental Industrial Hygienists (ACGIH) recommended Threshold Limit Values (TLV's) for individual PAH's. Concern for occupational exposure to PAH's as a class is reflected, however, in the federal occupational exposure limit for Coke Oven Emissions (29 CFR 1910.1029) of 0.15 milligram/cubic meter of air (mg/cu m) measured as the benzene-soluble fraction of total particulate matter (BSFTPM) and as a time weighted

average (TWA). In addition, the ACGIH (1976) has recommended a TLV-TWA of 0.20 mg/cu m (as BSFTPM) for both coal tar pitch volatiles (CTPV) and particulate polycyclic aromatic hydrocarbons (PPAH). Completed NIOSH criteria documents on asphalt fumes, and coal tar products also address PAH exposures, as will future criteria documents on the roofing industry, carbon black, and welding and brazing. It also should be noted that the criteria document on coal tar products contains a recommendation for a permissible exposure limit of 0.1 mg/cu m measured as the cyclohexane-soluble fraction of total particulate matter (CSFTPM).

The Federal coke oven standard, the ACGIH TLV's, and the NIOSH recommended standards, all deal with PAH's from particular sources. Different sources produce various amounts and mixtures of PAH's, and the individual PAH's have considerable ranges of toxicity. Adequate assessment of the hazards from occupational exposure to the general class of PAH's, and development of effective standards for worker protection probably will require their handling on a process basis.

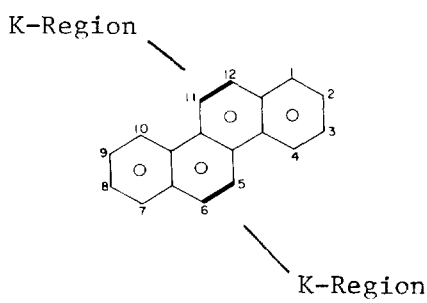
A review of the health hazards associated with chrysene and some of its derivatives, i.e., methylchrysene isomers, follows. General recommendations are included for control of occupational exposure in chrysene work areas, i.e., areas where chrysene is manufactured, used, or stored. Chrysene is merely one of many PAH's (which have wide ranges of toxicity) generated inadvertently in certain occupational environments, and the control recommendations included in this document may be applicable to

many PAH's, regardless of their source or fractional content. However, NIOSH recognizes that this will not necessarily be the case under all circumstances of PAH-exposure. The information and recommendations will aid the U.S. Department of Labor, industrial hygienists, occupational physicians, and employers in providing workers with protection from the hazards of exposure to chrysene. They also will aid workers in recognizing these hazards so that individual protective action may be taken.

I. PROPERTIES AND CHARACTERISTICS OF CHRYSENE

A. Identification

1. Synonyms: 1,2-benzophenanthrene
   1,2-benzphenanthrene
   benz (a) phenanthrene
   1,2,5,6-dibenzonaphthalene
2. CAS number: 000218019
3. Formula: C18H12
4. Molecular weight: 228.28



B. Physical/Chemical

1. Appearance and odor: colorless and odorless platelets, i.e., solid at STP in pure reagent form
2. Boiling point: 448 C (at 760 mm Hg)

3. Melting point: 254 C

4. Specific gravity: 1.274

5. Solubility: insoluble in water; slightly soluble in alcohol, ether, carbon disulfide, glacial acetic acid; soluble in benzene.

II. COMMON OPERATIONS, USE, AND OCCURRENCE

A. Workplace Environment

PAH's are perhaps the most ubiquitous of the carcinogenic agents that have been detected in the atmosphere. Chrysene, along with other PAH's, occurs most frequently as an adsorbate on airborne particulates resulting from the pyrolysis of carbonaceous materials (Filatova et al., 1972). Chrysene is sometimes encountered as a vapor. The yield of chrysene can be affected by the chemical and physical nature of the pyrolyzed compound, the pyrolysis temperature, the presence or absence of adequate oxygen for complete combustion, and the period of time during which the compound is exposed to heat. Experimental determinations have shown the optimal temperature for PAH formation to be around 700 C (Masuda et al., 1967). From an occupational health standpoint it is important to identify the sources of these compounds. Table I lists some of these sources for chrysene.

TABLE I. SOURCES AND CONCENTRATIONS OF CHRYSENE FOUND IN WORKPLACE ENVIRONMENTS

Source	Concentration*	Reference
Particulate matter, catalyst regeneration flue gas of an oil refinery	12,000 ppm	Sawicki et al., 1965
Coal tar pitches from coke oven production	7400 ppm and 10,000 ppm	Wallcave et al., 1971
Particulate matter, effluent of coal furnace	7000 ppm	Sawicki et al., 1965
Coal tar	2130 and 2860 ppm	Lijinsky et al., 1963
Creosote	940 and 1340 ppm	Lijinsky et al., 1963
Asphalt	34.0 ppm	Wallcave et al., 1971
Effluent from tar-fired retorts in a gas-producing plant	12.6 ppm	Cleary, 1963
Carbon black	3.0 ppm	Qazi and Nau, 1975
Soot from brick kiln exhaust	2.5 ppm	Cleary, 1963
Smoke from burning wood	0.58 ppm of sawdust burned	Rhee and Bratzler, 1968

TABLE I. SOURCES AND CONCENTRATIONS OF CHRYSENE FOUND IN WORKPLACE ENVIRONMENTS (continued)

Petrolatums	0.01 and 0.44 ppm	Lijinsky et al., 1963
Certain organic solvents derived from coal tar or petroleum:		Lijinsky and Raha, 1961
hexane-2	0.12 ppm	
benzene AR-1	0.0018 ppm	
iso-octane	0.0009 ppm	
Resinous substances from air in coke and metallurgical plants	----	Filatova et al., 1972
Extracts from bituminous coal prior to pyrolysis	----	Tye et al., 1966
Stack gases from sulfate pulping mill	----	Hendrickson et al., 1963
Extracts of processed rubber stoppers and automobile tires	----	Falk et al., 1951

*Unless otherwise stated, the units (ppm) refer to parts of chrysene per million parts of source material sampled, as a weight/weight ratio.

B. Occupational Areas of Concern

At present, it is believed that exposure to chrysene as an isolated chemical occurs only in specific occupations, i.e., in chrysene manufacture, laboratory experimentation, and perhaps in the synthesis of anthraquinone vat dyes. It was Holbro (1953) who suggested the use of chrysene as a starting material in the synthesis of dyestuffs, particularly of anthraquinone vat dyes, to which the results of his research showed chrysene imparted favorable properties regarding "affinity, shade, and fastness". While the scope of this report is limited primarily to chrysene as an isolated chemical, its occurrence with other PAH's in a wide range of occupational environments cannot be disregarded.

The following list of occupational areas, where worker exposure to chrysene and other PAH's is likely to occur, is separated into stationary-point and non-stationary point source categories; the former refers to inplace operations, i.e., where engineering controls could be expected to substantially reduce employee exposure, and the latter refers to outdoor or mobile operations where the application of engineering controls may be more difficult. In the latter case, however, it would be expected that natural factors (wind, etc.) would contribute to reductions in worker exposure. While this list is based on the occurrence of chrysene as it has been reported in the scientific literature, many other occupational exposure sources undoubtedly exist.

Stationary point sources

Aluminum reduction operations

Boiler-room operations

Carbon black manufacturing operations

Charcoal manufacturing operations

Coffee roasting operations

Coke oven operations (refer to Federal Standar
on Coke Oven Emissions)

Grill cooking operations

Iron foundry operations

"Liquid smoke" production

Municipal incinerator operations

Petroleum cracking operations

Rubber processing operations

Sintering mill operations

Smokehouse operations

Sulphate paper pulping mills

Wood preservative treatment processes

Non-stationary point sources

Acetylene-torch cutting and brazing operations

Airport runway operations

Bus terminal operations (boarding areas)

Diesel trucking operations

Furnace maintenance and repair operations

Gasoline station and automobile repair operations

Heavy equipment operations, e.g., tractors, cranes and earth-movers

Road paving and sealing operations

Roofing operations

Street sweeping operations

Telephone line repair and installation

Traffic control

Traffic tunnel repair

C. General Environment

Chrysene is found in the general environment primarily as a product of the pyrolysis of fossil fuels for energy production and the operation of diesel- and gasoline-fueled internal combustion engines.

Chrysene has been measured in the atmospheres of several major cities. In Sydney, Australia, a 12-month survey of the ambient PAH content of suspended particulate matter showed chrysene at concentrations ranging from 0.0002 to 0.0065 (u)g/cu m of air (Cleary and Sullivan, 1965). Chrysene was found in the atmosphere of Buffalo, New York, at concentrations of 0.082-0.116 (u)g/cu m of air, and in composite samples from the air of urban Cincinnati, Ohio at concentrations of 0.0032 and 0.006 (u)g/cu m of air (Conlee et al., 1967).

Studies have been conducted to determine the contribution of the gasoline engine to atmospheric levels of PAH's. Gasoline engine exhaust

tar was found to contain 175 ppm chrysene (Hoffman and Wynder, 1962). In another investigation, the PAH emissions from diesel- and gasoline-powered vehicles were compared (Sullivan and Cleary, 1964), and chrysene was found in a diesel-trafficked area at a concentration of 0.0032 (u)g/cu m of air; in the gasoline-trafficked area, chrysene was found at a concentration of 0.0034 (u)g/cu m of air.

In addition to the finding of chrysene in ambient air, its presence in soil and water has been substantiated. Although quantitative data are few, chrysene has been identified in soils in rural Massachusetts and Connecticut (Blumer, 1961), and in wells, rivers, lakes, and soils in Germany (Borneff, 1964; Borneff and Kunte, 1965). One investigator suggested that the occurrence of chrysene and other PAH's in rural soil is not due to fallout from polluted air but, rather, is the result of the natural pyrolytic transformation of plant organic matter to peat and lignite; or that alternatively, they occur as metabolites of organisms which reside in the soils (Blumer, 1961).

Chrysene also has been detected in certain food products: roasted coffee (Kuratsune and Hueper, 1960), spinach and tomatoes (Grimmer and Hildebrand, 1965), baker's yeast (Grimmer and Wilhelm, 1969), "liquid smoke" (Lijinsky and Shubik, 1965), smoked ham and barbecued beef (Malanoski et al., 1968), charcoal-broiled steak and chicken (Lijinsky and Ross, 1967), and electric-broiled fish (Masuda et al., 1966).

Of particular importance is the occurrence of chrysene in cigarette-smoke condensate. Chrysene has been found in cigarette-smoke condensate at a concentration of 0.06 (u)g per 100 cigarettes (Van Duuren, 1958). Cigarette smoke may add significantly to the total exposure to chrysene from both the general environment and work environment.

III. BIOLOGICAL EFFECTS OF EXPOSURE

A. Toxic Effects in Animals

Schmid et al. (1967) used mortality and body and organ weight changes in mice as measures of chrysene toxicity. Each of 10 male AKR and 10 male C57BL/6 mice was given a single i.p. injection of 7.5 mg chrysene dissolved in 1 ml of sesame oil. All animals were alive after 20 days of observation at which time they were killed. Autopsies revealed that none of the mice had experienced any significant change in body, spleen, or thymus weight. The investigators concluded that chrysene was not toxic as reflected by the aforementioned parameters and experimental conditions.

Gershbein (1958) tested the effects of chrysene on liver regeneration in partially hepatectomized adult male Sprague-Dawley rats. Single s.c. injections of 12.0 mg chrysene in peanut oil were given daily for the 11-day test period to each rat (320 mg/kg body weight). This treatment caused no significant change in the extent of liver regeneration from that in the corresponding controls.

Bernheim et al. (1953) found that a 0.3% solution of chrysene in benzene, when painted on the skins of white mice, inhibited fatty acid peroxide-induced thiobarbituric acid-color development on the epidermal side, but did not in the subcutaneous fat. The investigators attributed these results to inhibition of fatty acid oxidation. They noted that oxidized fatty acids are more effective inhibitors of certain enzymes and of bacterial growth than are the corresponding unoxidized acids, so that a carcinogen, by inhibiting peroxide formation, might produce conditions favorable for growth. They also suggested that inhibition of fatty acid oxidation may be a necessary, but certainly not a sufficient, condition for tumorigenesis.

B. Carcinogenicity in Animals

A number of experimental animal studies of the carcinogenic potential of chrysene have yielded varying results. Most of these studies have been conducted with mice, and various investigators have described chrysene as a weak carcinogen (Wynder and Hoffman, 1959); a cancer "initiator" (Hoffman et al., 1974); an "incomplete" carcinogen (Horton and Christian, 1974); and as a cancer "inhibitor" (Huggins et al., 1964). The following experimental results illustrate the variability in reported carcinogenic activity of chrysene.

Grover et al. (1975) tested the carcinogenicity of chrysene and chrysene 5,6-oxide (an epoxide), in newborn Swiss mice (Table II.A.), from a specific pathogen-free breeding colony. The mice were each injected

TABLE II. SUMMARY OF SELECTED EXPERIMENTS TO ASSESS THE CARCINOGENICITY OF CHRYSENE

A. MICE, newborn Swiss. Route of exposure: Subcutaneous injection
Grover et al., 1975)

Compound	dose per treatment	no. of treatments	total mice injected (M&F)	no. mice weaned (day 21) total, M,F	no. survivors at week 70	no. mice examined at termination of experiment (70-75 week) with: liver tumors	multiple liver tumors	lung tumors	multiple lung tumors	other tumors
Chrysene	100(u)g	3	104	51, 29, 22	27 M	13	6	1	1	0
					21 F	0	0	1	0	0
Chrysene 5,6-oxide	100(u)g	3	107	47, 24, 23	21 M	8	3	0	0	0
					20 F	1	0	0	0	0
PEG 400 (controls)	-	3	96	52, 34, 18	30 M	9	1	3	1	0
					15 F	0	0	1	0	0

B. MICE, C 57 black. Route of exposure: Subcutaneous injection
(Boyland and Sims, 1967)

Compound	number of injections x dose in mg	number of animals surviving until the appearance of the first tumor	sex	number of animals with tumors	time of first appearance of tumors (in days after first injection)
Chrysene	10 x 1	20	M	2	248 and 262
Chrysene 5,6-oxide	10 x 1	20	M	3	189, 284, and 303
Arachis oil (controls)	10 x 1	20	M	0	-

C. MICE, CD-1. Route of exposure: Skin application
(Scribner, 1973)

Compound	dose in (u) moles	dose of TPA in (u) moles	animals per group	tumor incidence, papillomas/mouse (% mice with tumors) (wk) 10	15	20	25	30	35	survivors 35 wk.
Chrysene	4.4	10	30	0.33 (20)	2.03 (60)	3.27 (73)	3.07 (77)	2.83 (67)	2.87 (73)	29
none	-	10	30	0 (0)	0 (0)	0 (0)	0.03 (3)	0 (0)	0 (0)	30

*TPA - tetradecanoyl phorbol acetate, applied, after chrysene, as a "promoter."

TABLE II: SUMMARY OF SELECTED EXPERIMENTS TO ASSESS THE CARCINOGENICITY OF CHRYSENE (continued)

D. MICE, ICR/HA Swiss. Route of exposure: Skin application
(Van Duuren et al., 1966)

primary (single dose)	secondary dose per animal (3 times weekly)	cumulative no. of mice with: papilloma	cancer	days to first: papilloma	cancer	survivors at end of experiment	weeks on test
Chrysene, 1 mg in 0.4 ml acetone	croton resin, 25(u)g in 0.1 ml acetone	16	2	125	411	10/20	63
Chrysene, 1 mg in 0.4 ml acetone	none	0	0	-	-	7/20	63
none	0.1 ml acetone	0	0	-	-	16/20	63
none	croton resin, 25(u)g in 0.1 ml acetone	5	1	237	411	13/20	63
none	none	0	0	-	-	70/100	63

E. MICE, C3H. Route of exposure: Skin application
(Horton and Christian, 1974)

Compound	vehicle	number and volume in (u)l applications per week	original no. mice	no. mice alive at 52 weeks	number of mice with tumors: carcinomas	papillomas	median time of tumor induction (wk)
1:1 (vol) Dodecane:Decalin	none	2 x 60	unknown	13	0	2	74
Chrysene, 0.15%	Decalin	2 x 60	20	12	0	1	76
Chrysene, 0.15%	1:1 (vol) Dodecane:Decalin	2 x 60	20	19	12	5	49

F. MICE, C57 black. Route of exposure: Subcutaneous injection
(Steiner and Falk, 1951) (Steiner and Falk, 1951)

Compound	amount of chemical	no. of mice	no. of mice surviving when first tumor appeared	no. of induced sarcomas	tumor yield (per cent)	minimum induction time (days)	average induction time (days)
Chrysene	5.0 mg in 0.5 cc tricaprylin	50	24	4	16.6	349	401
1,2-benzanthracene	5.0 mg in 0.5 cc tricaprylin	50	44	8	18.2	161	285
Chrysene plus 1,2-benzanthracene	2.5 mg each, in 0.5 cc tricaprylin	50	34	15	44.1	230	346

(s.c.) with 100(u)g of a test chemical, on each of the first 3 days of life. The test compounds were administered as 0.02 ml suspensions using polyethylene glycol (PEG) 400 as the vehicle. Surviving mice were weaned at 21 days of age and observed for 70-75 weeks. Of the 48 survivors at 70 weeks in the chrysene-treated group, liver tumors were observed in 13 (6 of which had multiple tumors) and lung tumors were seen in 2 (one of which had multiple tumors). Of the 45 chrysene 5,6-oxide-treated survivors at the end of 70-75 weeks, 9 had developed liver tumors (3 of which had multiple tumors). With the PEG 400 vehicle controls, of the 45 mice surviving to 70 weeks, 9 had liver tumors (1 of which was multiple) and 4 mice had lung tumors (1 of which was multiple). The investigators concluded that chrysene seemed to be a more active tumorigen than its epoxide, and that it increased the incidence of liver tumors above that in the controls, while the epoxide did not do so, and that neither chrysene nor the epoxide affected the incidence of lung tumors. The liver tumors ranged in appearance from well differentiated liver cell masses that were difficult to distinguish from normal liver tissues to those exhibiting pleomorphism, invasion, and metastasis. The increased incidence of liver tumors after exposure to chrysene, however, was not statistically significant (P=0.05).

Boyland and Sims (1967) conducted a similar experiment with chrysene and chrysene 5,6-oxide (Table II.B.). C57 black mice, 120 days old, were injected s.c. with 1.0 ml suspensions of arachis oil that contained 1.0 mg of either chrysene or chrysene 5,6-oxide, once/week, for 10 weeks. A third group, given only arachis oil, served as the control. After an observation period ranging from 60 to 80 weeks, 2 of the 20 chrysene-treated mice

surviving at the time of observation of the first tumor (initial number of animals treated was not stated) had developed injection-site tumors. A comparable number of animals (3 of 20) developed injection-site tumors from the epoxide but the time-to-tumor was longer by an average of 8.6 days. Because of the longer latency period for the epoxide, the investigators concluded that the parent PAH was a more active tumorigen. None of the control animals had tumors. Of the tumors induced by the active compounds, about 80% were spindle-cell sarcomas, about 12% were pleomorphic sarcomas and about 8% were squamous cell carcinomas.

Wynder and Hoffman (1959) applied a 1% solution of chrysene in acetone to the backs of female Swiss (Millerton) mice, three times weekly until the last animal died (sometime during the 13th month of treatment). Nine of the original 20 animals had developed papillomas and 8 had developed carcinomas, with the first tumor appearing after 8 months of treatment. From the original data it is not possible to determine with exactitude the number of animals with both papillomas and carcinomas. A decrease in lifespan was also noted; no controls were included in the experiment. It is unlikely, however, that such a high incidence of carcinomas would occur spontaneously in this strain of mouse.

Several investigators have shown chrysene to act as a cancer "initiator." Hoffman et al. (1974) tested the tumor-initiating activity of chrysene and of six of its methyl derivatives. The purities of the test substances were greater than 99.9 %. Ten doses of 0.1 ml of acetone each containing 0.1 mg of the test PAH (total dose, 1.0 mg) were applied on

alternate days to the shaved backs of each of 20 female Swiss albino mice (Ha/ICR/Mil). Application of the "promoting" substance, tetradecanoyl phorbol acetate (TPA) was begun 10 days following the last PAH-treatment. TPA, in doses of 2.5 (u)g in 0.1 ml of acetone, was applied three times a week for 20 weeks, for total doses of 0.15 mg. Acetone alone (10 doses of 0.1 ml) served as a control treatment and was reported to have produced negative results in a separate group of mice, although no quantitative data were given. In the chrysene-exposed group, after 20 weeks of TPA applications, 11 of the 18 surviving mice had a total of 19 tumors. The 3-methyl- and especially the 5-methylchrysene were even stronger tumor initiators than was chrysene, with 14 of 20 surviving mice sharing a total of 26 tumors, and 17 of 20 surviving mice sharing a total of 96 tumors, respectively. Each compound was concurrently bioassayed for "complete" carcinogenicity in mice of the same strain and sex. Solutions of 0.1 mg of each material in 0.1 ml acetone were applied under identical conditions to the shaved backs of the animals, three times weekly for the duration of the experiment. After 30 weeks, 5-methylchrysene showed high carcinogenic activity. Each of the 20 mice had tumors (with a total of 99 tumors), including 12 with 37 carcinomas, and 2 with multiple metastases to the lungs and spleen. Chrysene and the other five methylchrysenes showed no significant tumorigenic activity according to the investigators. Quantitative data for chrysene and for the control vehicle were not given.

Hecht et al. (1976) also tested 5-methylchrysene for "complete" carcinogenicity via two routes. Ten s.c. injections of 0.05 mg 5-methylchrysene in trioctanoin (0.1 ml each injection) were given to each of

25 male C-57 black mice at 2-week intervals, after which the animals were observed for 32 weeks. This exposure to 5-methylchrysene resulted in 24 fibrosarcomas in 22 animals, with the first tumor appearing at 25 weeks. Vehicle controls had no tumors. In addition, 5-methylchrysene was compared with benzo(a)pyrene (BaP) for tumor-initiating potential and "complete" carcinogenicity on mouse skin. For the tumor-initiating tests, ten initiator doses of 3 (u)g of 5-methylchrysene dissolved in acetone were applied to the skins of 20 female Swiss mice (Ha/ICR) during the second resting phase of the hair growth cycle. "Promotion" was accomplished by applying 0.1 ml of a 0.0025% solution of TPA in acetone thrice weekly for 20 weeks. Benzo(a)pyrene was tested similarly. The results indicated that 5-methylchrysene was a stronger tumor initiator than BaP; application of the former compound resulted in a total of 45 tumors in 20 mice (including 1 mouse with 1 carcinoma), whereas exposure to the latter resulted in a total of 6 tumors (no carcinomas) in 6 mice. The latency period for both groups was 5 weeks. None of the control mice had tumors. Each chemical was also applied thrice weekly at concentrations of 0.01% and 0.005% in 0.1 ml acetone to the shaved skins of 20 mice. After 55 weeks of exposure to 5-methylchrysene, 15 mice bore 38 tumors, including 10 mice with 12 carcinomas. Comparable results were seen in the BaP-treated mice. The control mice (solvent alone) developed no tumors. The investigators suggested that the demonstrated carcinogenicity of 5-methylchrysene may have resulted from the high electron densities at its 5-6 and 11-12 K-regions due to the electron-releasing effect of the methyl group; the high electron density might facilitate either metabolic activation to an

"ultimate" carcinogen or detoxification. (See additional discussion below.)

Scribner (1973) (Table II.C.), showed chrysene to produce multiple papillomas when used in small doses as an initiator. The backs of 30 female CD-1 mice, 8 weeks old, were shaved and treated 2 days later with chrysene in acetone (total chrysene dose, 4.4 (u) moles). One week later, a twice-weekly treatment with TPA was begun. At the end of 35 weeks, 73% of the 29 survivors had developed papillomas, whereas the control group (TPA only) had developed none.

Similar results had been obtained by Van Duuren et al. (1966) when, in order to test its cancer-initiating potential, they applied chrysene in doses of 1.0 mg (in 0.4 ml of acetone) to the shaved backs of 20 female ICR/HA Swiss strain mice (Table II.D). A known tumor promoter, croton resin, in doses of 25.0 mg (in 0.1 ml of acetone) was applied to the same mice beginning 13-21 days after the chrysene treatment and thrice weekly thereafter. This combined treatment resulted in papillomas in 16 of 20 mice and carcinomas in 2 of 20 mice, with the first observation of the former after 125 days and of the latter after 411 days of the 63-week experiment. Controls receiving only croton resin showed a much lower incidence of tumors (5 benign, 1 malignant). The authors stated that previous experimentation with this substance alone by other investigators had resulted in no malignancies. The investigators concluded that chrysene acts as a "notable" cancer- initiating substance. In the same experiment,

exposure to chrysene alone failed to result in the development of tumors in mice.

Other investigators have found evidence that chrysene may act synergistically with certain susbtances to induce tumorigenesis. Horton and Christian (1974) found that a 0.15% solution of chrysene in decahydronaphthalene (Decalin), a "non-carcinogenic" agent, when painted on the backs of 20 C3H male mice (60 (u)l doses, 2 x week), induced a papilloma in 1 of 12 surviving animals after 76 weeks of treatment (Table II.E.). However, when chrysene was applied in a 0.15% solution with a 50:50 Decalin: n-dodecane mixture (a known co-carcinogenic mixture) to the backs of 20 mice, 12 and 5 out of 19 surviving animals developed papillomas and carcinomas, respectively. The median time for tumor induction was 49 weeks. The effects of n-dodecane alone were not tested for. The authors classified chrysene as an "incomplete" carcinogen, i.e., one lacking the co-carcinogenic activity of a strong "complete" carcinogen.

Steiner and Falk (1951) studied the carcinogenic activity of chrysene in combination with 1,2-benzanthracene (Table II.F.). The actions of chrysene and of 1,2-benzanthracene were first tested separately. Fifty C57 black mice, 3-4 months old and in about equal numbers of males and females, were injected s.c. with 5.0 mg chrysene in 0.5 cc tricaprylin. There was a total of 4 injection-site sarcomas among the 24 surviving mice for a tumor incidence of 16.6 % compared with a 1.3 % incidence of tumors in the tricaprylin controls and a zero incidence in the uninjected controls. The

investigators considered the results to have shown that chrysene has a definite carcinogenic potential. A tumor incidence of 18.2 % was seen after the administration of 1,2-benzanthracene with the average induction time of 285 days. A mixed solution of the two PAH's (2.5 mg each) in tricaprylin was then administered to each of 50 mice in a like manner. The tumor incidence from this dose was 44.1%. Because this incidence was a little greater than the summation of the individual responses at full doses, the investigators concluded that the results were demonstrative of either summation or synergism.

In a follow-up study by Steiner (1955), a mixture of the "strong" carcinogen 1,2,5,6-dibenzanthrene with chrysene, when administered s.c. to approximately fifty C57BL mice, showed no significant additivity of effects, i.e., the incidence of tumors was the same as for either agent alone, and the question was raised by the investigator as to whether there had not been actual inhibition of tumorigenesis.

Huggins et al. (1964) attempted to detect any inhibitory effects of chrysene upon the action of a more potent carcinogen. In these experiments, 2 mg i.v. injections of 7,12-dimethylbenz(a)anthracene (7,12-DMBA) were given to each of 74 rats on days 50, 53, and 56 of their lives. Mammary tumors resulted in all 74 animals (100%). A separate group of animals was given (gastric intubation) single doses of chrysene (40 mg) followed by the same 7,12-DMBA treatment. These animals showed no significant change in the incidence of mammary tumors. However, in another group of test animals, multiple doses of chrysene (15 mg x 16 days) during

a period that overlapped i.v. injections of 7,12-DMBA greatly reduced the incidence of mammary tumors (60% vs 100%).

Riegel et al. (1951), painted a mixture of chrysene (15%) and methylcholanthrene (15%) in acetone on the shaved backs of CF1 mice (0.02 ml of test solution, twice weekly, for 31 weeks) and compared its carcinogenic activity with that of methylcholanthrene (15% in acetone) alone. In the group treated only with methylcholanthrene, 23 of 28 animals (82%) developed tumors after a mean latent period of 14.1 weeks. In the group painted with the chrysene/methylcholanthrene mixture, 17 of 20 animals (85%) developed tumors after a mean latent period of 13.8 weeks. Mean latent periods were calculated from the day that painting with the mixture was begun. From these results, the investigators concluded that chrysene had no significant inhibitory effect on the carcinogenicity of methylcholanthrene. Similar applications of 0.20% chrysene in acetone to 20 mice resulted in one tumor among 16 survivors at 31 weeks.

In addition to the positive animal test results, indirect evidence for the carcinogenicity of chrysene can be derived from results with two different in vitro test systems.

McCann et al. (1975) reported that both chrysene and chrysene-5,6-oxide were mutagenic in the Ames test. This test utilizes the organism, Salmonella typhimurium, to indicate DNA damage, and mammalian liver extracts for metabolic activation of the test chemical. Chrysene was tested on strain TA100 and resulted in 38 revertants per nanomole. The

investigators noted that the relationship between the mutagenic potency of a substance on a particular strain to its overall mutagenic potential on DNA in general, and to carcinogenic potency, remained to be determined.

Huberman et al. (1972) tested the effects of chrysene (15.0 (u)g/ml for 4 hours) on Syrian hamster embryo cells in culture and found definite dose dependent increases in the numbers of "malignant" transformations in exposed cell colonies. Also, it was found that chrysene 5,6-oxide (15.0 (u)g/ml) was even more active in producing transformations. Similar relationships were observed between other PAH's and their epoxides. For this reason, and because such epoxides had been shown to be metabolic intermediates of PAH's, the investigators postulated that the epoxides are possibly the ultimate carcinogenic forms of at least those PAH's that do not contain active methyl groups.

In a follow-up to the 1972 study of Huberman et al., Huberman and Sachs (1976) tested chrysene, in the presence of enzymes required for its metabolic activation, on Chinese hamster cells in culture (2-day treatment at 1(u)g/ml/day). Chrysene showed negligible mutagenic activity (9 mutants for chrysene vs 6 mutants for solvent alone, per 10,000 surviving cells).

Negative results were also obtained by Marquardt et al. (1972) who tested the ability of chrysene and its epoxide (1.0-10.0 (u)g/ml doses for each) to transform cells derived from mouse prostate.

According to other investigators, the primary process in cancer induction (by PAH's) involves binding to a cellular component, e.g., DNA, RNA, or protein, at the K-region of the PAH (Raha et al. 1973). Typically,

the K-region of a PAH is a highly reactive site. Experimentally measured bond lengths, chemical reactivities, and valence-bond and molecular orbital calculations all agree in assigning a higher electron density to the K-region than to any other bond in the PAH molecule (Herndon, 1973).

C. Toxic Effects in Humans

No reports concerning the toxicity of chrysene in humans were located. Considerations for any toxic effects of chrysene in humans would primarily be concerned with inhalation of particulate matter, since chrysene is encountered most frequently as an airborne particulate or as an adsorbate on airborne particulates. The occurrence of any biological effect would be closely related to the physical characteristics of the particle. Particle size predominantly determines the extent to which penetration of (and retention by) the tracheobronchial tree will occur, with maximum retention falling within an aerodynamic particle size range of 0.5 to 2.0 microns (Kotin and Falk, 1964). Ingestion and skin absorption must also be considered potential routes of exposure. Further, it should be noted that small amounts of chrysene-containing particles might be ingested following their inhalation as particles and mucus reflux from the bronchial tree (Hill et al., 1972).

No epidemiologic studies concerning the carcinogenicity of chrysene per se were located in the literature. The carcinogenic potential of chrysene in humans must therefore be estimated on the basis of animal studies or be deduced from experiences with mixed exposure to chrysene and other PAH's.

D. Summary of Carcinogenicity Data

While it is clear that exposure to PAH's is associated with increased risk for both lung and skin cancer in humans, the relative contribution of individual compounds is difficult to determine. Chrysene is a component in virtually all occupational exposures to PAH's. In order to assess the specific carcinogenic potential of chrysene, evidence based mainly on animal experiments, and supported by in vitro test results, must be utilized.

Evidence of chrysene's carcinogenic potential has come primarily from experiments in which the isolated chemical (and certain derivatives) was administered to animals by skin painting or by s.c. injection. Chrysene also has been shown to be active in some mammalian cell culture tests and in microbial assays for mutagenicity.

Certain generalizations can be made regarding the tumorigenic potential of chrysene. For instance, its tumorigenic potential seems to vary in a dose-dependent manner. Large doses and repeated applications, as well as lengthy induction periods, are usually required to obtain significant incidences of tumorigenesis, prompting many investigators to classify chrysene as a "weak" carcinogen. Further, it seems that chrysene may have a greater effect when acting in concert with other, more potent agents, i.e., as a tumor-initiator, a synergist, or an "incomplete" carcinogen, enhancing tumor formation but lacking the capacity for complete carcinogenesis. In one study, chrysene appeared to have inhibited the

activity of a more potent carcinogen, but this case was contradicted by other similar investigations that have produced evidence to the contrary. Thus, no generalization can be made regarding chrysene's possible tumor "inhibiting" properties. Tests on mammalian cells in culture also have produced conflicting results: some investigations have shown chrysene to produce malignant transformations in a dose-dependent fashion, while others have shown it to have a negligible effect. In microbial assays, however, chrysene has been mutagenic.

Chrysene's methyl derivatives and certain of their epoxides have been shown to possess carcinogenic potential. The 5,6 oxide of chrysene (an epoxide) was shown to be less active than chrysene in in vivo tests, but in in vitro tests on mammalian cell cultures and in microbial assays, the converse was seen. Because such epoxides have been shown to react covalently with cellular macromolecules in vitro, they have been postulated to be likely candidates for the role of the ultimate carcinogenic derivatives of PAH's (Huberman et al., 1972). Several possible explanations have been suggested for the lack of epoxide activity in vivo as opposed to in vitro. For instance, because of solubility and other factors the epoxide may be less able to enter the cell (Boyland and Sims, 1967), or possibly the high chemical reactivity of such epoxides could cause them to react nonspecifically in tissues (with extracellular keratin and other molecules) and hence be depleted before reaching and reacting with intracellular target macromolecules (Huberman et al., 1972). The 5-methylchrysene has been shown to be both a strong, "complete" carcinogen and a more active tumor "initiator" than the parent chrysene. One possible

explanation for the carcinogenic potential of this compound may lie in its metabolic activation by the formation of a carbonium ion on the methyl group (Marquardt et al., 1972).

Thus, there exists certain evidence indicating that chrysene and its derivatives have carcinogenic potential. Additional research is needed to elucidate the nature and extent of chrysene's carcinogenic activity and the mechanisms whereby such effects may be exerted. No experimental studies were found in the literature concerning the inhalation of chrysene as an adsorbate on airborne particulate matter, i.e., the mode of entry that represents one of the most probable routes of occupational exposure. Further, and most importantly, it should be noted that early studies which produced negative results for chrysene quite likely used inadequate numbers of animals with insufficient periods of observation (Steiner and Falk, 1951).

IV. CONTROL RECOMMENDATIONS FOR CHRYSENE

A. Engineering Controls

Engineering controls should receive primary emphasis for the reduction of occupational exposure to chrysene. Typically, such engineering controls include process enclosure. In addition, chrysene work areas, i.e., areas where chrysene is manufactured, used, or stored, should be isolated from all other work areas. Chrysene work areas should be maintained under negative pressure with respect to non-chrysene work areas. This may be

accomplished by providing continuous local exhaust ventilation so that air movement is always from non-chyrsene to chrysene work areas. Exhaust air from chrysene work areas, including laboratory gloveboxes, should not be discharged into any work area or into the general environment unless it is first decontaminated. One means to accomplish this is through the use of bag filters in conjunction with the ventilation system. All process equipment, including laboratory gloveboxes, should be inspected for leaks and other malfunctions on a regular basis. In addition, ventilation systems should be tested periodically for their effectiveness, e.g., air velocity, static pressure, and air volume.

B. Respiratory Protection

Use of respirators for protection against exposure to chrysene should be permitted only during the time period necessary to install feasible engineering controls. Two other exceptions would be during nonroutine maintenance and repair activities where engineering controls are not technologically feasible, and during emergencies. A type C supplied-air respirator operated in pressure-demand or other positive pressure modes, or self-contained breathing apparatus with a full facepiece operated in pressure-demand or other positive pressure modes would provide appropriate protection. Respirators should be selected from among those approved by the National Institute for Occupational Safety and Health or the Mining Enforcement and Safety Administration under the provisions of 30 CFR 11.

C. Protective Clothing

Employers should provide and ensure that all employees use appropriate protective clothing, e.g., coveralls and rubber or latex gloves, to prevent skin contact with chrysene during cleanup of spills and during those activities described in part B above.

D. Posting of Signs

Entrances to chrysene work areas should be posted with signs indicating:

DANGER

AUTHORIZED PERSONNEL ONLY

CANCER SUSPECT AGENT

E. Work Practices

Procedures for cleanup of spills and disposal of chemical waste should be instituted. Persons involved with cleanup activites should wear appropriate personal protective equipment and clothing as outlined above. Since chrysene crystals can be electrostatic, contaminated surfaces should be wetted with clean or soapy water followed by careful wiping with disposable towels. All materials collected (including disposable towels) should be placed in sealed containers for disposal in a secured sanitary landfill, or disposed of in an appropriate incinerator, i.e., one operating

at high enough temperatures and with sufficiently long contact times for complete combustion. General housekeeping and sanitation practices should also be established and enforced. Before leaving chrysene work areas at the end of each day, employees who have had direct dermal contact with chrysene, e.g., through handling, cleanup of spills, or accidents, should wash exposed skin areas with soap and water and discard contaminated clothing for laundering or disposal. Further, food storage, preparation, and consumption should be prohibited in chrysene work areas.

F. Informing Employees of the Hazard

Persons employed in chrysene work areas should be informed of the potential hazards of chrysene, including the results of animal carcinogenicity and mutagenicity tests.

G. Medical Surveillance

Medical surveillance for workers with potential for exposure to chrysene should consist of preplacement examinations and periodic evaluations on at least an annual basis.

The history and physical examinations should place emphasis on the smoking habits, skin, respiratory tract, and urinary system. Clinical testing should include chest x-rays and urinalysis with such other tests as sputum cytology carried out as considered appropriate by the responsible physician.

H. Recordkeeping and Availability of Records

Employers should keep accurate records of (1) all measurements taken to determine employee exposure to chrysene, (2) measurements demonstrating the effectiveness of mechanical ventilation, and (3) all data obtained from medical examinations which is pertinent to chrysene exposure. Pertinent records from medical examinations should be maintained for at least 30 years after the worker's employment has ended.

V. SAMPLING AND ANALYTICAL METHODS

A workplace environmental limit for chrysene is not recommended at this time. However, in the event that NIOSH should establish such a limit (either for chrysene or for the general class of PAH's) in the future, a validated sampling and analytical procedure would be required.

NIOSH has not validated a sampling method for chrysene. Chrysene is normally encountered as an airborne pollutant (usually adsorbed on airborne particulate matter, but sometimes as a vapor) along with numerous other PAH's and their derivatives. When chrysene is encountered as a solid or is adsorbed on particulate matter, a sampling method similar to the one described in the occupational standard for coke oven emissions (29 CFR 1910.1029) would be appropriate.

NIOSH has not validated a specific analytical procedure for chrysene. The choice of any particular method depends upon the sample source, the

amount collected, time constraints, financial constraints, or personal preferences. The occupational standard for coke oven emissions (29 CFR 1910.1029) utilizes the benzene-soluble fraction of total particulate matter (BSFTPM) as an indicator of worker exposure to PAH's. This procedure, however, does not identify individual PAH's. In order to identify and quantify individual PAH's, they must first be separated from any interfering compounds with which they occur.

Many reviews concerning the state of the art of PAH analysis have appeared in the literature, e.g., Sawicki, 1964; Sawicki et al., 1967; Rutzinger et al., 1973; Zander, 1975. In addition, Appendix A contains a list of some analytical methods which have been used to determine the PAH content of various compounds.