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FORMATION AND MANAGEMENT OF AN EXPERT TOXICOLOGICAL REVIEW TEAM--ETC(U)

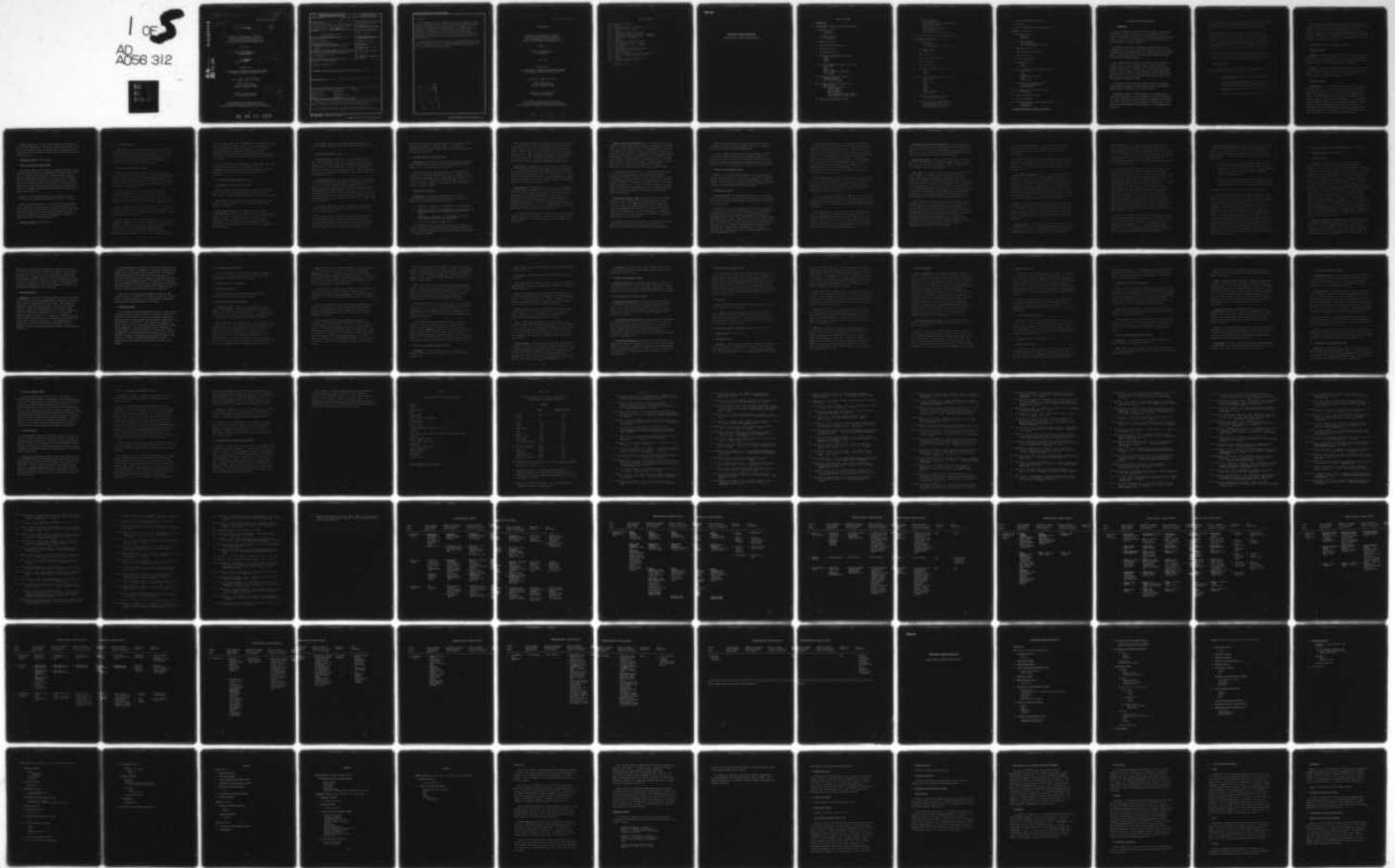
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6/ Formation and Management of an Expert
Toxicological Review Team for Literature
Search, Evaluation and Organization of
Currently Available Rapid Toxicological Tests

Volume II.

10/ Arthur J. Shanahan, Ph.D.
Program Manager

11/ April 1978

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Six compounds (benzene, cadmium, formaldehyde, phosphorus, phosgene, oxides of nitrogen) were chosen as models for analysis by a matrix format. Each matrix was supported by a review article. Panel members selected predictive endpoints from acute and subchronic data taken from the literature. The panel also recommended short-term tests relevant to the endpoints for each compound. Position papers were developed for: pharmacokinetics; behavioral toxicity; *in vitro* testing; reproductive assessment testing and a concept for toxicological testing. Bibliographies were prepared for the matrix reviews, each position paper, and one for the overall study.

Based on the analyses of the matrices, the position papers and their collective experience, the panel developed recommendations for short-term tests for a minimal toxicology screening program and pointed out gaps wherein additional research was required.

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FINAL REPORT

Formation and Management of an Expert
Toxicological Review Team for Literature
Search, Evaluation and Organization of
Currently Available Rapid Toxicological Tests

Volume II

Arthur J. Shanahan, Ph.D.
Program Manager

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EXPERIMENTAL BENZENE INTOXICATION

Backup Report to Benzene Toxicity Matrix

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EXPERIMENTAL BENZENE INTOXICATION

I. INTRODUCTION

Occupational diseases have been with us for several centuries, but the problem of industrial toxicity has increased markedly since the industrial revolution and recently, in the postwar era, especially in the plastics industry, where large quantities of benzene were being used as starting material for chemical synthesis.

The American Conference of Governmental Industrial Hygienists has recommended that workers not be exposed to atmospheric levels of benzene exceeding 25 ppm, while NIOSH suggests that exposure should not exceed 10 ppm as a time-weighted average of a 10-hour day or 40-hour work week, with no exposure exceeding 25 ppm.

The incidence of acute toxicity is low but can under conditions of stress be fatal. The most important effect of chronic exposure to benzene is depression of bone marrow. Both acute and chronic benzene exposure result in changes in the blood, central nervous system (CNS), vascular function, and liver, kidney and lung functions. Additionally, there is evidence to show that benzene causes birth defects and loss in body weight in experimental animals.

The symptoms of acute and chronic benzene toxicity are different. While benzene itself appears to cause acute toxicity, its metabolites appear to play an essential role in chronic toxicity.

Experimental evidence in animals suggests that, barring cases of hypersensitivity, there is in all likelihood a relationship between age, sex, route of administration, dose, duration of exposure, and the degree of severity of the toxic effects of benzene in various species. Thus, each of the above parameters becomes important in the in vitro testing system (1).

It is now well established that the toxicity of benzene is related to its solubility in the body lipids, and under chronic conditions of exposure, its accumulation is greatest in the fatty tissue (2).

For the purpose of this review, acute toxicity is defined as resulting from a single exposure (inhalation), injection intravenous (iv), subcutaneous (sc), intraperitoneal (ip), intramuscular (im), oral administration (po), or direct contact (by application or accidental contact). Subchronic toxicity results from repeated treatment during a 90-day period or less (short term tests), and chronic results from repeated treatment over a period of 2 years or more (long term tests).

The effects of benzene on in vitro cell and organ cultures and organ and tissue extracts, as well as its metabolism (in vivo) and studies on its mechanism of action, will be described under acute toxicity.

Information Sources

The information contained in the body of this backup document is basically derived from such current critical reviews as:

- o Occupational Exposure to Benzene, DHEW, No. (NIOSH)74-137 (NTIS TB 246-700), 1974
- o A Critical Evaluation of Benzene Toxicity, 1977, Editors, S. Laskin and B.D. Goldstein, N.Y.U., Institute of Environmental Medicine, for API, Contract U-150-14 (PS-7)
- o Benzene Health Effects Assessment, 1977, EPA. External Review Draft
- o Mutagenicity Studies with Benzene, J. Lyon, 1975.

The specific points of information noted in the backup document were checked by reading the pertinent literature citation. In addition, a few pertinent citations that were identified through a Tracor Jitco literature search are included. The information contained in the backup document is summarized in the appended matrix. The term "none reported", which appears in the matrix, means that no literature reference related to that particular item was identified.

II. ACUTE TOXICITY (including in vitro observations)

1. Hematologic Effects

Leucopenia. Single sc injections of benzene (1 ml/kg) into rabbits produced a rapid fall in the number of leucocytes in the peripheral blood within 24 hours of treatment (2). Female rabbits were found to be more sensitive than males to the leucopenic effect of benzene (3).

Hemolysis. Exposure to high concentrations (10,000 ppm) of benzene resulted in high blood levels of the toxin in rabbits (20-30 mg/100 ml), and this produced a decrease in red blood cells (RBC) and an increase in the RBC resistance to saponin and hemolysis (4).

2. Bone Marrow Changes

Erythropoiesis. In an attempt to detect early effects of benzene on RBC production, Lee et al. (5, 6) used the incorporation of ⁵⁹Fe into the hemoglobin of maturing red cells as a measure of RBC synthesis in the mouse. They found that single sc doses of benzene (440-2200 mg/kg) reduced ⁵⁹Fe incorporation into erythrocytes at a time when leucocyte production was not affected. Using this technique, it was possible to show, in agreement with Steinberg (7) and Moeschlin and Speck (8) who used the radioautographic method, that the early stage of red cell maturation, involving pronormoblast and normoblasts, was more sensitive to benzene toxicity, than were the stem cells, (colony-forming cells, CFC), reticulocytes, or the process of hemoglobin synthesis.

However, Uyeki et al. (9) have recently shown that mice exposed to benzene vapors (4680 ppm for 8 hours) show a significant depletion of the CFC of the bone marrow 24 hours following exposure, indicating that the precursor cells of the hemic cell renewal system are sensitive to benzene inhalation in mice.

3. Immunologic Response - None reported.

4. Central Nervous System (CNS) Effects

The narcotic threshold concentration for laboratory animals is about 4000 ppm. Concentrations above 10,000 ppm result in death within a short time. For example, rabbits exposed to 35,000-45,000 ppm of benzene developed light anesthetic reactions at 3.7 minutes (min) followed by excitation and tremors (5.0 min), loss of papillary reflex to light (6.5 min), loss of blinking reflex to tactual stimuli (11.4 min), papillary contraction (12 min), involuntary blinking (15.6 min), and death (38.2 min) (10). Low doses (0.1 mg) given iv resulted in excitation, severe hemolysis, and death (11).

Gerarde (2) noted that effects such as breathlessness, nervous irritability, and unsteady gait may persist for a period of 2-3 weeks.

Orally, benzene was more toxic to 14-day-old rats and young adults than to older males. The oral LD₅₀'s for the newborn, young, and adult male rats were 1.0, 3.8, and 5.6 mg/kg, respectively (12). Higher doses resulted in paralysis (hind limb), petechial bleeding (urinary tract, eyes, nose), and mild, acute gastritis accompanied by stripping of epithelial cells of the stomach lining (2, 13).

5. Behavioral Effects - See CNS Effects.

6. Cardiovascular Effects

Nahum and Hoff (14) exposed cats and monkeys to air saturated with benzene vapor and recorded ventricular extrasystole or periods of ventricular tachycardia, which occasionally terminated in ventricular fibrillation. The postulated mechanism is the rapid release of the adrenal hormone, epi- and norepinephrine, into the bloodstream, sensitizing the myocardium to the action of benzene.

7. Biochemical and Histochemical Changes

Spinal Cord. A histochemical assay of the spinal cord of mice exposed for 40 minutes to 60 mg/l of benzene in air (18,780 ppm) revealed a decrease in the activity of succinic dehydrogenase, NADH-diaphorase, alkaline phosphatase, 5-nucleotidase, acid phosphatase, and thiolacetic esterase. There was also an elevation of deoxyribonuclease II in the white matter of the spinal cord (15).

Succinic dehydrogenase is directly associated with benzene metabolism, but the functions of the other enzymes remain to be elucidated. Jonck et al. (15) suggest that benzene selectively influences oxidation in neurons, and they postulate that the changes could result from a direct lesion on the lipoprotein membrane of the cell.

Brain. Kadyrov and Safarov (16) exposed rabbits to 35 mg/l and 0.35 mg/l of benzene and measured the activity of glutamic decarboxylase in various areas of the brain. The activity of the enzyme was significantly increased at both concentrations.

Kidney. Enzymatic activity was studied in kidney slices of mice exposed to 3750 ppm benzene (12 mg/l) for 40 minutes; some were killed immediately and some were killed one hour later. Histochemical studies showed increased succinate dehydrogenase and NADH₂-diaphorase (respiratory enzymes) and Ca-formol-ATPase and alkaline and acid phosphatases (active transport enzymes) activity. However, the activity

in all the enzymes, except the acid phosphatase, was decreased at 3 hours and increased at 12 hours post exposure. The acid phosphatase activity did not decrease until 12 hours after exposure (17). The authors considered that benzene initially activates the processes of intracellular respiration and active transport and then depresses them. A subsidence of the poisoning symptoms was observed in the final phase of the experiment.

The above enzymatic studies demonstrate the connection between enzyme activity and benzene toxicity. A few enzymes, such as the dehydrogenases, have been found to be directly responsible for benzene metabolism, but the functions of most of the others remain to be elucidated.

8. Effect on Body Weight, Organs and Tissues

Body Weight. No adverse effects reported.

Skin. The defatting effect of benzene is well known. Withey and Hall (13) and Gerarde (2) showed that direct contact with high concentrations of benzene leads to hemorrhage and destruction of the skin.

Fatty Tissues. Withey and Hall (13) noted destruction of the soft tissues of the internal organs in rats, which resulted from lipid solubility of benzene.

Spleen, Thymus and Lymph Nodes. Benzene produced a marked reticuloendothelial response in the lymph node, spleen, thymus and bone marrow of rats (18). Single sc injections of 0.4 ml/kg resulted in decreased thrombocyte formation (120 hours), increased number of macrophages in the lymph node and thymus (16 hours), and marked proliferation of reticuloendothelial cells in the spleen, accompanied by aberrant morphology.

Other Organs. High doses of benzene produced hemorrhage in the brain, pleurae, pericardium, urinary tract, and mucous membranes of rats (2).

9. Cytologic and Cytogenetic Effects

Nuclear Aberrations. Rondanelli et al. (19, 20) observed giant nuclei among erythroid precursor cells of humans and newts exposed in vitro to 135 microgram/ml of benzene. Observations over several hours showed that mitotic abnormalities were the result of unequal nuclear division, polynucleated cells, and atypical nuclei. Benzene induction of giant nuclei occurred through polyploidy, as seen in preparations from exposed humans (21-25).

Chromosomal Aberrations. An increased incidence of chromatid breaks and gaps was observed in cultured human leucocyte and HeLa cells at 1.1 or 2.2×10^{-3} M benzene after brief exposures to benzene (26, 27). Another study in which the peripheral blood lymphocytes were stimulated by phytohemagglutinin (PHA) exposed to benzene for 72 hours resulted in both numerical and structural alterations in chromosomes (28). In this study, aneuploidy was seven times more frequent in treated cultures, and chromosomal breaks were seen in 11% of the treated cells as compared with 1% of the controls.

In one other study in which rats were injected sc (2 ml/kg) with benzene, chromatid breaks and gaps were observed at 12 and 24 hours in cells of the bone marrow (29).

Since chromatid gaps are difficult to interpret because their rates vary significantly with the interpreter and the preparation, their inclusion as chromosomal aberrations has been questioned. For example in the study where toluene-tested controls were used (30), significant increases in aberrations were also found in the controls, but the

benzene-treated animals in this group showed a greater number of breaks than gaps as aberrations. Exchange figures, i.e., ring forms, translocations, and dicentrics, which result from abnormal repair after breakage, were rarely seen.

10. The Molecular Site of Benzene Toxicity

DNA Synthesis. Moeschlin and Speck (8) injected rabbits with single doses (2 mg/kg) of pure benzene and found that DNA-synthesis in bone marrow cells is severely inhibited at this dose.

Some investigators have used incorporation of ^{14}C -thymidine into the nucleus as a measure of DNA synthesis. By this technique a decrease in DNA synthesis was demonstrated in human lymphocytes and HeLa cells (26, 27) in culture, following brief exposures to small doses (1.1 or 2.2 $\times 10^{-3}\text{M}$) of benzene. Inhibition of DNA synthesis means that benzene is acting as a "mitotic poison".

RNA and Protein Synthesis

Polyribosomes. In vivo and in vitro studies on rat liver polyribosomes and soluble RNA synthesis showed that single doses of benzene (0.52-5.63 mM) given ip produced (31, 32):

- a. Inhibition of in vitro protein synthesis in the polyribosomes
- b. Disaggregation of liver polyribosomes with ribosomal monomer and dimers and appearance of an intermediate peak not found in controls
- c. Inhibition of incorporation of labeled RNA precursor into polyribosomes but not into total hepatic RNA
- d. An increase in soluble (4S) RNA (31, 32).

These observations were interpreted to mean that the primary toxic action of benzene is at translation and that the defect specifically is at the level of the ribosome associated within RNA, namely the inhibition of initiation of synthesis.

Other studies that support the above observation are those of Lee et al. (5), who found decreased ^{59}Fe incorporation into mouse bone marrow after benzene injection (sc), suggesting that benzene affects heme synthesis, which is necessary for the initiation of synthesis of most, if not all, proteins. Hemin (50 mM) was found to prevent benzene-induced heme inhibition in rabbit and human reticulocytes (33), and benzene effects were found to be associated with reversible conversion of polyribosomes of smaller aggregates, predominantly monoribosomes.

Heme Synthesis. Freedman et al. (34) studied the incorporation of L-2- ^{14}C glycine and L- ^{14}C leucine into rabbit reticulocytes suspended in media containing benzene (0.113 M). They found a 50% inhibition in incorporation of both precursors. When 4- ^{14}C aminolevulinic acid (ALA) was used, no inhibition was observed. Since ALA measures heme synthesis beyond ALA synthetase, the authors concluded that benzene inhibits heme synthesis at or before this enzymatic step.

ALA-Synthetase. ALA-synthetase plays a key role in erythropoiesis. Levere and Gidari (35) summarized the data showing that erythropoietin and 5 B-H steroids stimulate erythropoiesis, heme and globin synthesis in bone marrow cells by inducing ALA synthetase. Recently Glass et al. (36), using murine bone marrow erythroid precursors, showed that erythropoietin stimulates ALA synthetase and heme synthesis before it stimulates globin synthesis. It was also shown that heme synthesis was maximal in the earliest precursor cells and decreased with cell maturity, while globin synthesis increased with cell maturity.

It is postulated that benzene, by acting on or before ALA synthetase, prevents initiation of heme synthesis and depression of heme-controlled repressor (HCR), which in turn are necessary for globin synthesis and erythropoiesis.

Aplastic Anemia and Heme Synthesis. It is believed that very early inhibition of bone marrow precursors leads to the development of aplastic anemia. Since ALA synthetase is the rate limiting enzyme, it along with other enzymes in the pathway will have to be inhibited. Robinson and Climenko (37) exposed rabbits to a nonfatal dose of benzene and recorded a concentration of benzene in the RBC of up to 25-30 mg/100 ml of blood. On the basis of a 20-fold increase in concentration, a value of 500-600 mg/100 l would be expected for the marrow. In the in vitro reticulocyte studies (33, 34), concentrations of 438 mg/100 ml were used. Conceivably, these studies reflected what occurs in vivo in humans.

The studies described above relate to acute benzene exposure, and most cases of aplastic anemia are caused by prolonged exposure. Furthermore these studies do not take into account the possible effects of environmental pollutants such as alcohol and lead, which inhibit heme and protein synthesis in reticulocytes (38-42) and cause a sideroblastic anemia in humans (43, 44). In cultures of rabbit leucocytes, lead also produces polyribosomal disaggregation, which is reversed or prevented by hemin (39) and formation of HCR, which in turn block initiation of protein synthesis (45).

Heme Synthesis and Cyclic AMP. In intact reticulocytes in vitro, epinephrine (10^{-6} M), teophylline (10^{-4} M) and dibutyryl cyclic-AMP (10^{-4} M) all elevate cyclic-AMP concentrations within one minute of incubation. These results suggest that reticulocytes contain adenyl cyclase and phosphodiesterase systems (36). Benzene itself does not change the cyclic AMP levels in the cells, but when it is administered along with either of the above compounds, it stimulates cyclic AMP and prevents heme and protein inhibition.

This means that in intact reticulocytes, cyclic-AMP elevates benzene inhibition of heme synthesis which then allows inactivation of heme-controlled repressor, thus permitting normal protein synthesis.

Further investigation into the relationships of heme synthesis, cyclic-AMP, and pyridoxine metabolism might prove to be of therapeutic value in the treatment of benzene hematotoxicity.

It must be emphasized that the above in vitro findings in animal cells cannot be directly extrapolated to occupational exposure situations. However, it should be noted that the rabbit reticulocyte, which is extensively used as a model of hemoglobin synthesis, has proven extremely useful in the identification of agents toxic to humans (36).

11. Embryonic and Teratogenic Effects

Watanabe et al. (47) injected pregnant mice sc with single doses of benzene (3 mg/kg) on days 11-15 of gestation. The fetuses, delivered by cesarian section on day 19 of gestation, showed anomalies of the palate and jaw. Increased maternal body weight and decreased maternal WBC count were noted in dams with and without malformed fetuses.

12. Metabolism in Animals

In vivo Metabolism. Few investigations on benzene toxicity involve the use of species other than the rabbit, rat, mouse and dog. Regardless of the route of administration, benzene is eliminated both in the expired air and in the urine.

William and his co-workers found that 21% of a dose of benzene given po to rabbits was excreted mainly as phenols (phenol, catechol, quinol, and hydroxyquinol) and a small amount as trans-trans-muconic acid (nonaromatic material) (48). The excreted phenols were conjugated either as glucuronides or as ethereal sulfates (49). Over 95% of the phenol and 60% of the hydroxyquinol, catechol, and quinol were eliminated during the first two days. The elimination of hydroxyquinol was maximal on the third day, which suggested that dihydroxyphenols were subsequent oxidation products of phenols and that the trihydroxyphenol was an even later oxidation product (49).

More accurate and quantitative determinations were performed when radioactive labeled benzene became available. Parke and Williams (50) administered ^{14}C -benzene (0.34-0.5 mg/kg po) to rabbits and recovered 84-89% of the dose as radioactivity in expired air, urine, feces, and body tissues. In the expired air, 43% was recovered as unchanged benzene and 1.5% as $^{14}\text{CO}_2$, with elimination beginning 12-18 hours after administration and continuing for several days. The urine contained 34.5% of the radioactivity, with phenol accounting for 23.5% and the remainder consisting of hydroquinone (4.8%), catechol (2.2%), hydroxyhydroquinone (0.3%), trans-trans-muconic acid (1.3%), and phenylmercapturic acid (0.5%). The feces and body tissues contained 5-10% of the dose and the bile 1%.

Benzene metabolism has also been studied in rats. Cornish and Ryan (51) gave ip injections (88 mg/kg) and found a 23% yield of phenol in the urine, 70% of which was conjugated as sulfates, 17% conjugated as glucuronides, and the rest were free phenols. A higher percentage of organic sulfates was observed in another study (52), in which the metabolism was complete by 8 hours (53).

In dogs treated with 10-100 mg/kg iv, 55-80% of the dose was excreted in the urine as a mixture of phenylglucuronides and phenylsulfates. Cats and pigs showed similar metabolism, but the goat predominantly excreted phenylsulfate (75%), the remainder being glucuronide (25%) and about only 1% phenol (54).

Thus it appears that, regardless of the route of administration, benzene is eliminated both in the expired air and in the urine. The expired air predominantly consists of unchanged benzene and CO_2 . In the urine, phenol represents the largest quantity among the conjugated metabolic oxidation products of benzene, accompanied by smaller amounts of catechol, hydroquinol, and hydroxyhydroquinol. The liver appears to be the major site of both oxidation and conjugation.

Mixed Function Oxidase and Cytochrome P450. Results from several studies indicate that benzene is hydroxylated by cytochrome P450 and the mixed function oxidase in the liver microsomes of the rat, rabbit, and mouse (55-57). Benzene reacts with cytochrome P450 and P448 in much the same way as other substrates for mixed function oxidase.

Benzene Hydroxylation. The mechanism of hydroxylation of benzene by mixed function oxidase has not yet been determined. Studies by Jerina and co-workers (58 to 60) suggested that the reactions probably occur via the formation of arene oxide intermediates.

Arene Oxides. The products of arene oxide degradation result from either enzymatic or nonenzymatic reactions. Nonenzymatically, they undergo isomerization to form phenols; enzymatically, they may be hydrated to a dihydrodiol by the action of epoxide hydrase, followed by a subsequent reduction to catechol. The significance of the latter pathway is not understood as yet since very little catechol is formed in the course of benzene metabolism. Another enzymatic reaction is the transfer of glutathione to arene oxides by the enzyme, arene oxide-glutathione transferase. The glutathione conjugates are readily metabolized to premercaptapuric acid followed by dehydration to mercapturic acid (59).

Perhaps the most significant reaction of arene oxide with cellular nucleophiles (nucleic acids and proteins) is that which could result in cellular damage indicative of benzene toxicity and carcinogenesis. The stability of a particular arene oxide will also influence the extent of its nonenzymatic reaction with intracellular nucleophiles. Although the proposal is attractive that arene oxides are the bioactivated intermediates responsible for binding and for the cytotoxic and carcinogenic effects of polycyclic hydrocarbons, the possibility that metabolites other than arene oxides are the active agents in carcinogenesis should certainly not be excluded.

The nature and the binding site of arene oxides, the structural parameters and the kinetics that influence their activity, and their binding capacity to specific target molecules are still under investigation.

In summary, the metabolic fate of benzene can be visualized as the formation of an arene oxide, followed by the rearrangement to the less chemically active phenol, the interaction of the oxide with cellular nucleophiles, or the enzymatic conversion to either dihydrodiol or a mercapturic acid.

Rate of Benzene Metabolism. The rate of benzene metabolism can be determined by the dose of benzene and by compounds that stimulate or inhibit benzene metabolism. Gerarde and Ahlstrom (52) and Snyder (61) demonstrated a dose dependency for the rate of benzene metabolism in the rat and mouse, respectively. Pretreatment of rats with phenobarbital (64) and toluene (53) inhibits benzene toxicity. Because benzene is metabolized via the hepatic microsomal mixed function oxidase (55), it is to be expected that the compounds that stimulate the activity of that system might increase the rate of benzene metabolism, while those that react with cytochrome P450 might inhibit benzene metabolism.

Norpoth et al. (63) exposed rats to 450 ppm of benzene vapors for 10 days and found an increase in cytochrome P450 levels (65%), and Drew et al. (64) found an increase in microsomal benzene metabolism following exposure of rats to 4000 ppm for 4 hours/day for 3 days. However, the failure of DMSO to similarly increase cytochrome P450 levels suggests that other factors may play a role in determining the rate of benzene metabolism.

Enzyme Induction. In addition, parenteral administration of benzene also increased the rate of zoxazolamine hydroxylation and p-nitrobenzoic acid reduction in rats (65). Therefore benzene appears to function as a microsomal stimulant that can increase the rate of drug metabolism

without increasing P450. Induction by benzene is characterized by an increase in metabolism and in the incorporation of amino acids into microsomal protein within 24 hours of a single dose, but no proliferation of the smooth endoplasmic reticulum (SER) was observed at that time (65, 66). After 1 or 2 weeks of benzene treatment, SER showed proliferation and the metabolism of benzene remained slightly elevated. No relationship was found between the degree of proliferation of SER and the rate of benzene metabolism.

Since enzyme induction by benzene involves protein synthesis, the data suggest that at least two different types of protein may be synthesized to account for increases in the benzene metabolic rate. The observation that increased benzene metabolism, following induction with benzene was not accompanied by raised cytochrome P450 levels is consistent with induced synthesis of a minor component of the microsomal cytochrome P450 population which is responsible for benzene hydroxylation. This small elevation in cytochrome P450 level would become apparent only when measuring binding spectra and not when measuring total cytochrome P450 levels. On the other hand, the observations of Norpoth et al. (63) that cytochrome P450 levels do increase in rats exposed to benzene vapor indicate that the increase produced by benzene may not be sufficient to be detectable within 24 hours. Several studies have shown that the rate of metabolism was more closely associated with the optical density changes in the binding spectra than with cytochrome P450 content of the microsomes following enzyme induction (67, 68).

There is evidence that the relationship between the rate of benzene metabolism and the binding spectrum may be due to the induced synthesis of a so-called "binding protein", which facilitates the binding of the substrate to the cytochrome (69). Thus the rate-limiting step in hydroxylation of benzene is the rate at which the enzyme substrate complex is reduced. A protein that increases binding will hasten the rate of reduction and thereby stimulate benzene metabolism.

Benzene Metabolism and Toxicity. Very few attempts have been made to correlate benzene metabolism with benzene toxicity. Following the studies of Parke and Williams (70) in which phenol and polyhydroxylated phenols were identified as benzene metabolites, Dustin (71) suggested that the metabolites might be responsible for benzene toxicity through two mechanisms:

- (1) The quinone-yielding metabolites (catechol, quinol, pyrogallol) could react directly with chromosomes and interfere with mitosis (erythropoietic system) or produce an arrest in maturation (bone marrow cells) or both, and could also inhibit DNA and RNA synthesis (hemopoietic cells).
- (2) Cell division and maturation could be inhibited through benzene detoxification via sulfo-conjugation, with subsequent depletion of glutathione in bone marrow leading to bone marrow depression.

No evidence has yet been developed to support either suggestion.

In vitro studies showed that phenol, catechol and hydroquinol are mitotic toxins. Nomiyama (72) administered benzene metabolite to rats at dose levels similar to those observed in metabolic studies and found that only the catechols depressed bone marrow by causing anemia and leucopenia. Ikeda (73) exposed rats to benzene in air at 1000 ppm for 7 hours/day, 5 days a week and found that the order of increasing sensitivity based on leucocyte depression was: young females, adult females, and finally males. He evaluated aryl-4 hydroxylase, ethereal sulfate, and glucuronide-forming enzymes and concluded that the best correlation between benzene toxicity and metabolism would be made between the rate of ethereal sulfate formation and toxicity. No correlation between metabolism and toxicity for the other enzymes was found. Other studies, in which rats treated with phenobarbital metabolized more benzene and were resistant to benzene-induced leucopenia, indicated that phenobarbital may either have detoxified benzene or hastened the removal of a toxic intermediate (74).

13. Possible Leukemogenic and Tumorigenic Effects - None Reported.

III. SUBCHRONIC TOXICITY (Short Term Tests)

1. Hematologic Effects

Leucopenia. Four reports have been selected to exemplify subchronic effects of benzene inhalation in laboratory animals. Deichmann et al. (75) exposed rats to varying concentrations of benzene in air (44-65 ppm) and found that the time for development of significant leucopenia was dose- and time-related. Thus exposure to 61 ppm or more induced significant leucopenia between after 2-4 weeks of exposure, 44 and 47 ppm induced moderate but definite leucopenia after 5-8 weeks of exposure, and 15-29 ppm did not induce any changes in the hemopoietic tissue after 3-7 months exposure. The number of red blood cells and the hemoglobin concentration in circulating blood were not affected by any of the exposures. Guinea pigs given 23 exposures in 32 days developed mild leucopenia (76). In a study where rats, guinea pigs, and dogs were exposed (817 mg/m^3) for 30 exposures in a 6-week period to benzene vapor, a decrease in leucocytes occurred in the rat and an increase in the dog. No change was found in the guinea pig (77). Other effects besides leucopenia included slight anemia, thrombopenia, and progressive depletion of myelopoietic cells in bone marrow (78). Leucopenia was also seen in animals given benzene by the sc route (1 mg/kg for 3 weeks) (2). Rotter (79) observed a prolongation of prothrombin time, accompanied by a decrease in serum proteins in guinea pigs exposed to benzene vapors (4 mg/l) for 6 hours daily for 5 weeks.

Sex-Related Changes in Leucocyte Count. Sex differences in benzene poisoning have been observed in the rat. Female rats exposed to 10,000 ppm daily for 20 days responded with a 60% decrease in the total leucocyte count in the peripheral blood. Under similar experimental conditions, the males showed only 30% decrease (80, 100). Male rats feminized by castration or injected with 1.0 mg/kg of estrogen reacted

more severely than rats given androgen (81). Conversely, female rats masculinized by ovariectomy or testosterone injections showed increased leucocyte counts for the first 30 days, followed by a rapid decrease thereafter (81). Although Ito did not analyze his data statistically, his experiments and those of others (75) showed unequivocally that female rats were more susceptible to benzene than males. It was suggested that the greater susceptibility of the female to benzene could be due to the high affinity of benzene for fatty tissue (82).

2. Bone Marrow Changes

Hypoplasia. Sc injections of varying amounts (0.5-3.0 ml) of benzene (in olive oil) to guinea pigs produced initial leucocytosis, followed by mild leucopenia and granulocytopenia in 6-8 days. These changes were progressive, and animals that survived for 15 days or more developed profound leucopenia and granulocytopenia. Other cellular changes included the appearance of "primitive" (Q-cells of aplastic anemia) and giant cells. There was a marked shift to the left of the myeloid elements, suggesting impairment of cell maturation. Abnormal cytoplasmic basophilia was a conspicuous feature in both myeloid and erythroid cells in the marrow of benzene-treated animals. These abnormalities are suggestive of a dissociation of nuclear and cytoplasmic growth and maturation, possibly resulting from a metabolic block to some biochemical level (83).

The above observation, coupled with the fact that a lag period of 6 to 8 days is necessary for development of leucopenia, indicated that the primary effect of benzene was on the bone marrow (83). The leucopenia and granulocytopenia were accounted for by the natural death of the circulating leucocytes, which were inadequately replenished by the bone marrow due to toxic damage by benzene. The results of this experiment were in disagreement with those of Latta and Davies (84), who postulated that benzene exerts toxic effects primarily on the mature leucocytes in peripheral blood, and the bone marrow is affected secondarily.

A rapid fall in femoral marrow nucleated cell counts was also observed in rats (1 or 2 mg/kg for 3-5 weeks) by Koike et al. (85) and (1 ml/kg for 2 weeks) Gerarde (2). In the latter study, leucopenia and involution of the spleen and thymus were also noted.

3. Immunologic Response

It has been shown that the complementary potential of native serum is decreased in rabbits receiving a sc dose of 0.5 ml/kg of benzene every other day for 40 days (86). In another study, rabbits treated with benzene lacked the serum complement activity against iv administration of increasing doses of vaccine (87). These observations suggest that benzene inhibits the tissue and humoral factors that control the immune defense mechanisms. In a third experiment in which leucopenia was induced through sc injection of benzene (0.3-1.0 ml/kg for 5-10 days), the appearance of the leukoagglutinin type antibody was noted in the blood of those rabbits that showed leucocyte counts of 3500 per 1 mm³ of blood or less (88). Tikhachek and Frash (89) also noted a potentiation of the autoimmune process in rabbits injected sc with benzene.

4. Central Nervous System Effects

No central nervous system effects were observed by Deichmann et al. (75) at any of the benzene concentrations they used (15-831 ppm) to intermittently expose rats over a 90 day period.

5. Behavioral Effects - None Reported.

6. Cardiovascular Effects

Rats did not show any abnormal cardiovascular response to intermittent exposure to benzene (15-831 ppm) (75).

7. Biochemical and Histochemical Changes

A considerable number of studies have been done on the levels of enzymes and intermediates in blood and organs with the objective of finding subtle changes from benzene poisoning. It is now believed that the toxic effects of benzene are primarily on the enzymes that control its oxidation in the body, such as the peroxidases and catalases.

Brain. Muzyka (90) injected male rabbits sc with 0.1 and 1.0 ml/kg of benzene daily for 12 days and measured the delta-aminolevulinic acid, porphobilinogen, copro- and protoporphyrin in the grey matter of the brain. He noted that the direct action of increasing benzene concentrations upon the secreted enzymatic preparation resulted in a gradual fall of its activity, with an attendant increase in delta-aminolevulinic acid levels. From this, it was concluded that the influence of benzene on porphyrins biosynthesis in the grey matter of the brain involves induction of the delta-aminolevulinic acid synthesis.

Serum. One of the earlier studies on catalase activity in blood of rats injected daily with 1 or 2 ml of benzene/kg was conducted by Hasegawa and Sato (91). A 60-80% decrease in activity was observed within 10 days and this declined still further by 3 weeks. The decrease was definitely not due to phenol. Since the physiological action of this enzyme is still uncertain, the significance of these results is not interpretable in terms of benzene metabolism.

In another study, repeated exposure of rabbits to benzene resulted in increased serum aldolase levels in 10 days. Glutamic oxalacetic transaminase and glutamic pyruvic transaminase levels were not affected. Again the role of aldolase in benzene metabolism remains to be elucidated (92).

Rotter (79) exposed guinea pigs to benzene vapors (6 hours/day for 5 weeks) and found increases in aspartate and alanine aminotransferase activities throughout the test period. Aldolase activity in this experiment remained unchanged, but cholinesterase activity decreased during the first week.

Rozera (93) examined the alkaline phosphatase activity in erythrocytes and the concentration of phenol in the blood of rabbits given intramuscular (im) injections of 0.5 ml/kg of a 30% oil emulsion of benzene. He found the serum phosphatase activity was inhibited. The inhibition occurred earlier and lasted longer in the erythrocytes than in the leucocytes. The blood levels of phenol were increased initially but returned to normal by 30-60 days following exposure. The author suggested that inhibition of the alkaline phosphatase activity was due to the products of oxidation of benzene (phenols) rather than to benzene itself.

Frash and Karaulov (94) injected rabbits sc daily with 0.5 ml/kg of benzene for 2-3 weeks and rats with 2 ml/kg for 4 weeks, then determined the RNA, the acid phosphatase, succinate-dehydrogenase, and lymphopoietic activity of the blood serum histochemically. They observed a decrease in lymphocyte counts, a depression in RNA synthesis, and a decrease in activity of both enzymes.

Liver. Ikeda (73) exposed rats of different ages to 1000 ppm of benzene, 7 hours a day, 5 days a week for 60 days and found an increase in aryl-4-hydroxylase and UDP-glucuronyl-transferase activity. Both enzymes were involved in benzene metabolism. Other liver enzymes concerned with sulfating activity (sulfate adenytransferase, adenytransferase, aryl-sulfotransferase) were not affected.

Histochemical studies on liver and kidney slices from mice exposed to 24 mg/l for 6 hours a day for 2 weeks showed damage to the mitochondria, and this was reflected in depression of succinic and lactic acid dehydrogenase, glucose-6-phosphate dehydrogenase, and NADH₂-tetrazole reductase activity. The effect of benzene on alkaline phosphatase was inconsistent (95,96,97).

The microsomal mixed function oxidase system is a relatively non-specific enzyme system that is primarily responsible for the metabolism of many xenobiotic compounds (98). Activity requires molecular oxygen and NADPH, which revolve round a series of reactions of a heme-containing protein, cytochrome P450. Studies on benzene metabolism strongly suggest that the mixed function oxidase is benzene hydroxylase (55, 99). Further details of its role in benzene metabolism in experimental animals are discussed under acute toxicity, Section 11.

8. Effects on Body Weight, Organs and Tissues

Body Weight. The rate of growth in rats, as indicated by the increase in body weight of the animals, was not affected by benzene exposure (75).

Skin. Changes in the skin were similar to those described following acute exposure (2, 13).

Fatty Tissues. The effects were the same as described for acute studies (13).

Lung. Rats exposed to 31 ppm for 7 hours a day for 90 of 126 days showed pathological changes in the lung which resembled those of chronic bronchopneumonia (75).

Spleen. Noeske and Mentens (101) observed a marked decrease in myeloid parenchyma and an increase in lymphoid tissue following exposure of rats to sc injections of benzene for 3 weeks. Deichmann et al. (75) observed significant hemosiderosis in benzene-exposed rats (31 ppm, 7 hours a day for 90 of 126 days) as compared with controls.

Kidney. Mild nephritis, with convoluted tubule abnormalities, was found by McCord et al. (102) following exposure of rats to benzene. However, Deichmann et al. (75) exposed rats to varying benzene concentrations and noted that the kidneys were normal.

Liver. Selling (103) found fatty infiltration, hepatitis and necrosis following benzene inhalation (1000 ppm) in rats, but Deichmann et al. (75) found livers of rats exposed to 31 ppm to be normal. Rozera et al. (104), who gave rabbits benzene im (1 ml of a 30% solution in oil), identified functional changes in the liver, which they suggested were secondary to the hematologic disturbance resulting from benzene administration.

Endocrine Organs. Iannaccone and Ciccchella (105) treated rats with 1 ml/kg of benzene daily for 22 days and found morphological changes in the anterior pituitary, thyroid, thymus and adrenal. An increase in ACTH and TSH production was also observed. There was increased functional activity in the cells of the Isles of Langerhans (pancreas), the medullary cells of the adrenal, and the follicular cells of the thyroid. None of these changes were observed by Deichmann et al. (75).

Other Organs. The heart, testes, ovary and gastrointestinal tract were normal in rats exposed to benzene vapor (31 ppm, 7 hours a day), subchronically (90 days) (75).

9. Cytologic and Cytogenetic Changes

Chromosomal Aberrations. Chromosomal aberrations were observed in the bone marrow of rats injected sc with 1000 or 2000 mg/kg of benzene for 12 days (30, 106). In both studies, Heps, chromatic and isochromatic breaks were recorded at metaphase in more than 50% of the cells examined.

10. The Molecular Site of Benzene Toxicity

Radioautographic Measurements. Moeschlin and Speck (8) measured ¹⁴C-thymidine in corporation into DNA of rabbit bone marrow following daily sc injections of benzene (0.3 ml/kg/day) for 1-9 weeks. This treatment resulted in severe pancytopenia. Hypoplasticity ranged from mild to severe. White blood cells were reduced in number, and DNA synthesis was inhibited.

In Vivo Radioactive Incorporation. Similar studies were performed by Kissling and Speck (107), where the incorporation of ³H-thymidine and ³H-cytidine into DNA and RNA of the bone marrow cells of rabbits was measured following benzene administration (0.2 ml/kg/day for 6-12 weeks). Marked hypocellularity and decreased incorporation of radiolabeled precursors indicated disturbance and inhibition of both DNA and RNA of bone marrow cells.

In Vivo Colony Forming Assay. Speck and Kissling (108) used in in vivo colony-forming assay technique to study the effect of benzene on the hemopoietic stem cells. A high dose of benzene was used (300 mg/kg/day), and the treatment was extended over 2 weeks. The results indicated that the primary effect of benzene was on the stem cell and not on the differentiated cell.

11. Embryonic and Teratogenic Effects

In one reproductive study, female rats were exposed continuously to 0.3-209.7 ppm of benzene vapor for 10-15 days prior to impregnation. The animals exposed to 209.7 ppm failed to become pregnant, and at the other doses the number of offspring obtained per female on day 22 of gestation was inversely related to benzene intoxication. Fetal weight was not affected in any of the treated groups, but the organ/body weight ratio for the 19.8 ppm group was significantly higher than controls. From these results, it was concluded that the parenchymatous organs were affected by benzene poisoning (109).

12. Metabolism

In vivo metabolism of benzene during subchronic treatment is similar to that described for acute treatment.

In summary, subchronic benzene toxicity appears to be related to the levels and amounts of benzene metabolites present in the animals. Treatments that increase the rate of benzene metabolism also increase the rate elimination of the metabolites, thus decreasing the total exposure and elevating the resulting toxicity.

13. Possible Leukemogenic and Tumorigenic Effects - None Reported.

IV. CHRONIC TOXICITY (Long Term Tests)

1. Hematologic Effects

Leucopenia. Four reports were selected to exemplify the relationship between dose, exposure time and degree of toxicity. Latta and Davies (84) administered benzene to rats sc at 2-4 mg/kg/day, while Deichmann et al. (75) exposed rats to benzene in air and observed toxic effects in the

range of 65-831 ppm. Hough et al. (110) exposed dogs to 600-1100 ppm, whereas Selling (103) gave rabbits benzene sc at a dose of 1 mg/kg. Although an initial transitory leucocytosis was frequently observed, the result in each case, regardless of the species or administration route, was leucopenia. At lower doses more time was required to achieve the effect, but the final result was the same. The most striking effect was neutropenia accompanied by lymphocytosis, which disappeared as the benzene continued to attack the myeloid tissue.

Latta and Davis (84) found the lymphoid tissue to be more sensitive to benzene than the myeloid tissue in rats. In rabbits, the opposite was true (103). The neutropenia, characterized by a shift to the left of the myeloid element, suggests that the leucocyte maturation is affected. The leucopenia can occur quite rapidly and the cell counts usually reach extremely low levels prior to death.

Hormonal and other cyclic factors must also be taken into account when evaluating cell counts. Although female rats are more prone to develop leucopenia following benzene intoxication, studies by Farris (111) clearly show that there is a significant decrease in leucocyte number at the peak of the estrus cycle with counts returning to normal during the diestral interval.

Platelets. A number of investigators have presented evidence of decreased platelet function associated with benzene hematotoxicity in humans (112, 113, 114, 115). This appears to be due to intrinsic platelet abnormalities that enhance the effect of any decrease in absolute platelet number. The presence of purpura or other bleeding manifestations may be due to such an effect on platelet function. In addition, there is some suggestion that abnormalities of coagulation factors, in addition to decreased platelet function, may also be present in benzene toxicity (112, 114).

2. Bone Marrow Changes

Aplasia - Hyperplasia. The sequence of hemopoietic changes in rabbits following sc injections of benzene was studied by Selling (103), using the routine cell counting technique. The results showed that the lymphoid tissues were more susceptible to benzene toxicity, as indicated by a rapid decrease in lymphocytes and a relatively stable number of erythrocytes in the peripheral blood. But histopathological examination of the bone marrow showed that all cell types - erythrocytes, granulocytes, thrombocytes and lymphocytes - were affected. Since the granulocytic leucocytes have a shorter life than the erythrocytes, a slight inhibition of leucocyte production will be rapidly reflected in the peripheral leucocyte count (85, 116), whereas inhibition of erythrocyte production in myeloid tissue will not be detected by peripheral erythrocyte counts. Although aplasia was the predominant condition, Selling (103) also showed the co-existence of hyperplasia. This was the result of regeneration that began within 3-4 days of treatment, with formation of groups of cells containing large lymphocytes, granulocytes, or erythroblasts.

Reticuloendoplasmocytic Reaction. Low doses of benzene given sc (0.05 - 0.1 mg/kg) produced a marked reticuloendoplasmocytic reaction in the bone marrow (89).

Among the recent studies evaluating relatively large groups of individuals occupationally exposed to benzene is that of Aksoy et al. (117). Anemia and leucopenia, with or without thrombocytopenia, were the most common findings. The bone marrow findings, which ranged from slightly hypocellular to hypercellular, were in keeping with the many studies of benzene-induced pancytopenia in animals. The benzene-induced pancytopenia is very similar to the so-called idiopathic aplastic anemia.

3. Immunologic Effects

Animal experiments have shown that severe leucopenia is accompanied by the absence of antibacterial bodies, which results in increased susceptibility to infection. Simmonds and Jones (118) found increased mortality in rabbits injected with benzene (1 mg/kg, sc); they succumbed to spontaneous infections readily and showed decreased amounts of agglutinins and opsonins. Alekseeva and Zorina (119) and Tikhachek (89) treated rabbits with sc injections of benzene (0.05 - 0.1 mg/kg) weekly and observed the presence of autoantibodies in their plasma at the end of 2 years.

In humans, occupational exposure to benzene led to a decrease in circulating granulocytes (granulocytopenia) (121) and to a lowered resistance to infection. In addition, the serum levels of immunoglobulins (120) have been reported to be altered in early benzene hematotoxicity.

4. Central Nervous System Effects

Repeated exposures of rats to 1000 ppm of benzene for 7 hours daily, 5 days a week for 28 weeks did not cause any central nervous system depression (122). However, effects on the central nervous system, including changes in cerebral circulation (123) and alterations recorded by electroencephalographs (124), have been reported in humans exposed to benzene in occupational settings.

5. Behavioral Effects - None Reported.

6. Cardiovascular Effects

No adverse effects were reported in rats exposed to 47 ppm (7 hr/day on 180 days over a total of 245 days) or 15 ppm (5 day/week for 154 of 215 days) of benzene (75). However, there are unconfirmed suggestions in the literature that the human cardiovascular system may be adversely

affected by benzene (126, 127). Monaenkova and Zorina (127) showed that during benzene poisoning there is a tendency toward increased cardiac output. Circulation was accelerated, and peripheral resistance and arterial pressure were decreased.

7. Biochemical and Histochemical Changes

Brain. Muzyka (90) injected male rabbits sc with 0.1 and 1.0 ml/kg of benzene daily for 5 months and assayed the porphobilinogen, copro- and protoporphyrin and delta-aminolevulinic acid in the brain (grey matter). He observed a dose-related decrease in enzyme activity (synthetase) and increase in delta-aminolevulinic acid. From these observations, he concluded that the effect of benzene on brain porphyrin synthesis involved induction of the delta-aminolevulinic acid synthesis.

Serum. Quantitative changes in several serum enzymes have been reported by Hanka (128) and Lob (129) in humans exposed to benzene over long periods (occupational exposure).

Frash and Karaulov (94) injected rabbits with 0.1 ml/kg of benzene 3 times a week for 13 months and measured the RNA, acid phosphatase, and succinate dehydrogenase activity in the lymphocytes. They observed a decrease in all three parameters. The number of lymphocytes was also reduced.

8. Effects on Body Weight, Organs and Tissues

Body Weight. Rats chronically exposed to benzene vapor showed a normal growth rate as compared with controls (75).

Lung. Exposure of rats to 15 ppm, 7 hours a day, 5 days a week on 154 of 215 days produced pulmonary effects similar to those described for subchronic studies (75).

Skin. Dermal contact with liquid benzene may cause erythema and blistering of the skin, and a dry, scaly dermatitis may develop during chronic treatment (2).

Spleen. Tikhachek and Frash (89) noted a marked reticuloplasmocytic reaction in the spleen of rabbits injected sc with benzene (0.05-0.1 mg/kg), while Wolf et al. (76) found degenerative changes in this organ following benzene inhalation (rabbit, 80-88 ppm, 5 days a week) and po administration (0.05-0.1 mg/kg, 5 days a week) for 6 months. Deichmann et al. (75) noted hyperemia and a "more marked" hemosiderosis in rats exposed to benzene vapors (15 and 47 ppm, 7 hours a day for 154 and 180 days, respectively) than in controls. The incidence and severity of the abnormality were higher in females than in males but were not dose-related.

Gastrointestinal Tract. No adverse effects were noted in the gastrointestinal tract of rats exposed to chronic inhalation of benzene vapors (15 ppm, 7 hours a day, 5 days a week for 154 of 215 days) (75). However, there are unconfirmed reports that suggest that the human gastrointestinal system may be adversely affected by chronic benzene exposure (130, 131).

Testes. In laboratory animals, (rats, guinea pigs, rabbits, monkeys) chronic (6 months) inhalation of 80-88 ppm of benzene in air daily for 5 days a week or po administration of 1 mg/kg daily for 5 days a week resulted in testicular degeneration, and retardation and depression of the maturation of the germinal epithelium (76).

Other Organs. The heart, liver, endocrine glands, urogenital organs and lymph nodes were not adversely affected in rats exposed to 15 ppm of benzene (7 hours a day, 5 days a week on 154 of 45 days) (75).

9. Cytologic and Cytogenetic Changes

Chromosomal Aberrations. Chronic treatment of rabbits with benzene (0.2 ml/kg/day sc) resulted in inhibition of cell proliferation as evidenced by a reduced number of cells, a decrease in the labeling of myeloid precursor cells, and a reduction in the number of mature normoblastic cells in the bone marrow (132, 133).

Trisomy (Human). In contrast to the paucity of experimental data, there is abundant information on exposed human populations and case reports on leukemia patients. Due to the variability of the exposure pattern (concentrations, duration, other clinical complications), these data are hard to interpret. Nevertheless, certain trends appear amidst this confused mass of data, which relate to the appearance of additional chromosomes (134, 135, 20, 25). In two cases, the additional chromosomes were identified as members of the C group (136), which according to one reviewer, does not constitute evidence of benzene etiology (137).

The C group anomaly or trisomy has been found in "pre-leukemia" (pancytopenic) patients (138). The persistence of abnormal chromosomes long after exposure, and the occurrence of tetraploidy and polyploidy have also been reported (21, 24, 25, 138).

However, the chromosomal aberrations produced by benzene appear to be non-specific and unrelated to aberrations associated with various forms of leukemia. The dose-dependent relationship between exposure to benzene and amount of chromosomal damage has not been demonstrated.

10. The Molecular Site of Benzene Toxicity

DNA Synthesis. DNA inhibition was observed in rat bone marrow in vivo following chronic benzene inhalation (78). The number of nucleated cells declined and uptake of radiolabeled thymidine (a precursor of DNA) was decreased. These changes in DNA synthesis suggest that the mutagenic action of benzene could involve interference with mitosis.

11. Effect on Pregnancy (Human)

Male-female differences in susceptibility to benzene have been observed in humans by a number of investigators. The greater sensitivity of females is compatible with the hormonal influence on benzene toxicity (80, 81) and is supported by animal studies (see Section II, 1). Of possible pertinence are reports of benzene hemotoxicity during pregnancy (139). In this study two sisters who were chronically exposed to benzene during pregnancy developed pancytopenia and hypoplastic bone marrow. One recovered rapidly post-partum, the other had a spontaneous abortion and later died of acute leukemia during a subsequent pregnancy. Idiopathic aplastic anemia of pregnancy is a recognized condition but is very rare. Forni et al. (140) reported benzene-induced hemotoxicity in females exposed to benzene during pregnancy; normal babies were delivered.

12. Metabolism (Human)

The metabolism and elimination of benzene in humans follow the same paths as in animals; however, the distribution of benzene in humans has not been thoroughly investigated. The major difference between man and animals is related to the conjugation of the final metabolites. Humans and dogs show preference for sulfate formation, especially at low levels of exposure. Glucuronide formation is observed only when the sulfonation route is heavily used.

Studies on human volunteers exposed to benzene vapors (47-110 ppm for 2 hrs) showed that 30-50% of the absorbed benzene was eliminated through the lungs and only 0.1-0.2% through the kidneys. The remaining dose was metabolized. The rate of benzene elimination was highest during the first hour. In another investigation, 28-34% of the given dose (6000 ppm) was retained and absorbed in the blood. Approximately 55-60% of the benzene in the blood became fixed within the bone marrow, fatty tissues, and the liver (141).

13. Possible Leukemogenic and Tumorigenic Effects

Leukemogenic. Leukemia is known to occur spontaneously in some strains of mice and can be experimentally induced by a variety of chemicals in others.

Lignac (142) produced leukemia (lymphoblastoma) in mice by *sc* injections of benzene (0.001 ml benzene in 0.1 ml olive oil) for 17-21 weeks. However, the failure to provide control data and the diagnostic criteria used to evaluate the experiments leave this report open to question. Amiel's (143) attempt to duplicate Lignac's results in a different mouse strain was not successful, and the animals treated subcutaneously throughout their lifetime failed to develop either aplastic anemia or leukemia. Ward et al. (144) treated C57BL/6N mice for 54 weeks and they were sacrificed 104 weeks from the initiation of the experiment, increasing the dosage schedule from 450 mg/kg to 118 g/kg over the experimental period. Although benzene was fatal to some animals, there was no statistically significant increase in the incidence of neoplastic disease in the treated over the control groups.

Attempts by other investigators to produce leukemia via oral dosing (145), skin painting with benzene (146), and inhalation (145, 147) failed to produce conclusive evidence of leukemia in rats, mice, rabbits, and guinea pigs.

In contrast, with respect to benzene-associated leukemia in humans, evidence from industries that use benzene heavily indicates a direct relationship between benzene exposure and the development of leukemia. In every instance, leukemia was associated with a severe pancytopenic condition (148). However, the fact that in some instances there is a long delay between the cessation of exposure to benzene and the onset of leukemia (149, 150, 151) raises the questions of whether benzene or its toxic metabolites directly act as carcinogens on bone marrow hematopoietic cells, or is acute leukemia a secondary manifestation of

benzene-induced pancytopenia, expressed through unrelated aberrations in the bone marrow-producing stem cells that are susceptible to mutagenic change (preleukemic state). Morphologic abnormalities of the preleukemic state include bizarre RBC precursors (erythroleukemic state). It is possible that leukemogenic effects of benzene are expressed through interaction with other factors (genetic, environmental, etc) (148).

Tumorigenic. Hiraki et al. (152) injected Swiss mice sc with 0.1 ml of a 1% solution of benzene and reported that the treated animals developed transplantable subcutaneous sarcomas. The validity of their findings is questionable since they did not use adequate controls.

The possible role of arene oxides in carcinogenesis is discussed under acute toxicity (p. 13). However, it must be emphasized that although it is possible that arene oxides are the bioactivated intermediate responsible for the cytotoxicity and carcinogenicity of benzene, the possibility that other metabolites could play an active role cannot be ignored.

V. SIGNIFICANT PHYSICAL AND CHEMICAL PROPERTIES

Benzene is a clear, colorless, noncorrosive, highly flammable liquid with a strong, rather pleasant odor. Its physical properties are given in Table 1. Today, it is obtained primarily from the petroleum industry where it is produced as a petrochemical from paraffinic hydrocarbons. It is also recovered from the gases and coal tar in coke oven operations. The major impurities in commercial benzene (benzol) are toluene and xylene, although the commercial form may also be contaminated with phenol, thiophene, carbon disulfide, acetyl nitrile, pyridine, and other substances. "Benzol 90" contains from 80-85% benzene, 13-15% toluene, and 2-3% xylene. The "90" designation refers to the percent of total liquid, by volume, which distills below 100° C.

The solubility of benzene in animal (rabbit) tissues, expressed as partition coefficients (numerically equal to Ostwald solubility coefficients) was determined by Sato et al. (153). Table 2 shows the results of these experiments, which were conducted at 37° C. The authors noted the relatively high partition coefficient between fat-blood and suggested that the coefficient between tissues-blood would depend, at least in part, upon the fat content in the given tissue.

Table 1

Significant Physical Properties of Benzene

Formula

C₆H₆

Formula Weight

78.1

Boiling Point

80.1° C (176° F) at 760 mm Hg

Melting Point

5.5° C (42° F)

Specific Gravity

0.87865 g/ml at 20° C (68° F) 4° C (39.2° F)

Solubility

0.06% in water, mixes freely with alcohol, ether and most organic solvents.

Explosive Range for Vapor

1.4 - 7.1% by volume in air

Flash Point

-12 to -10° C (10.4-14° F)

Ignition Temperature

490° C (914° F)

Vapor Density

2.7 (Air = 1.0)

Derived from references 154 and 155.

Table 2 (153)

Partition Coefficients of Benzene for Body Fluids,
Tissue Homogenates and Organic Materials

BENZENE

		MEAN	STANDARD DEVIATION
A	Blood	10.70	1.35
	Plasma	5.4	0.33
B	Liver	1.61	0.18
	Kidney	1.13	0.28
	Brain, whole	1.93	0.50
	Lung	1.25	0.31
	Heart	1.44	0.38
	Muscle, femoral	1.08	0.17
	Bone marrow	16.18	2.45
	Fat, retroperitoneal	58.53	11.87
C	Lecithin, from egg	196.42	9.30
	Triolein	535.68	31.14
	Cholesterol	20.95	0.85
	Cholesterol oleate	83.65	4.49
	Human fat, peritoneal	406.22	10.10

A: Fluid-air partition coefficients. The figures are means and standard deviations of 5 rabbits.

B: Tissue-blood partition coefficients, which are expressed as tissue-air partition coefficient/blood-air partition coefficient. The figures are means and standard deviations of blood-air partition coefficients of 5 rabbits.

C: Material-air partition coefficients. The figures are means and standard deviations of 5 determinations.

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Benzene Toxicity - Matrix

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
1. Hematologic Effects	<ul style="list-style-type: none"> o <u>Leucocytes.</u> Significant decrease at 24 hr; female more sensitive than male (2,3). o Hemolysis (4). 	<ul style="list-style-type: none"> o <u>Leucopenia.</u> Appearance dose and sex related (75-82). o Thrombopenia (78). o Prolonged bleeding and clotting time (79). 	<ul style="list-style-type: none"> o <u>Leucopenia:</u> Appearance dose and sex related (75, 84, 103,110). o Neutropenia (103). o <u>Platelets:</u> Decreased number, abnormal function (111-115). o Purpura (111, 113). 	Sig dec Leu (No
2. Bone Marrow Changes	<ul style="list-style-type: none"> o Significant reduction in the precursor cells of the hemic cell renewal system (9). o Hemoglobin synthesis decreased (5-8). 	<ul style="list-style-type: none"> o <u>Hypoplasia:</u> Leucopenia, granulocytopenia, giant cells, "Q-type" cells impaired myeloid cell maturation (83-85). o Stem cells of hemotopoietic system affected (108). 	<ul style="list-style-type: none"> o <u>Aplasia:</u> Decreased RBC, WBC, granulocytes, thrombocytes, lymphocytes (85, 89, 103,116). o Anemia (117). o Fibrosis (human). o <u>Hyperplasia:</u> focal regeneration of lymphocytes, granulocytes and RBC (103). 	Sig Red Pre Cel cel sys
3. Immunologic Effect	<ul style="list-style-type: none"> o None Reported^a 	<ul style="list-style-type: none"> o Decreased serum complement activity against vaccine (86-89). o Decrease in agglutinins (86-89). 	<ul style="list-style-type: none"> o Decreased serum complement activity against infection (118-121). o Decrease in agglutinins and opsonins (118-121). 	(S de co co wi in re th

benzene Toxicity - Matrix

Treatment Groups,	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Dose related (78). Feeding time	<ul style="list-style-type: none"> o <u>Leucopenia:</u> Appearance dose and sex related (75, 84, 103,110). o Neutropenia (103). o <u>Platelets:</u> Decreased number, abnormal function (111-115). o Purpura (111, 113). 	Significant decrease in Leucocytes (Non-Specific)	<ol style="list-style-type: none"> 1. Complete CBC (RBC, WC HG, Hct Differential Platelets) 2. Red cell survival 3. Clotting and bleeding times
Anemia, Hemolysis and reticulocytosis	<ul style="list-style-type: none"> o <u>Aplasia:</u> Decreased RBC, WBC, granulocytes, thrombocytes, lymphocytes (85, 89, 103,116). o Anemia (117). o Fibrosis (human). o <u>Hyperplasia:</u> focal regeneration of lymphocytes, granulocytes and RBC (103). 	Significant Reduction in Precursor Cells-Hemic cell renewal system	<ol style="list-style-type: none"> 1. <u>In Vitro</u> cytotoxicity 2. Bone marrow differential (turn over and cycle rates)
Serum antibodies	<ul style="list-style-type: none"> o Decreased serum complement activity against infection (118-121). o Decrease in agglutinins and opsonins (118-121). 	(Subchronic) decreased serum complement - correlation with increase in reticuloendo- thelial cells	<ol style="list-style-type: none"> 1. Serum complement depression test 2. Depressed synthesis tests (globulins and interferon) - electrophoresis

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pr En
7. Biochemical and Histochemical Effects	<ul style="list-style-type: none"> o <u>Brain.</u> <u>Elevated</u> glutamic decarboxylase activity (16). o <u>Spinal Cord.</u> <u>Elevated</u> deoxyribonuclease (15). <u>Depressed</u> succinic dehydro- genase activity, NADH-diaphorase, alkaline and acid phosphatases, 5'nucleotidase and thiolacetic esterase activity (15). o Serum. None reported. 	<ul style="list-style-type: none"> o <u>Brain.</u> <u>Elevated</u> aminolevulinic synthetase activity (90). o <u>Spinal Cord.</u> <u>Elevated</u> aminolevulinic synthetase (90). o <u>Serum.</u> <u>Elevated</u> aldolase (92), aspartic and alanine amino- transferase (79) activity. <u>Depressed</u> catalase (91), alkaline (93) and acid (94) phosphatases, succinate de- hydrogenase (94) and cholinesterase (79) activity. <u>Depressed</u> RNA synthesis (94). 	<ul style="list-style-type: none"> o <u>Brain.</u> <u>Elevated</u> aminolevulinic synthetase (90). o <u>Spinal Cord.</u> <u>Elevated</u> aminolevulinic acid synthetase (90). o <u>Serum.</u> <u>Elevated</u> lactic dehydrogenase in humans exposed to benzene in occupational settings (133, 134). <u>Depressed</u> RNA synthesis (94). 	<ul style="list-style-type: none"> 1. 2. 3. 4.

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pr En
4. Central System (CNS)	<ul style="list-style-type: none"> o Stimulation followed by depression (10, 11). o Paralysis, petechial bleeding (12, 13). 	<ul style="list-style-type: none"> o No adverse effects noted under experimental conditions (75). 	<ul style="list-style-type: none"> o No adverse Nervous effects noted under experimental conditions (75,122) but in humans from occupational setting, changes were observed in cerebral circulation (123) and electroencephalographs (124). 	No
5. Behavior Effects	<ul style="list-style-type: none"> o See CNS effects 	<ul style="list-style-type: none"> o None reported. 	<ul style="list-style-type: none"> o None reported. 	No
6. Cardiovascular Effects	<ul style="list-style-type: none"> o Ventricular fibrillation (14). o Hemorrhagic pericardium (2). 	<ul style="list-style-type: none"> o No adverse effects reported under experimental conditions (75). 	<ul style="list-style-type: none"> o No adverse effects reported under experimental conditions (75) but in humans benzene exposure caused increased cardiac output combined with a drop in peripheral resistance and a decline in arterial pressure. Death often occurred due to heart failure (125-127). 	No

Benzene Toxicity - Matrix (Cont'd)

Treatment tests, days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Adverse effects reported under experimental conditions (75).	o No adverse Nervous effects noted under experimental conditions (75,122) but in humans from occupational setting, changes were observed in cerebral circulation (123) and electro- encephalographs (124).	None	None
Adverse effects reported.	o None reported.	None	1. Activity-wheel running test (related to blood picture)
Adverse effects reported under experimental conditions (75).	o No adverse effects reported under experimental conditions (75) but in humans benzene exposure caused increased cardiac output combined with a drop in peripheral resistance and a de- cline in arterial pressure. Death often occurred due to heart failure (125-127).	None	None

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints
7. Biochemical and Histochemical Effects (Cont.)	o <u>Kidney.</u> Elevated succinic dehydrogenase, NADH ₂ -diaphorase, ca-formol-ATPase, alkaline and acid phosphatase activity (17).	o <u>Kidney.</u> Depressed succinic and lactic dehydrogenase, glucose-6-phosphate dehydrogenase (95-97).	o <u>Kidney.</u> None reported.	
	o <u>Liver.</u> Elevated aryl-4-hydroxylase, UDP-glucuronyl transferase activity (73), cytochrome P-450 (63), aminopyrine dimethylase (63). Depressed succinic and lactic dehydrogenase, glucose-6-phosphate dehydrogenase and NADH tetrazole reductase (95-97).	o <u>Liver.</u> Same as in acute studies.	o <u>Liver.</u> None reported.	

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoint
8. Body Weight, Organs and Tissues	o <u>Body weight.</u> No adverse effect reported (13).	o <u>Body weight</u> increased (75, 82, 100).	o <u>Body weight</u> increased (75, 82, 100).	1. Endo chan
	o <u>Skin.</u> Hemorrhage (2, 13).	o <u>Skin.</u> Erythema, blistering, hemorrhage (2).	o <u>Skin.</u> Erythema, blistering, dry scaly dermatitis (2, 126).	2. Sple incr R.E. corr with immu effe
	o <u>Fatty tissue</u> - destruction (13).	o <u>Fatty tissue</u> bioaccumulation (2).	o <u>Fatty tissue</u> bioaccumulation (2).	3. Roug endo reti (RE)
	o <u>Lung and pleurae.</u> hemorrhage (2).	o <u>Lung</u> - changes resembled those seen in chronic bronchopneumonia (75).	o <u>Lung</u> same as in subchronic.	4. (Sub Live infi
	o <u>Spleen</u> , increased proliferation of reticulo-endothelial cells (18).	o <u>Spleen</u> , decreased myeloid parenchyma and increased lymphoid tissue (101); hemosiderosis (75).	o <u>Spleen</u> , marked reticuloplasmocytic reaction (105), degenerative changes (76), hyperemia and hemosiderosis (75).	5. Hemo derm
	o <u>Lymph nodes</u> , decreased thrombocytes and increased macrophage (18).	o <u>Lymph nodes.</u> No adverse effect reported (75).	o <u>Lymph nodes.</u> No adverse effect reported (75).	
	o <u>Kidney.</u> None reported.	o <u>Kidney</u> , mild nephritis, convoluted tubular abnormalities (102).	o <u>Kidney.</u> No adverse effect reported (75).	
	o <u>Liver.</u> None reported.	o <u>Liver</u> , fatty infiltration, hepatitis, necrosis (75), functional changes (104).	o <u>Liver.</u> Same as in subchronic.	

Gene Toxicity - Matrix (Cont'd)

Treatment tests, (ys)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Weight increased (100).	o <u>Body weight</u> increased (75, 82,100).	1. Endocrine changes	1. Hematological and histological tests
Erythema, (2).	o <u>Skin.</u> Erythema, blistering, dry scaly dermatitis (2, 126).	2. Spleen increased R.E.C. - correlation with immunologic effects	2. Electron microscope for RER
Issue (ulation)	o <u>Fatty tissue</u> bioaccumulation (2).	3. Rough endoplasmic reticulum (RER)	3. Decreased protein synthesis test
Changes (d those chronic pneumonia)	o <u>Lung</u> same as in subchronic.	4. (Subchronic) Liver -fatty infiltration	4. 90-Day percutaneous toxicity test hexane or acetone controls
decreased parenchyma (eased tissue hemosiderosis)	o <u>Spleen</u> , marked reticuloplasmocytic reaction (105), degenerative changes (76), hyperemia and hemosiderosis (75).	5. Hemorrhagic dermatitis	
Nodes. No effect (75).	o <u>Lymph nodes.</u> No adverse effect reported (75).		
mild (s, convoluted abnormalities)	o <u>Kidney.</u> No adverse effect reported (75).		
fatty (ation, (s, necrosis functional (104).	o <u>Liver.</u> Same as in subchronic.		

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pred Endp
8. Body Weight, Organs and Tissues (Cont.)	<ul style="list-style-type: none"> o <u>Endocrine organs.</u> Decreased thrombocytes and increased macrophages in thymus (18). o <u>Gastrointestinal tract.</u> Stripping of gastric mucosa and bleeding (13). o <u>Testis.</u> None reported. 	<ul style="list-style-type: none"> o <u>Endocrine organs,</u> morphological and functional changes in pituitary, adrenal, thyroid, isles of Langerhans (pancreas) and thymus (105). o <u>Gastrointestinal tract.</u> No adverse effects observed (75). o <u>Testis.</u> No adverse effects observed (75). 	<ul style="list-style-type: none"> o <u>Endocrine organs.</u> No adverse effects observed (75). o <u>Gastrointestinal tract.</u> No adverse effects observed under experimental conditions (75), but effects were observed in humans during occupational exposure (130, 131). o <u>Testis.</u> Testicular degeneration, depressed maturation of germinal epithelium (76). 	

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints
9. Cytologic and Cytogenetic Effects	o Chromosomal aberrations (26-30).	o Chromosomal aberrations (30, 106).	o Chromosomal aberrations (132, 133).	Chromosomal aberrations
10. Molecular Effects	o <u>DNA synthesis</u> , inhibited (8, 26, 27). o <u>RNA and protein synthesis</u> , inhibited (31, 32). o <u>Heme synthesis</u> , inhibited (5, 33-36). o Aplastic anemia (37-45). o Cyclic-AMP unchanged (46).	o <u>DNA synthesis</u> inhibited (8, 107). o <u>RNA synthesis</u> inhibited (8, 107).	o <u>DNA synthesis</u> inhibited (80).	DNA, RNA, Protein synthesis inhibition
11. Embryonic and Teratogenic Effects	o Embryonic death (46). o Fetal abnormalities (46).	o High embryotoxicity (90). o Lowered organ/body weight ratio (90).	o None reported under experimental conditions but in humans absence of pregnancy was observed following exposure to benzene (59,60, 138, 139).	1. Embryonic Death 2. Fetal abnormalities

1

Chronic Toxicity - Matrix (Cont'd)

Treatment Tests, ()	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
1 s (30,	o Chromosomal aberrations (132, 133).	Chromosomal aberrations	1. Sister chromatid exchange 2. Chromosome breaks and exchanges
sis (8, 107).	o <u>DNA synthesis</u> inhibited (80).	DNA, RNA, Protein synthesis inhibited	1. Thymidine - Uridine uptake tests (<u>in vitro</u>) (EPA Screening level tests)
sis (8, 107).			
toxicity gan/body io (90).	o None reported under experimental conditions but in humans absence of pregnancy was observed following exposure to benzene (59,60, 138, 139).	1. Embryonic Death 2. Fetal abnor- malities	1. Embryotoxicity screening tests 2. One generation mouse test

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in</u> <u>vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pred. Endp
12. Metabolism	<ul style="list-style-type: none"> <li data-bbox="464 596 743 827">o Excretion products are primarily phenol and small amounts of trans-trans-muconic acid (48-54). <li data-bbox="464 949 743 1583">o The metabolic fate of benzene can be visualized as the formation of an intermediate, <u>arene oxide</u>, followed by a rearrangement to the less chemically active phenol, the interaction of the oxide with cellular nucleophiles or the enzymatic conversion to either dihydrodiol or a mercapturic acid (55-60). 	<ul style="list-style-type: none"> <li data-bbox="787 596 1049 680">o Metabolism as described under acute studies. 	<ul style="list-style-type: none"> <li data-bbox="1166 596 1500 940">o Metabolism in humans essentially same as in experimental animals (see acute studies) except sulphonation is the predominant form of conjugation at low concentrations of benzene and glucuronide at high doses of benzene. <li data-bbox="1166 949 1500 1121">o Highest concentrations of benzene found in blood, bone marrow, fatty tissue and liver (141). 	Aren Form

benzene Toxicity - Matrix (Cont'd)

Treatment in tests, days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
ism as ed under studies.	<ul style="list-style-type: none"> o Metabolism in humans essentially same as in experimental animals (see acute studies) except sulphonation is the predominant form of conjugation at low concentrations of benzene and glucuronide at high doses of benzene. o Highest concentrations of benzene found in blood, bone marrow, fatty tissue and liver (141). 	Arene Oxide Formation	<ol style="list-style-type: none"> 1. Pharmacokinetic studies (absorption, distribution, excretion, body-burden) 2. Induction of P450 and RER 3. Extent of Co-Valent binding (liver/kidney)-labelled compounds

2

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	F E
12. Metabolism (Cont.)	<ul style="list-style-type: none">o Enzyme induction (63-69).o Proliferation of smooth endo- plasmic reticulum (66).o Suggested mechanism of benzene toxicity through metabolites which interfere with mitosis and cellular maturation and inhibit DNA and RNA synthesis (71-74).			

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
13. Leukemogenic and Tumorigenic Effect	o None reported.	o None reported.	<ul style="list-style-type: none"> <li data-bbox="1177 531 1528 709">o Experimental data reported to show that benzene produces leukemia in animals were inclusive (142-147). <li data-bbox="1177 709 1528 825">o In <u>humans</u> benzene is considered as a suspect leukemogen (148). <li data-bbox="1177 825 1528 972">o Experimental data to show that benzene produced sarcomas were inconclusive (152). <li data-bbox="1177 972 1528 1379">o It was proposed that arene oxides are the bioactivated intermediates responsible for the cytotoxic and carcinogenic effect of benzene. However, the possibility that metabolites other than arene oxides are active agents in carcinogenesis cannot be ruled out (55-60). 	Non

1

Benzene Toxicity - Matrix (Cont'd)

Chronic Treatment Short term tests, to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None reported.	<ul style="list-style-type: none">o Experimental data reported to show that benzene produces leukemia in animals were inconclusive (142-147).o In <u>humans</u> benzene is considered as a suspect leukemogen (148).o Experimental data to show that benzene produced sarcomas were inconclusive (152).o It was proposed that arene oxides are the bioactivated intermediates responsible for the cytotoxic and carcinogenic effect of benzene. However, the possibility that metabolites other than arene oxides are active agents in carcinogenesis cannot be ruled out (55-60).	None	<ol style="list-style-type: none">1. Sister chromatin exchange2. Leucocyte-chromosome damage test (Brewer and Evans)

Benzene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in</u> <u>vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
14. Physical Chemical Properties				Non

^aNone reported, means that no literature was identified.

Benzene Toxicity - Matrix (Cont'd)

Chronic Treatment Short term tests, (90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
		None	<ol style="list-style-type: none"> 1. Chemical structure relationship to known carcinogens examined should be 2. Identify volume-use characteristics of compound

Identified.

Tracor Jitco

EXPERIMENTAL CADMIUM INTOXICATION

Backup Report to Cadmium Toxicity Matrix

EXPERIMENTAL CADMIUM INTOXICATION

INTRODUCTION

ACUTE TOXICITY (Including In Vitro Studies)

1. Hematologic Effects

Hemolysis

2. Bone Marrow Changes

3. Immunologic Response

4. Central Nervous System (CNS) Effects

Cerebrum and Cerebellum
Spinal Ganglia

5. Behavioral Effects

6. Cardiovascular Effects

Hypertension

7. Biochemical and Histochemical Changes

Metallothionein
Mitochondria - Liver, Pulmonary Alveolar Macrophages
Glucogenesis
Other Effects
Collagen
Serum Albumin and Globulin

8. Effects on Tissues and Organs

Kidney
Lung
Pancreas
Macrophages
Gonads

9. Cytologic and Cytogenetic Effects

Dominant Lethal Mutations
Chromosome Rearrangements

10. The Molecular Site of Cadmium Toxicity

RNA-Polymerase and Protein Synthesis
Nucleic Acid and Protein Synthesis

11. Reproductive and Teratogenic Effects

Gonads

Testes
Ovaries
Behavior

Teratogenesis
Reproductive Performance

12. Cadmium Metabolism

Absorption

Skin
Respiratory Tract
Gastrointestinal Tract

Transport

Intravenous Route
Intraperitoneal Route
Subcutaneous Route

Tissue Distribution and Retention

Oral Intake
Liver
Kidneys

Injection Route
Liver
Kidneys

Placental Transfer
Experimental Animals
Human Beings

Excretion

Urinary Excretion
Excretion Via the Alimentary Tract
Hair
Milk
Saliva

Biological Half-Time

13. Carcinogenesis

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

1. Hematologic Effects

Anemia

2. Bone Marrow Changes

3. Immunologic Response

4. Central Nervous System Effects

5. Behavioral Effects

6. Cardiovascular Effects

Rabbits

Rats

7. Biochemical and Histochemical Changes

Liver and Kidney Enzymes

Cyclic AMP

Mitochondria

Collagen

8. Effects on Tissues and Organs

Adrenals

Pancreas

Liver

Bone

9. Cytologic and Cytogenetic Changes

10. The Molecular Site of Cadmium Toxicity

11. Reproductive and Teratogenic Effects

Fetal Deaths

Fetal Malformations

Prolonged Estrus

12. Cadmium Metabolism

Absorption: Gastrointestinal Tract
Transport

Tissue Distribution and Retention
Liver, Kidney, Pancreas, Spleen
Embryo and Placenta

Excretion
Urine
Alimentary Tract

Biological Half-Time

13. Carcinogenesis

CHRONIC TOXICITY (Long-term tests, 2-year or lifetime treatment)

1. Hematologic Effects

Anemia
Hemoglobin
Erythrocytes
Reticulocytosis
Eosinophils

2. Bone Marrow Changes

Hyperplasia

3. Immunologic Response

Antibody Producing Cells
Immune Response

4. Central Nervous System (CNS) Effects

Cerebral Cortex (Humans)
Motor and Optical Chronaxy (Experimental Animals)

5. Behavioral Effects

6. Cardiovascular Effects

Hypertension

7. Biochemical and Histochemical Changes

Liver

8. Effects on Tissues and Organs

Kidney
Teeth
Bone
Experimental Animals
Testes

9. Cytologic and Cytogenetic Changes

10. The Molecular Site of Cadmium Toxicity

11. Reproductive Effects

Testes
Reproductive Performance
Rats
Monkeys

12. Cadmium Metabolism

Absorption
Transport
Tissue Distribution and Retention
Liver, Kidney, Pancreas, Spleen

Excretion
Urine
Alimentary Tract

Biological Half-Time

13. Carcinogenesis

Injection-Route
Oral Route

SIGNIFICANT PHYSICAL AND CHEMICAL PROPERTIES

ADDENDUM I

ACUTE TOXICITY

1. Hematologic Effects
2. Bone Marrow Changes
4. Central Nervous System (CNS) Effects
7. Biochemical and Histochemical Effects
8. Effects on Tissues and Organs
 Liver
9. Cytologic and Cytogenetic Effects
10. Molecular Effects

SUBCHRONIC TOXICITY

8. Effects on Tissues and Organs
 Bone
 Thyroid
12. Cadmium Metabolism
 Transport

CHRONIC TOXICITY

11. Reproductive and Teratogenic Effects
13. Carcinogenesis

ADDENDUM 2

ACUTE TOXICITY (Including in vitro studies)

7. Biochemical and Histochemical Changes

Liver glycogen
Blood glucose
Serum urea
Cyclic AMP
Adenylate Cyclase
Adenosine-3'5'-monophosphate phosphodiesterase (PDE)

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

3. Immunologic Response

B Lymphocyte effect

5. Behavioral Effects

Locomotor activity

7. Biochemical and Histochemical Changes

Brain biogenic amines
Tyrosine hydroxylase
Tryptophan hydroxylase
5-Hydroxyindoleacetic acid
Catechol-O-methyl transferase
Dopamine
Norepinephrine
Pyruvate carboxylase
Phosphoenolpyruvate carboxykinase
Fructose-1,6-diphosphatase
Glucose-6-phosphatase
Reduced insulin values in blood
Hyperglycemia

8. Effects on Organs and Tissues

Adrenal hypertrophy
Testicular atrophy

ADDENDUM 2

CHRONIC TOXICITY (Long-term tests, 2 years, or lifetime treatment)

5. Behavioral Effects

Learning disability

8. Effects on Tissues and Organs

Olfactory apparatus (anosmia)

Teeth

Bone

Lungs

Kidneys

Proteinuria

Renal stones

INTRODUCTION

Chronic illness due to occupational exposure to cadmium was reported as early as 1920. Nutritional deficiencies, associated with World War II, may have contributed to bone disease (osteomalacia) in cadmium workers in France. Cadmium is also believed to be involved in Itai-Itai disease in Japan.

The definitions of "cadmium," "action level," and "occupational exposure to cadmium" used in this report are those established by the National Institute for Occupational Safety and Health. "Cadmium" refers to elemental cadmium and all cadmium compounds. An "action level" is half the time-weighted average concentration environmental limit of cadmium. "Occupational exposure to cadmium" refers to exposure to cadmium at a concentration greater than the action level (1).

Workers should not be exposed to cadmium concentrations greater than "40 micrograms per cubic meter of air (40 ug Cd/m^3) determined as a time-weighted average (TWA) exposure concentration for up to a 10-hour workday, 40-hour work week, or at a ceiling concentration greater than 200 ug Cd/m^3 for any 15-minute sampling period" (1).

Cadmium compounds have an adverse effect on many organs and body systems. In experimental animals, damage or functional effects have been detected in the liver, gonads, adrenals, bone, and, probably most importantly, in the kidney. The hematopoietic, cardiovascular, and central nervous systems are also affected. In addition, birth defects, abnormal behavior, and possibly cancer, have been produced (1,2).

Cadmium toxicity can be induced in laboratory animals (rats, mice, rabbits, guinea pigs, hamsters, gerbils, dogs, cats, calves, and monkeys) via inhalation, intravenous (iv), intraperitoneal (ip), and subcutaneous (sc) injections, oral administration (po), or by direct contact with the skin (1,2).

Acute toxicity studies in animals have indicated that cadmium is an extremely toxic metal. The inhalation LC₅₀ of cadmium oxide fumes in several species of common laboratory animals ranged from 700 mg/m³/10 min in the mouse to 4000 mg/m³/10 min in the dog. Oral LD₅₀ values for cadmium oxide and cadmium chloride in the rat of 72 mg/kg and 88 mg/kg, respectively, have been reported. The LDLo (lowest published lethal dose) of cadmium chloride when administered to guinea pigs via the skin was 233 mg/kg (3).

Specific effects of cadmium and cadmium compounds on the various organs and body systems in experimental animals will be discussed in the following sections on (I) Acute Toxicity, (II) Subchronic Toxicity, and (III) Chronic Toxicity Studies. Acute toxicity in this report is limited to toxic effects following administration of a single dose. Subchronic toxicity refers to toxicity observed in short-term tests of less than 90 days. Chronic toxicity includes toxic effects occurring in long-term tests, two-year or lifetime treatment.

INFORMATION SOURCES

The information contained in the body of this back-up document (Experimental Cadmium Intoxication) was derived mainly from such critical current reviews as:

- o Occupational Exposure to Cadmium, HEW Publ. No. (NIOSH) 76-192, National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1976.
- o Cadmium in the Environment, 2nd Edition, Friberg, L., M. Piscator, G.F. Nordberg, and T. Kjellstrom, CRC Press, Cleveland, Ohio, 1974.
- o Occupational Diseases, Revised Edition, Key, M.M. (ed.) DHEW Publ. No. (NIOSH) 77-181.

In addition, some pertinent research reports, identified through a Tracor Jitco literature search, were included.

The information contained in the back-up document is summarized in the appended matrix. The term "None Reported", which appears in the matrix, indicates that no literature reference related to that particular item was identified.

ACUTE TOXICITY (Including In Vitro Observations)

1. Hematologic Effects

Information on acute hematologic effects of cadmium in experimental animals is meagre. In an early study, a hemolytic effect was noted following a single injection of cadmium sulfate (approx. 25 mg Cd/kg) into a dog (4). Friberg et al. (2) stated that dose-response relationships involved in acute effects of cadmium on the hematopoietic system cannot be established because of lack of data and the overshadowing effects of lung edema and the general toxemia.

2. Bone Marrow Changes

No data reported in literature surveyed to date.

3. Immunologic Response

No data in literature surveyed to date.

4. Central Nervous System (CNS) Effects

Neurotoxic effects from cadmium chloride have been observed in rats, mice, rabbits, guinea pigs, and golden hamsters. Hemorrhagic damage in the cerebellum and cerebrum of young rats and rabbits was found following single sc doses of 10 mg/kg and 20 mg/kg, respectively (5). Acute hemorrhagic lesions of Gasserian and sensory spinal ganglia were detected in guinea pigs, golden hamsters and mice after 10 mg/kg sc injections, and in rats given 2 mg/animal sc (6). In vitro exposure of dorsal root ganglia from Wistar rat embryos (19-21 days) to 4×10^{-4} M cadmium chloride from one day to three weeks revealed degeneration of mitochondria and unmyelinated axons, as well as glycogen deposits in neuronal perikarya; the latter effect was dose-related. The authors concluded that cadmium ions are direct pathogens for nerve tissues (7).

5. Behavioral Effects

No data in literature surveyed to date.

6. Cardiovascular Effects

Schroeder and Buckman (8) found rats given one ip injection of cadmium acetate (1.5 mg Cd/kg) developed hypertension.

7. Biochemical and Histochemical Changes

Metallothionein

Thionein, a low molecular weight protein rich in sulfhydryl groups which binds certain heavy metals to form metallothioneins, has been found in man and a number of animal species and is believed to play a role in cadmium metabolism and toxicology (1).

Webb (9) has shown that thionein is induced by Cd (II) and certain other heavy metals and that cycloheximide inhibits its synthesis. This suggests, according to the author, that induction of thionein is controlled at the translational level rather than at the transcriptional level. Metallothionein can protect mouse testes from injury by cadmium (10) but cadmium-thionein, on the other hand, was found to cause more damage to renal tubules than cadmium chloride in CBA mice via the venous or subcutaneous routes, and it was fatal at lower doses (11).

Mitochondria - Liver, Pulmonary Alveolar Macrophages

Low levels of cadmium (II) have been found to uncouple phosphorylation coupled to aerobic oxidation of succinate or reduced diphosphopyridine nucleotide. In rat liver mitochondria in vitro, uncoupling of phosphorylation was detected with the oxidation of both succinate and citrate at concentrations as low as 5 uM Cd (II). The uncoupling effect was completely reversed, however, by the chelating agent, ethylene diamine tetracetic acid (12,13). Phosphorylation and respiration in mitochondria of pulmonary alveolar macrophages were also abolished with 5-10 um Cd (II) (14). The uncoupling mechanism seems to involve the binding of cadmium to dithiol groups of the enzymes. For example, a sulfhydryl enzyme, succinoxidase was inhibited 50% by 7 uM cadmium (15). Metal-sensitive sites, other than SH groups, were also found to be affected by cadmium in a study on the inhibition of alpha-oxoglutarate dehydrogenase by heavy metals (9).

Glucogenesis

Acute effects of cadmium (II) on glucose metabolism have been studied both in vitro and in vivo. In one study, glucogenesis by rat kidney cortex slices was found to be inhibited by cadmium (16). In vivo, however, there was a significant increase in glucose concentration in the testes when rats were given 0.04 mmole/kg Cd (II) sc. This was accompanied by a marked decrease in glucose-6-phosphate and adenosine triphosphate at 2 hours. Glycogen and total high energy phosphate were considerably reduced, while lactate was increased after 4 hours. The authors stated that the reduction in high energy metabolites might be related to the ischemic or anoxic state of the testes (17).

Other Effects

The low molecular weight protein, thionein, is able to bind both cadmium and zinc, and consequently, the two metals are transported together in the body. Also, cadmium seems to have the ability to exchange with zinc in zinc-dependent enzymes and thus affect enzyme activity (2). For example, Ribas-Ozonas et al. (18) discovered that Cd (II) brought about a significant decrease in the activity of alkaline phosphatase (a zinc-containing enzyme) in both the kidney and prostate gland in guinea pigs. Acid phosphatase and esterase activities were unaffected.

Collagen

Helgeland (19) determined the effect of cadmium incubated with rabbit dental pulp in vitro on the incorporation of ^{14}C -proline into the trichloroacetic acid (TCA)-soluble pool, total protein, collagen, and high-molecular weight material released into the medium. Cadmium caused an increase in the amount of ^{14}C -activity in the TCA-soluble pool, but further incorporation into total protein and collagen was markedly inhibited. The proportion of ^{14}C -labeled protein and hydroxyproline recoverable from the medium also was markedly reduced by cadmium. Cadmium reduced the proportion of protein-bound ^{14}C -activity converted into ^{14}C -hydroxyproline by about 50% at 20 μM and inhibited the hydroxylation by about 75% at 80 μM . The proportions of ^{14}C -labeled protein and collagen recovered from the medium were reduced by about 50% and 70%, respectively, by 20 μM cadmium. At 80 μM , cadmium reduced the proportion of total protein-bound ^{14}C -activity of the medium by about 70% and the amount of ^{14}C -hydroxyproline by 90%.

Serum Albumin and Globulin

Axelsson and Piscator (20) found a reduction in serum albumin and an increase in alpha- and beta-globulins in rabbits (Belgian Giant) given 0.25 mg/kg Cd (II) sc.

8. Effects on Tissues and Organs

Kidney

Cadmium acetate, 0.03 m-mole/kg, injected into rats sc on the 21st day of pregnancy caused 40% mortality within the first 24 hours. In 80% of the animals, bilateral hemorrhagic renal necrosis was found (21). Tubular lesions of the nephrotic type developed in rats given a single sc injection of cadmium chloride (10 mg Cd/kg) (22). Subcutaneous doses of 0.02 mM/kg cadmium chloride, however, did not cause kidney damage in this species although testicular necrosis occurred (23). Cats exposed to cadmium oxide fumes, 18 mg/m³ Cd gradually reduced to 4 mg/m³ Cd over a period of 24 hours, showed a moderate amount of fat in the renal tubular epithelium when sacrificed at 5 to 9 days. It was estimated that between 6 and 12 mg of cadmium had been inhaled by the animals (24). Nordberg et al. (11) reported that cadmium-thionein also was toxic for the kidney. In a study with CBA mice, this complex was lethal at lower doses and caused more damage to renal tubules than cadmium chloride when the preparations were administered iv or sc (11). Metallothionein can protect mouse testes, however, from cadmium injury (10).

Lung

Inhalation of cadmium oxide fumes at concentrations greater than 1/4 LD₅₀ can cause acute symptoms and permanent lung damage in experimental animals. In rats, inhalation of finely dispersed cadmium oxide or cadmium chloride aerosols resulted in acute cadmium poisoning, manifested by an initial acute pulmonary edema (first 24 hours), proliferative interstitial pneumonitis (3rd to 10th days), and finally, permanent lung damage in the form of perivascular and peribronchial fibrosis (25).

Pancreas

Ghafghazi and Mennear (26) reported that in response to glucose, tolbutamide, and potassium, insulin secretion by isolated rat pancreata was inhibited following perfusion with 1×10^{-3} and 5×10^{-4} M Cd.

Macrophages

Waters et al. (27) investigated the cytotoxic effect of cadmium in an in vitro test employing rabbit alveolar macrophages as the indicator system. Cadmium chloride at 0.099 mM Cd (II) reduced cell viability (without cell lysis) to 50% of control values in 20 hours. Specific activity of acid phosphatase, a lysosomal indicator enzyme, was also reduced to 50% in 20 hours by a concentration of 0.205 mM Cd (II).

Gonads - See Reproductive and Teratogenic Effects.

9. Cytologic and Cytogenetic Effects

Gilliavod and Leonard (28) found no increase in dominant lethal mutations from cadmium chloride (1.75 mg/kg) administered ip to male BALB/c mice. There were no chromosomal rearrangements in dividing spermatocytes removed from animals sacrificed three months after cadmium administration, or in their male offspring.

10. The Molecular Site of Cadmium Toxicity

RNA-Polymerase and Protein Synthesis

Hidalgo et al. (29) found that cadmium inhibited both RNA-polymerase and protein synthesis in the liver in female albino rats given 20 umol cadmium chloride kg/rat ip. RNA-polymerase inhibition was maximal at 1 1/2-2 hours and increased continuously thereafter. Maximum inhibition of cytoplasmic protein synthesis occurred one hour after cadmium injection, then recovered and increased from 7-16 hours. Soon after exposure, cadmium apparently depresses both RNA and protein synthesis by separate mechanisms, since maximum inhibition of protein synthesis precedes maximal RNA synthesis inhibition.

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Nucleic Acid and Protein Synthesis

Stoll et al. (30) studied the effect of cadmium on nucleic acid and protein synthesis in the liver following ip administration of cadmium acetate to male Sprague-Dawley-derived rats. The authors found that cadmium both enhanced and inhibited RNA synthesis in vivo as well as in vitro, depending on the dose and time. The effect appeared to be correlated with the concentration of the cadmium ion in the liver. In isolated hepatic nuclei, RNA synthesis was inhibited at concentrations as low as 5×10^{-8} M Cd^{2+} . DNA synthesis was decreased in partially hepatectomized rats at a level of 0.34 or 1.70 mg Cd^{2+} /kg administered ip. It was suggested that cadmium may interact directly with DNA on the basis of its ability to alter the melting profile of calf thymus DNA. Cadmium administration caused a trend in vivo toward enhancement of mRNA activity, whereas polyuridylic acid-directed amino acid incorporation into protein was inhibited. A concentration-related decrease in mRNA and polyuridylic acid-directed incorporation was observed when cadmium was added to the microsomal system; an increased incorporation was noted only at the lowest cadmium concentration (10^{-8} M). At concentrations greater than 10^{-6} M, there was a decrease in mRNA activity.

11. Reproductive and Teratogenic Effects

Gonads Testes. Complete testicular necrosis occurred in mice given 1.1 mg Cd/kg sc. Doses of 0.25-0.5 mg/kg, however, caused little or no damage (10). In a study involving 80 rats, Parizek and Zahor (31) found severe damage to all testicular tissue as early as 24-48 hours after sc injection of 1 ml of 0.03 M cadmium chloride. Edema and capillary stasis were observed 2-4 hours after injection. Mason et al. (32) found a dose-response relationship in a study involving testicular damage in rats given single doses of cadmium chloride sc. The lowest dose, 0.57 mg CdCl_2 /kg had no effect; at 0.85 mg CdCl_2 /kg damage to the testes was observed in 32% of the animals; at 1.1 mg CdCl_2 /kg, the incidence was 90%, and at 1.4 mg CdCl_2 /kg, 100% of the rats were affected. Parizek (33) noted a decrease in the weight of the accessory sex organs, seminal

vesicles, and prostate glands, plus replacement of testicular tissue by eosinophilic material in rats and mice injected sc with cadmium chloride at a level 0.04 mmol/kg. After reviewing the results of studies on testicular necrosis from Cd (II), Friberg et al. (2) postulated that damage to the germinal epithelium is probably secondary to the vascular damage caused by cadmium.

Gunn et al. (34) found that 0.012 mmol cadmium chloride/kg administered sc was the minimal dose needed to produce complete necrosis of all seminiferous tubules by 6 days in mature male C.D. Charles River mice. Testicular damage was blocked completely by BAL (0.5 mmol/kg) if the dithiol was given 6 hours before the cadmium injection. Vascular damage to the testis was prevented by cysteine (6 mmol/kg) when the thiol was administered before or after cadmium was injected. Cadmium chloride administered to male mice ip at 1.35-7.0 mg/kg by Epstein et al. (35) resulted in a reduced rate of pregnancy, suggesting that Cd (II) may have caused testicular dysfunction prior to mating. There were no early fetal deaths or preimplantation losses attributable to cadmium.

Ovaries. Discovery of the severe effect of cadmium on the male gonads, stimulated investigation of possible effects on ovaries. Studies with rats and gerbils have shown that Cd (II) has a sterilizing effect on the ovaries of these species.

Kar et al. (36) reported acute changes in ovaries of prepubertal rats (6-8 weeks old), involving mass atresia of all follicles within 48 hours when cadmium chloride was administered sc in a single dose of 10 mg/kg. According to the authors, the atresia observed might be due to "interference with pituitary factors". Massive hemorrhage, accompanied by necrosis, was observed in the ovaries of all rats given cadmium chloride or acetate sc at a dosage of 0.02-0.04 mmol/kg, in a study conducted by Parizek et al. (37). The animals had been injected 3-6 months earlier (5 days of age) with either testosterone propionate or

19-nortestosterone phenylpropionate and then treated with cadmium after a persistent estrus had developed. Kaul and Ramaswami (38) observed severe, but reversible, effects on the ovaries of both mature and immature female gerbils given a single sc injection of cadmium chloride. Mature animals were given 0.45 mg; immature gerbils received 0.22 mg. Extensive hemorrhages and widespread atresia were found in all animals. A gradual reduction in ovarian weights also occurred in the adult animals. Follicular atresia might be due to alterations in the blood supply rather than from a direct effect of cadmium on germ cells. Development of placental necrosis in pregnant rats treated with cadmium lends support to this hypothesis (1).

Behavior. Madlafousek et al. (39) discovered that cadmium had a behavioral effect on male rats. Injection of cadmium chloride at a dosage sufficient to cause complete testicular necrosis and permanent sterility (0.04 mmol/kg), caused a temporary loss of copulatory activity within 3 weeks in most animals tested. The effect gradually subsided, with only slight impairment of sexual behavior after 2 months. Administration of androgens prevented the effect.

Teratogenesis

Teratogenic effects have been induced in rats, mice, and hamsters by a single injection of cadmium salts (sc, ip, or iv) during the 7-11 day period of gestation. Barr (40) found that cadmium chloride (16 μM Cd^{++} /kg) was teratogenic when administered ip to Wistar rats on day 9, 10, or 11 of gestation. Doses greater than 22 μM Cd^{++} /kg were often fatal to pregnant rats. Susceptibility was greatest when the compound was administered on day 9. Anophthalmia or microphthalmia, dysplastic or absent ears, and hydrocephaly were the most prominent malformations mentioned. Subcutaneous injections of cadmium chloride were not teratogenic in either stock of rats employed in the study.

A dose-related increase in the malformation rate was found by Ishizu et al. (41) in pregnant mice given cadmium chloride sc at levels of 0.63, 2.5, and 5 mg/kg on day 7 of gestation. The highest 'no effect' dose was 0.33 mg/kg. Exencephaly was the most common malformation found.

Absence of tail, vaginal atresia, and skeletal malformations were also observed. Some dams aborted. Amniotic fluids were hemorrhagic. Cadmium concentrations were significantly increased in the placenta but not in the fetus.

Fetal abnormalities in cartilage formation and delayed ossification, as well as cleft lips and palates, were induced in pregnant hamsters given cadmium sulfate (2 mg/kg) iv on the 8th day of gestation in a study conducted by Mulvihill et al. (42). The authors attributed the cleft malformations to "a mesodermal deficiency rather than to a delay in shelf transposition."

Reproductive Performance

Dixon et al. (43) studied the effect of cadmium on reproductive performance in Sprague-Dawley rats using the serial mating technique. Male rats given a single oral dose of 6.25, 12.5, or 25 mg Cd/kg in the form of cadmium chloride were housed with an untreated female for ten 7-day periods. The females were sacrificed 9 days after the end of the breeding period (9-16 days pregnant) and examined for numbers of reabsorptions and viable fetuses. The authors concluded that cadmium, at the concentrations tested, was without significant reproductive toxicity.

12. Cadmium Metabolism

The fate of cadmium in laboratory animals has been investigated after exposure via dermal, respiratory, gastrointestinal, or parenteral routes.

Absorption

Skin. Data on absorption of cadmium through the skin apparently are very meagre. A single report by Wahlberg (44) indicated that guinea pigs absorbed only 1.8% of 2 ml of an aqueous solution of cadmium chloride (0.239 M) via this route in 5 hours.

Respiratory Tract. Dogs exposed to a cadmium chloride aerosol showed a rapid clearance from the lungs during the first 2 weeks. Long-term clearance appeared to be slow, since no further decrease was observed after 10 weeks (45). Friberg et al. (2) have estimated that about 10-40% of cadmium inhaled by laboratory animals is absorbed.

Gastrointestinal Tract. Rats given about 6.6 mg/kg of ^{115}Cd as the nitrate apparently absorbed about 1-2% after 24 hours, as indicated by 98-99% found in stomach, gut, and feces (46). Monkeys (Saimiri sciureus), intubated with 0.17 mg Cd/kg-1.7 mg Cd/kg BW as $^{115}\text{CdCl}_2$, retained an average of 2.9% of the amount given 10 days after ingestion, as determined by whole body measurement (47). When the dose was reduced to 1 ug/kg BW, the average whole body retention after 10 days was 1% (48). A considerable amount of unabsorbed cadmium is eliminated via the feces as late as between the 5th and 10th day after ingestion (2).

Transport

Cadmium is found in the blood following exposure via air, gastrointestinal tract, or injection. In most studies on clearance of cadmium from the blood of experimental animals, exposure was via injection.

Intravenous Route. The clearance of cadmium from plasma is characterized by an initial rapid phase followed by a slower phase. Using dogs, most of the radioactive cadmium administered as the nitrate disappeared from the plasma within the first 30 minutes; thereafter the concentration remained approximately the same for the duration of the 2-hour measurement (50).

Intraperitoneal Route. Maximum blood levels are reached within a very short time after an intraperitoneal injection of soluble cadmium compounds. For example, Johnson and Miller (51) found that the maximum concentration was reached after 5 minutes in rats given ^{109}Cd ip as the chloride. After 80 minutes, the blood level was only 12% of the maximum value, indicating rapid clearance from the blood.

Subcutaneous Route. Cadmium appears in the blood soon after subcutaneous injection of a soluble cadmium compound and reaches maximum levels within 10-60 minutes, depending on the amount injected. Most of the element is found at this time in the albumin or larger protein fractions (2). During the first 6 hours after a single sc injection, of ^{109}Cd , the plasma contained per unit volume more radioactivity than did the blood cells. In later periods the radioactivity in the plasma declined to low levels, whereas in the blood cells it increased (52).

In human beings, a mean blood-Cd concentration of 3.4 ng/ml was found in healthy subjects with normal blood pressure, as compared with a mean of 11.1 ng/ml in untreated hypertensive patients. Blood contains about 0.1% of the total body burden of cadmium (53).

Tissue Distribution and Retention

Oral Intake. In rats given 115 cadmium nitrate orally, the liver contained the greatest total amount of cadmium 8 hours after administration. The maximum concentration was reached in both organs at 72 hours, following which there seemed to be a slow decrease over the remainder of the 360-hour observation period (46). Friberg et al. (2) noted a study by Miller et al. (54) who gave goats a single oral dose of 109 cadmium chloride and observed that after 14 days the concentration in the kidneys was more than twice as high as that in the liver.

Injection Route. Distribution of cadmium following injection varies considerably depending on the dose and the time. After uptake to the blood, the liver-kidney distribution pattern is generally the same as that found after oral administration. Initially, most of the cadmium goes to the liver and relatively small amounts to the kidneys. Later, kidney levels increase and eventually become higher than liver levels (2). For example, Burch and Walsh (55) gave dogs an iv injection of 115m cadmium nitrate (0.32 to 0.4 mg Cd/kg) and found that the concentration in the liver during the first 24 hours was considerably greater than that in the kidneys. After 20-30 days, the concentration in the kidneys was 50 to 100% that of the liver levels.

Placental Transfer

Experimental Animals. Animal experiments have indicated that the placenta is a barrier against the transfer of cadmium when small doses are given. When the doses are large, cadmium may "destroy" the placental barrier and enter the fetus (2). Berlin and Ullberg (56) gave pregnant mice single iv injections of $\text{Cd}^{109}\text{Cl}_2$ (8 μc , carrier-free). Animals sacrificed on the 18th day following conception showed no cadmium in the fetus, although a special uptake of the element was found in some parts of the placenta. On the other hand, Ferm et al. (57) found significant amounts of cadmium in both the placenta and fetus in pregnant hamsters after administration of a single iv dose of $^{109}\text{CdCl}_2 - \text{CdSO}_4$ (carrier-free) on the 8th day of gestation.

Friberg et al. (2) stated that the liver and kidney appear to be the two organs of greatest interest with regard to cadmium storage. Indeed, prior to the occurrence of renal damage, 50-75% of the total body burden of cadmium is estimated to be in these two organs in experimental animals. In these two organs, the pancreas and spleen also store relatively large amounts; some cadmium will be found in most other compartments of the body. The accumulation in the liver and kidneys appears, at least, to be mainly dependent on the storage of cadmium in the metal-binding protein, thionein.

Humans. Chaube et al. (58) found Cd concentrations of the following order in fetuses derived from Japanese mothers: whole embryo, 0.032-0.101 ug/g wet tissue in 57% of embryos; in 80% of livers, a mean level of 0.113 ug/g; in 28% of kidneys, mean 0.05 ug/g; in 17% of brains, mean 0.14 ug/g. Whole embryo concentrations were determined in first trimester embryos. Liver, kidney, and brain determinations were made on fetuses of the second trimester. None of the mothers had Itai-Itai disease or resided in an area where it had been reported.

Excretion

Animal studies have shown that after a single exposure cadmium may be excreted via the urine, feces, bile, hair, or saliva (2).

Urinary Excretion. Lucius et al. (52) found that in rats, around 1% of a single sc injection of ^{109}Cd cadmium solution was excreted in the urine after one week. In another study in rats, Shaikh and Lucius (59) found a daily excretion in the urine of only 0.003 to 0.007% of the dose administered. In this study, however, $^{109}\text{CdCl}_2$ (carrier-free) and Zn chloride Zn65 were administered simultaneously. After analysis of a number of other studies, Friberg et al. (2) asserted that no conclusions could be drawn with regard to urinary excretion of cadmium following a single exposure.

Excretion Via the Alimentary Tract. Decker et al. (46) gave rats a single iv dose of ^{115}Cd (0.63 mg/kg) and found that after 24 hours, 7.3% was present in the feces. After 72 hours, 18.5% of the dose had been eliminated by this route.

Hair. Truhaut and Boudene (60) reported high concentrations of Cd in the hair of rats and rabbits injected with cadmium and suggested that analysis of the hair could be of value for estimating cadmium levels in workers exposed to the metal or its compounds. Berlin and Ullberg (56) found that cadmium also accumulated in the hair of mice following a single dose of ^{109}Cd cadmium.

Milk. Friberg et al. (2) calculated, from the data of Lucis et al. (61), that excretion of cadmium in the milk was less than 0.05% per gram of milk per day following a single sc postpartum injection of ^{109}Cd together with ^{65}Zn to female rats. In a study by Tanaka et al. (62), sucklings of female mice not given radioactive cadmium were suckled by female mice that had been given radioactive cadmium before delivery. After 14 days, 0.3% of the dose given the sucklers was found in the sucklings, as determined by whole body measurements. Since some cadmium must have been excreted via the feces of the sucklings, the total amount excreted in the sucklers' milk must have been greater than 0.3% (2).

Saliva. Driezen et al. (63) found average concentrations of cadmium in marmoset saliva after stimulation with pilocarpine and mecholyl to be 4.4 ug% and 7.8 ug %, respectively, with a maximum of 34.4 ug% (pilocarpine).

Biological Half-Time

The estimated biological half-time of cadmium following a single exposure is about 200 days in rats and mice, 400 days in dogs, and 1.5 years in squirrel monkeys. If renal dysfunction occurs, the biological half-time will be changed considerably because of the greater excretion of cadmium (2).

13. Carcinogenesis

No data in literature surveyed to date.

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

1. Hematologic Effects

Several studies in experimental animals have revealed that cadmium causes anemia. Friberg (64) suggested that the type of anemia induced by cadmium was mainly hypochromic, and he studied the effect of iron or liver in male rabbits given daily sc injections of 0.65 mg/kg Cd (6 days/wk) over a period of 70 days. Even large amounts of iron, however, failed to completely prevent anemia, indicating that the condition was due in part to an alteration in iron utilization. Hemoglobin values were significantly greater in the group receiving iron than in other groups. In another study, the effect of dimercaprol on cadmium intoxication was investigated. Rabbits given 0.65 ug Cd/kg, as cadmium sulfate sc, 6 days per week for 10 weeks, were treated 3 times each day with either 4 or 12 mg/kg sc injections of the drug. A control group received cadmium sulfate only. A significant and progressive decrease in blood hemoglobin occurred, although erythrocyte counts decreased significantly only in the high dose dimercaprol animals. The medication also seemed to worsen other effects of cadmium poisoning, i.e., proteinuria, weight loss, and lethality. Four animals receiving the higher dose of dimercaprol and 3 given only cadmium sulfate died during the experiment. All animals showed splenic fibrosis, hepatic cirrhosis, and nephrosis at autopsy. Cadmium concentrations of the organs studied did not differ from group to group; values were 60, 30, and 25 mg/100 g wet tissue for liver, kidneys, and spleens, respectively (65).

2. Bone Marrow Changes

There were no subchronic studies regarding the effect of cadmium on bone marrow in experimental animals in the literature surveyed.

In a study by Friberg (66), bone marrow examinations of 19 workers exposed to cadmium revealed no pathologic changes.

3. Immunologic Response

Rabbits given 300 ppm cadmium chloride in the drinking water for 70 days had significantly lower neutralizing antibody titers than did the controls, after inoculation with pseudorabies virus. Antibody titers were determined by the plaque assay method (67).

4. Central Nervous System (CNS) Effects

No data reported in literature surveyed to date.

5. Behavioral Effects

No data reported in literature surveyed to date.

6. Cardiovascular Effects

Rabbits

Thind et al. (68) induced hypertension in rabbits by giving weekly ip injections of cadmium acetate (2 mg/kg) for 7 weeks. The authors suggested a decrease in vascular responsiveness to angiotensin as playing a role in the pathogenesis of cadmium hypertension. The mortality rate over the 7-week period was 25%.

Rats

Porter et al. (69) found no evidence of an elevation in blood pressure in female Sprague-Dawley-derived rats given cadmium acetate ip at 2 mg Cd/kg, followed by a second dose of 1 mg Cd/mg ip 3 weeks later. Aortic strips from cadmium-treated rats showed a diminished reactivity to angiotensin, epinephrine, barium, and tyramine. The authors concluded "that cadmium desensitizes rat vasculature to vasoconstrictors and vasodilators independently of its ability to cause hypertension." Fowler et al. (70) observed that oral administration of cadmium caused

constriction of the smaller, and mild dilation of the larger, renal arteries, as well as a fibrosis of peritubular capillaries in male rats. The animals were treated with cadmium chloride in the drinking water at levels of 0.0 to 200 ppm Cd (II) for 6 or 12 weeks. The diets contained normal (0.7%) or low (0.1%) calcium. Cadmium concentrations in the kidneys of animals on a low calcium diet were higher than those on the normal diet. In addition to the blood vessel effects observed, cadmium also caused an increased BUN but little tubule cell damage.

7. Biochemical and Histochemical Changes

Liver and Kidney Enzymes

Short-term studies in rats revealed that cadmium increased the activity of four glucose-synthesizing enzymes in both liver and kidney. The test animals were given daily ip injections of cadmium chloride (1 mg/kg) over a period of 45 days; controls received saline. An increase in blood glucose and a decrease in liver glycogen were correlated with the increased enzyme activity. Even when cadmium injections were discontinued for 28 days, increases in glucose synthesis in liver and kidney cortex were not reversed (71). According to NIOSH (1), these data suggest a possible biochemical basis for some of the toxic effects caused by cadmium. They pointed out, however, that the results are in direct contrast with those of Rutman et al. (16) who found that cadmium strongly inhibited glucose formation by rat kidney cortex in vitro.

Vigliani (72) noted a severe reduction in kidney leucine aminopeptidase activity in mice given 50 ug/day of cadmium over a period of 5 days.

Cyclic Adenosine Monophosphate (AMP)

The effect of cadmium on AMP in the testes and prostate of rats given daily ip injections of 1 mg/kg of cadmium chloride over a period of 45 days was investigated to ascertain whether testicular damage was related to changes in this mediator. Decreases in the weights of the testes and prostate, as well as decreased body weight, were observed in the treated rats. Cyclic AMP levels in the testes remained unchanged (adenyl cyclase was significantly increased but this was offset by a concomitant increase in phosphodiesterase activity). The activities of cyclic AMP-dependent and AMP-independent forms of testicular protein kinase were decreased, but the binding of cyclic AMP to protein kinase was not affected. The results were the opposite in the prostate gland: there was a decrease in cyclic AMP (attributed to a decrease in activity of adenylyl cyclase), increased cyclic AMP binding to prostatic protein kinase, and increased cyclic AMP-dependent protein kinase activity (73).

Mitochondria

Sporn et al. (74) found evidence of altered phosphorylation (uncoupling) in liver mitochondria in rats given 10 ppm of Cd (II), as cadmium chloride, in the food over a period of 60 days.

Collagen

Panigrahy and Patnaik (75) studied the effect of cadmium chloride on collagen in the garden lizard. Immature male animals given two 25 ug ip doses of CdCl₂ 7 days apart showed increases in the solubility of collagen and in the soluble/insoluble collagen ratio in bone, cartilage, and tendon, when sacrificed 2 weeks after the first injection. There was a decrease, however, in the total collagen content. The authors suggested that the cadmium salt prevented collagen cross-linking and may have interfered with collagen metabolism.

8. Effects on Tissues and Organs

Adrenals

The adrenal glands are adversely affected by cadmium. Singhal et al. (76) injected rats daily with 1 mg/kg cadmium chloride ip for 45 days and found increases in adrenal weights and norepinephrine and epinephrine levels, as well as increased adrenal tyrosine hydroxylase activity. Adrenal weights were still somewhat greater than normal 28 days after the last injection, although norepinephrine and epinephrine levels, as well as tyrosine hydroxylase activity, had returned to normal.

Pancreas

Ithakissios et al. (77) found that ip injections of cadmium acetate solution in rats at 0.5 mg Cd/kg every other day for 70 doses caused a decrease in insulin secretion and immunoreactive insulin (IRI) release when the pancreata were perfused with buffered glucose. Levels of 0.25-0.5 mg Cd/kg resulted in reduced excretion of radioactive $^{14}\text{CO}_2$ from the lungs and increased excretion of radioactive carbon and glucose in the urine. There were no significant changes, in plasma glucose or insulinogenic index (ratio of plasma IRI to plasma glucose).

Liver

Prodan (24, 78) reported fatty infiltration of the liver in cats exposed to cadmium oxide fumes or dust, but not cadmium sulfide, for brief periods of time.

Bone

Rabbits injected with 1 mg cadmium sulfate daily for two months showed a decrease in calcium serum levels and decreased mineral content in bone (79).

9. Cytologic and Cytogenetic Changes

No data reported in literature surveyed to date.

10. The Molecular Site of Cadmium Toxicity

No data reported in literature surveyed to date.

11. Reproductive and Teratogenic Effects

A dose-related increase in fetal deaths, rate of fetal anomalies (micrognathia, cleft palate, clubfoot, and small lungs) and a decrease in fetal weights were observed in CD-strain rats by Chernoff (80) after administration of 4-12 mg/kg cadmium chloride on 4 consecutive days beginning on day 13, 14, 15, or 16 of gestation. The author concluded that a decrease in lung size in fetuses from rats injected at 8 mg/kg on days 14-17 was definitely a specific retardation. It was uncertain whether the fetal anomalies were the result of a direct action of cadmium on the fetus or from placental effects or maternal effects, singly, or in combination. Tsvetkova (81, 84) found an effect on the estrus cycle in female rats exposed to cadmium sulfate. Neonates from exposed dams were smaller and weighed less than offspring of control animals and the cadmium content of the liver was 2.5 times as high as in the controls.

12. Cadmium Metabolism

Absorption: Gastrointestinal Tract

Miller et al. (82) estimated an 18% absorption of daily oral doses of cadmium (3.0 g/day) administered to cows over a period of 2 weeks on the basis of finding 82% of the dosage excreted in the feces. Friberg et al. (2) stated that this figure should be regarded with caution since it has been shown that cows in cadmium-contaminated areas of Sweden show renal

levels around 1-2 ug Cd/g wet weight which indicates a low absorption. These authorities concluded that most animal studies indicate an absorption of about 2% of ingested cadmium, although there are large individual variations. They pointed out, however, that a low calcium or a low protein intake will increase cadmium absorption by a factor of 2 or more.

Transport

Friberg (64) administered 115 cadmium sulfate sc (0.65 mg Cd/kg) to rabbits 6 days a week and found a steady increase in blood levels up to a maximum of more than 2 ug/ml after about 60 days.

Tissue Distribution and Retention

Liver, Kidney, Pancreas, Spleen. Friberg (83) gave sc injections of 115 cadmium sulfate (0.65 mg Cd/kg) to rabbits 6 days a week for 4 weeks in one group and for 10 weeks in a second group. After 4 weeks, mean cadmium levels in liver, kidney, pancreas, and spleen were 1160, 600, 75, and 45 ug/g (dry wt.), respectively. After 10 weeks, the corresponding values were 1480, 1000, 193, and 180 ug/g.

Embryo and Placenta. In pregnant rats exposed from the day of conception to cadmium oxide dust in a concentration of approximately 3 mg/m³, the livers of the embryos after 22 days had a cadmium content more than twice that of the controls (81). Parizek (85) found that sc administration of 0.04 mmol Cd(II)/kg in the form of cadmium chloride, acetate, or lactate to pregnant rats caused complete destruction of the pars foetalis resulting in delivery of the dead conceptus or resorption. In most cases the placental changes were accompanied by hemorrhage.

Excretion

Urine. Friberg (83) administered 115 cadmium sulfate sc to rabbits in daily doses of 0.65 mg Cd/kg and followed the excretion of the isotope in the urine for 10-12 weeks. During the first 6-7 weeks, the daily excretion of cadmium was very small, less than 1% of the daily dose. About the 8th week, however, there was a sharp rise in the excretion rate reaching a level of approximately 100 times that of the amount excreted during the first weeks. This rise was found to coincide with the appearance of proteinuria in the animals.

Alimentary tract. Ceresa (79) found the mean fecal excretion rate of cadmium to be 1.8% of the injected amount in five rabbits given about 5.5 mg Cd/kg daily (as the sulfate) by the subcutaneous route. The amount excreted in the urine over the exposure period was slightly less than that eliminated in the feces. All of the animals died 7 to 9 days after the first injection.

Biological Half-Time

No data reported in literature surveyed to date.

13. Carcinogenesis

No data reported in literature surveyed to date.

CHRONIC TOXICITY (Long-term tests, 2-years, or lifetime treatment)

1. Hematologic Effects

Fitzhugh and Meiller (86) gave rats cadmium (cadmium chloride) in their diets at levels of 15-135 ppm for up to 6 months. At 135 ppm, there was pronounced anemia, low hemoglobin, and RBC counts as low as 2 million. There was also a reduction in growth rate and in some cases early death. At 45 ppm, some animals had no blood changes after one year and at 15 ppm, only one animal had marked anemia. Wilson et al. (87) also found that rats developed anemia after 2-3 months when fed a diet containing as little as 0.0062% of cadmium as cadmium chloride. Increases in reticulocytes and eosinophils were also noted in a group eating a diet with 0.0125% cadmium as cadmium chloride for 3 to 7 months. As little as 0.0016% of cadmium in the form of cadmium chloride in the diet caused bleaching of the enamel of the incisor teeth.

2. Bone Marrow Changes

In a study by Wilson et al. (87) described above, the authors also found hyperplasia of the bone marrow after feeding cadmium in the diet at concentration of 0.0125% cadmium chloride for 3-7 months. Bone marrow changes were not found in 19 workers studied by Friberg (66).

3. Immunologic Response

Koller et al. (88) exposed Swiss Webster mice to cadmium chloride at levels of 3 or 300 ppm (cadmium) in deionized water for a period of 70 days, and then ascertained an immune response by ip inoculation of the mice with sheep RBC antigen (SRBC) at varying periods of time after cadmium exposure. Cadmium levels as low as 3 ppm caused a considerable reduction in the number of spleen cells producing 19S or 7S antibodies. The primary immune response (19S antibody) varied according to the interval between cadmium treatment and administration of antigen. A reduction was noted at the 300 ppm level for as long as 42 days after cadmium exposure. Secondary immune responses (7S antibodies) were also reduced at both cadmium exposure levels over the same time period.

4. Central Nervous System (CNS) Effects

Humans

Vorob'yeva (1, 89), in a study of 160 workers exposed to cadmium oxide (cadmium concentrations of 0.1 to 24 mg/cu m in the workroom air), found prolongation of the chronaxy of cutaneous sensory nerves in many cases, as well as optical chronaxy, which was proportional to years of exposure to cadmium. Major complaints were headache, dizziness, irritability, depression, and sleep disturbances. Motor chronaxy changes were also noted. In workers with less than 5 years exposure, muscle chronaxy was reduced; in workers with greater than 5 years exposure to cadmium, it was increased. Signs of change in the functional state of the cerebral cortex were also observed, although the author stated that further research was needed before definite conclusions about the effects of cadmium on the brain could be drawn.

Experimental Animals

Friberg et al. (2) stated that long-term studies with small doses of cadmium to investigate effects on the nervous system have not been reported.

5. Behavioral Effects

No data in literature surveyed to date.

6. Cardiovascular Effects

Schroeder (90) found that trace amounts of cadmium (5 ug/ml) given to rats in their drinking water caused hypertension after approximately one year; the incidence increased with age. The incidence was higher in females than in males, although the mortality rate was greater in males with hypertension; the median lifespan was shortened in both sexes. Soft

water increased the hypertensive effect of cadmium. In contrast to the findings of Schroeder (90) above, Lener and Bibr (91) were unable to detect hypertension when female Wistar rats were given cadmium in the drinking water (5 ppm) over a period of 16 months. The authors state that there are factors in food which decrease the hypertensogenic effect of cadmium ions.

7. Biochemical and Histochemical Changes

Sporn et al. (74) observed changes in liver enzymes in rats given cadmium (as cadmium chloride) in the drinking water at a concentration of 1 mg/liter over a period of 11 months. There was an increase in phosphorylase and a decrease in aldolase activity, indicating alterations in hepatic carbohydrate metabolism. Mitochondrial oxidative phosphorylation was unaffected, even when cadmium administration was extended to 335 days.

8. Effects on Tissues and Organs

Kidney

Wilson et al. (87) fed cadmium chloride to rats in the diet in concentrations of 0.0031-0.05% cadmium over a period of 100 days. Histological examination revealed slight tubular changes but no glomerular alterations in the animals exposed to 0.025 and 0.05% dietary cadmium. Renal tubular damage was also observed in rabbits (Belgian Giant) given cadmium chloride sc at 0.25 mg cadmium per kg 5 days a week for 11-29 weeks, according to Axelsson and Piscator (92). The tubular damage was localized in the proximal segment and microscopic changes were also found in the glomeruli. Cadmium deposition was mainly in the renal cortex (proximal segment of tubules and distal tubules but not in collecting tubules, glomeruli, or stroma). Following the development of proteinuria, significant increases in amino acid excretion in the urine occurred. After renal damage, excretion of cadmium increased greatly and eventually exceeded amounts being administered each day.

Teeth

Bleaching of the incisor teeth was observed by Fitzhugh and Meiller (86) when 3-week old rats were given cadmium (cadmium chloride) at levels as low as 15 ppm in the diet over a period of 6 months. Ginn and Volker (93) also noted decreased pigmentation of incisor enamel in rats given 50 ppm of cadmium (cadmium chloride) in food or drinking water for 150 days in a study on the possible relationship of cadmium administration to dental caries. In this regard, it was found that administration of cadmium in the water apparently increased susceptibility to caries. There was also a reduction in blood hemoglobin, and the authors suggested that blood and enamel effects may have resulted from interaction of cadmium with iron-containing proteins. In another experiment with rats given 0.004% cadmium chloride in the drinking water during the period of calcification of the molars, there was no increase in numbers of carious lesions but there was an increase in the rate of their progression which was attributed to cadmium intake (94).

Bone (Human)

Itai-Itai disease, which occurs in certain areas of Japan, has been attributed to cadmium in water and crops polluted by industrial waste. The disease appeared like osteomalacia, with painful joints and bones in the back and legs. A dietary deficiency (low calcium and protein), as well as low estrogen levels, may also have had a bearing on the osteoporosis (1).

Experimental Animals

Shimizu et al. (95) administered $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ to adult Wistar rats at a level of 245 ppm in the drinking water for 100 days and observed a marked retardation in growth and a slight decrease in the weight of the femur, as well as an abnormal proportion of water and calcium in bone. According to the authors, the abnormal proportion of water in the bone suggested that normal calcification or remodeling might have been impaired by cadmium. The ratio of calcium to hydroxyproline in both sexes was also higher in cadmium-treated animals than in the controls.

Testes - See Reproductive Effects

9. Cytologic and Cytogenetic Changes

No data reported in literature surveyed to date.

10. The Molecular Site of Cadmium Toxicity

No data reported in literature surveyed to date.

11. Reproductive Effects

Testes

Nordberg (10) found only slight testicular changes in CBA mice given 0.5 mg Cd/kg as cadmium chloride sc 5 days/week for 6 months, and no significant changes in the testes at a dosage of 0.25 mg Cd/kg. The lower dosage, however, caused a decrease in total protein excretion (96). Similarly, rabbits given cadmium chloride sc 5 days/week for 24 weeks at 0.25 mg Cd/kg showed no macroscopic or microscopic evidence of testicular changes, although marked damage to the kidneys was found (97).

Reproductive Performance

Rats. Wills et al. (98) conducted a four-generation study with rats on the effect of cadmium chloride, administered in the feed (both sexes) at levels of 33 or 73 ppb, on reproductive performance. Monogamous matings were established and continued until 4 litters/couple were delivered. Some animals sacrificed from each generation, all fatalities, and those killed at the end of the study were examined macroscopically and, where observations indicated, microscopically. At the 73 ppb level, there was a deficit in weight gain, a slight decrease in longevity, and a slight increase in fertility. At the 33 ppb level, a slight deficit in weight gain and a slight increase in longevity were noted. No significant macroscopic or microscopic changes were found in any of the 276 rats examined. The authors concluded that feeding Cd (II) at 33 ppb actually may have been beneficial.

Monkeys. Wills et al. (98) also investigated the effect of cadmium on reproduction in monkeys in an abbreviated study. Three female animals fed cadmium chloride in a sweetened beverage at 1.5 or 3.0 ug/kg/day for 18 months showed no adverse effects from the compound (one control monkey and one animal on the lower dosage died after 6 months from a disease, according to the authors, that was not related to Cd (II) ingestion). At the end of the treatment, the three survivors were mated with untreated males. One female at each of the cadmium dose levels delivered one infant which nursed and developed normally and appeared to have no abnormalities.

In the reproduction study by Dixon et al. (43), discussed in the Acute Toxicity section, rats were tested also for effects of cadmium on reproductive performance after exposure to cadmium levels of 0.001, 0.01, or 0.1 mg/liter in the drinking water for 90 days (estimated daily dose, 14 ug/kg). Forced breeding studies, as well as examination of reproductive organs and a variety of clinical chemistry tests, failed to reveal any significant reproductive toxic effects of cadmium.

12. Cadmium Metabolism

Absorption

Decker et al. (99) gave cadmium to rats in the drinking water at levels ranging from 0.1 ppm to 10 ppm for 6 to 12 months and determined the amount retained in the kidneys and liver. Since these organs accumulate most the cadmium (50 to 75%), it was estimated that the total body retention was less than 1% (2).

Transport

In mice given daily sc injections of 109 cadmium chloride (0.025 mg Cd/kg BW) 5 days per week for 18 weeks, Nordberg (100) found that the mean blood level and whole body retention at the 21st week were 48.4 ng/g and 61.1 ug, respectively.

Tissue Distribution and Retention

Distribution of cadmium among various organs in rabbits after long-term exposure was studied by Stowe et al. (101). The animals were given 160 ug/g as the chloride in the drinking water for 200 days, the mean exposure being 14.9 mg Cd/kg BW per day. Mean cadmium concentrations in liver, kidney, pancreas, and spleen were 188, 170, 29, and 10 ug/g wet weight, respectively. Renal tubular damage was detected by histopathological examination.

Excretion

Urine. Axelsson and Piscator (92) gave rabbits 0.25 mg Cd/kg as cadmium chloride sc for 5 days a week over a period of 6 months in an excretion study. During the first 4 months, there was an insignificant excretion of cadmium in the urine. However, a sudden rise in the excretory rate then occurred and in some animals daily excretion exceeded the daily dose. An increase in urinary protein paralleled the increased urinary excretion of cadmium.

Biological Half-Time

The half-time of radioactive cadmium in mice after a single injection, followed by daily doses of non-radioactive cadmium for 25 weeks, was found to be 2 years (2).

13. Carcinogenesis

Injection Route

Heath and Webb (102) induced rhabdomyosarcomata in rats by im injection of powdered cadmium metal and maintained the tumors for at least 6 years by transplantation (75 transplants). Most of the metal that was incorporated intracellularly by the primary tumor was bound by the nuclear fraction. Later studies revealed that at least 50% of the nuclear fraction was bound by the nucleoli (103). Kazantzis and Hanbury (104) observed sarcomata in the rat after injection of a single dose of cadmium sulfide. In this study, *metastatic tumors also were found in regional lymph nodes and lungs*. A number of other workers have induced injection-site sarcomas in rats with cadmium compounds. Gunn et al. (105) found pleomorphic sarcomas at the site of injection of four sc injections of 0.17 mg cadmium chloride in 3 of 30 male Wistar rats 12-16 months later. The same authors also induced interstitial cell tumors of the testes in rats and mice by a single sc injection of 0.03 mmol/kg of cadmium chloride (106).

Oral Route

Kanisawa and Schroeder (107) gave Long-Evans rats cadmium acetate in the drinking water at 5 ppm (Cd ion) from weaning until death, a period of 4 years. The Cd (II)-fed rats, however, did not have a statistically significant increase in tumors above the incidence in the untreated controls at the 0.05 level.

SIGNIFICANT PHYSICAL AND CHEMICAL PROPERTIES

Cadmium is a soft, ductile, silvery-white metal. Its physical properties are given in Table 1. It occurs with zinc, lead-zinc, and copper-lead-zinc ores in nature and is obtained as a by-product in the refining of these ores. Cadmium is difficult to separate completely from zinc and small amounts are found in commercial zinc compounds. It readily forms alloys with most heavy metals. It is precipitated from solutions by metallic zinc. Cadmium is slowly oxidized superficially by moist air and reacts readily with mineral acids and relatively slowly with many organic acids to form salts.

The distribution and fate of cadmium in the environment has not been investigated extensively. Air normally contains about 0.001 ug/m^3 . In the vicinity of cadmium-emitting factories, concentrations of 0.1 to 0.5 ug/m^3 have been reported. The normal concentration in water is less than 1 ng/g . Liver and kidney from animals and shellfish are typical foodstuffs that normally may contain levels greater than 0.05 ug/g . Rice and wheat may have concentrations as high as 1 ug/g derived from cadmium-contaminated soil or water.

Table 1

Significant Physical Properties of Cadmium

Atomic number	48
Atomic weight	112.40
Outer electron configuration	4d(10)5s(2)
Melting point	320.9 C, 609.7 F
Boiling point	765 C, 1409 F
Density	8.642 g/cc

Solubility of cadmium compounds in water (0-25 C):

Soluble at more than 100 g/100 cc

Cadmium chlorate, chloride, nitrate

Soluble at 50-90 g/100 cc

Cadmium bromide, iodide, sulfate

Soluble at 1-10 g/100 cc

Cadmium benzoate, cyanide, fluoride, lactate

Insoluble

Cadmium carbonate, hydroxide, oxide, selenide, sulfide

From: Occupational Exposure to Cadmium, 1976 (1)

EXPERIMENTAL CADMIUM INTOXICATION

ADDENDUM 1

ACUTE TOXICITY (including in vitro observations)

1. Hematologic Effects

Cadmium produces cytopathic effects in erythrocytes and platelets in vitro that are similar to those seen with other heavy metals. The underlying mechanism appears to be a toxic effect on sulfhydryl enzymes of the blood cells (108).

2. Bone Marrow Changes

In vivo, it seems possible that cadmium is transported to the bone marrow where it inhibits hemoglobin synthesis and is partly incorporated into the molecule (109).

4. Central Nervous System (CNS) Effects

Cadmium stearate inhibited outgrowth of rat cerebellar cells in vitro and caused degeneration at a concentration of 0.58×10^{-6} M, according to Kasuya et al. (110). The minimal inhibitory level for nerve fibers, glial cells, and fibroblasts was found to be about 2.3×10^{-6} M.

7. Biochemical and Histochemical Effects

The mechanism of cadmium toxicity has been the subject of much study. Buell (111) called attention to the fact that many of the 2,000 or more enzymes that regulate the great number of chemical reactions in cells are influenced or affected by zinc or other trace elements and suggested that cadmium toxicity may result from its replacement of zinc or reaction with -SH groups in certain enzymes. Other reviewers have pointed out that necrosis of the testis, subcutaneous sarcomas, and other

toxic conditions induced by cadmium can be partially or totally prevented by treatment with zinc, selenium, cobalt, thiol compounds, or estrogen (112, 113).

8. Effects on Tissues and Organs

Liver

Kajikawa et al. (114) exposed Chang's liver cells to cadmium chloride for 72 hours in vitro and found a decrease in cell numbers and a reduced proliferative rate at concentrations as low as 0.01 ppm. Complete reduction in growth occurred at a level of 1.0 ppm. The authors stated that these results were the same as those obtained in an unspecified animal test for cadmium toxicity.

9. Cytologic and Cytogenetic Effects

Shiraishi et al. (115) observed chromosomal aberrations in human leucocytes exposed in vitro at 37° C to cadmium sulfide added to the culture fluid during the last 8 and 4 hours of incubation to a final concentration of 6.2×10^{-2} ug/ml. Chromatid and isochromatid breaks, translocation, and dicentric chromosomes were found. No chromosome aberrations were noted in untreated control cell cultures. The authors concluded that it seems highly possible that cadmium sulfide is mutagenic.

Peripheral leucocytes from seven Itai-Itai disease patients also showed a significantly higher incidence of chromosomal abnormalities than those from controls. Chromatid breaks were especially common (116).

Rohr and Bauchinger (117) found that the mitotic index was significantly reduced in Chinese hamster fibroblasts ("HY") exposed in vitro to cadmium sulfate (10^{-6} mol/l) for 16 hours. Stickiness and pycnosis of chromosomes occurred at 10^{-4} mol/l. A stathmokinetic effect was observed after 3 hours at levels of 10^{-5} and 10^{-4} mol/l. The

mitotic index increased, mitoses were blocked in metaphase and "initial C-mitoses" and "C-mitoses" were observed. Structural chromosome aberrations (chromatid type mainly) were observed more than 12 hours after exposure for one hour to 10^{-4} mol/l cadmium sulphate.

The effect of cadmium and mercury on cellular organelles in C3H mouse L strain fibroblasts in vitro was studied by Kawahara et al. (118) with the electron microscope. Cadmium chloride at a concentration of 15.6 ug produced severe adverse changes in the cultures maintained at 37° C for 24 hours. The ribosomes disappeared, mitochondria showed varying degrees of destruction, and the endoplasmic reticulum was swollen and beadlike. The nucleus appeared to be actually structureless and its membranes were wavy and irregular.

10. Molecular Effects

Gantayat and Patnaik (119) found decreases in RNA, DNA, and RNA/DNA ratio in the testes of rats seven days after the intratesticular injection at a single dose of cadmium chloride consisting of 0.25 mg/100 g body weight. The authors pointed out, however, that the changes observed might have been secondary to ischaemia caused by cadmium, which in turn may lead to a reduction in metabolites required for nucleic acid synthesis.

Murray and Flessel (120) studied the effect of cadmium and several other biologically-important metals on the mixing curves of synthetic polyribonucleotides in vitro. Cadmium chloride at 10^{-3} M brought about changes suggestive of base mispairing between polyribonucleosinic acid (poly(I)) and polyribocytidylic-uridylic acid (poly(C,U)). A significant decrease in hypochromicity, indicating a decrease in the extent of interaction of the polynucleotide chains, as well as a small shift in the overall stoichiometry of complex formation, was found. The authors discussed the possibility that direct metal ion-nucleic acid interaction may be involved in metal carcinogenesis at least in some cases.

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

8. Effects on Tissues and Organs

Bone

Yoshiki et al. (121) observed retardation of bone growth as well as osteoporotic changes as early as three weeks, during treatment of weanling male rats with 10 to 300 ppm cadmium (in the form of cadmium chloride) in the diet. Kidney damage, on the other hand, did not occur until the seventh week. There were no osteomalacic changes at any time during the 12-week exposure period. The authors stated that the results obtained suggest that cadmium may act primarily on bone, rather than secondarily through disturbance to the kidneys.

Thyroid

Der et al. (122), in a short report, stated that cadmium had a deleterious effect on thyroid function in male Sprague-Dawley rats given daily injections of 50 or 250 ug Cd over a period of 60-70 days.

12. Cadmium Metabolism

Transport

The metal-binding protein, thionein, is involved along with the blood in the transport of cadmium and a variety of other metals in the animal body. Sargent and Metz (123) pointed out that the accumulation of cadmium in the kidney and liver is dependent on this transport mechanism. The protein moiety of the cadmium-thionein complex passes through the glomeruli into the urine, resulting in a gradual concentration of cadmium in the renal tissue. Serious kidney impairment occurs before the concentration in the cortex reaches 200 ppm.

CHRONIC TOXICITY (Long-term tests, 2-year or lifetime treatment)

11. Reproductive and Teratogenic Effects

Schroeder and Mitchener (124) discovered a marked adverse effect of cadmium on the reproductive performance of mice (Charles River CD Strain), resulting in loss of the strain in two generations, when a soluble salt was administered in the drinking water at a level of 10 ppm (element). A congenital abnormality, sharp angulation of distal third of the tail, was found. A high percentage of the progeny died before weaning and 13% of those surviving beyond weaning showed growth retardation.

13. Carcinogenesis

Kendrey and Roe (112) reported that teratomas or Sertoli-cell adenomas have been induced in experimental animals by the subcutaneous, intramuscular, or intratesticular injection of cadmium.

EXPERIMENTAL CADMIUM INTOXICATION

ADDENDUM 2

ACUTE TOXICITY (including in vitro studies)

7. Biochemical and Histochemical Changes

A single ip dose of cadmium chloride (60 mg/kg), injected into rats sacrificed one hour later, lowered hepatic glycogen levels and significantly increased the concentration of blood glucose, serum urea, and hepatic cyclic AMP (cAMP) to 415%, 232%, and 206% of control values, respectively. The treatment also increased both the basal and fluoride-activated adenylate cyclase (AC) as well as cAMP levels and decreased the activity of hepatic adenosine-3',5'-monophosphate phosphodiesterase (PDE) activity (76).

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

3. Immunologic Response

Cadmium (30 ppm) administered in the form of cadmium chloride to Swiss Webster (CBA/J) mice in the drinking water for 10 weeks significantly decreased the ability of bone-marrow derived splenic B lymphocytes to form rosettes using an erythrocyte (E)-antibody (A)-complement (C) (EAC) assay. Splenic B cells have surface receptors for the activated third component of complement (C'3 receptor). Sheep RBC sensitized to antibody and complement (EAC complexes) bind to the C'3 receptor on B cells to form the rosette. Only B cells formed rosettes by the EAC technique. A dosage of 300 ppm cadmium did not cause inhibition of EAC rosettes, but the authors explained that this demonstrated the variable response of animals to different levels of cadmium observed so often in their laboratory. The direct inhibitory effect of cadmium on B cells could be detrimental to health since the B cell is the precursor of antibody-producing cells (125).

5. Behavioral Effects

Cadmium chloride solution administered to newborn Sprague-Dawley rats at a dosage of 10 ug/100 g by intubation daily for 30 days significantly increased spontaneous locomotor activity by 37%. Striated tyrosine hydroxylase and mid-brain tryptophan hydroxylase were also increased. The concentration of 5-hydroxyindoleacetic acid was elevated in the mid-brain region only. The authors stated that the results suggest that exposure to cadmium in early life increased the synthesis and physiological utilization of these supposed transmitters, which in turn probably caused the increased spontaneous locomotor activity. Increasing the daily cadmium chloride dose to 100 ug/100 g for 30 days did not increase locomotor activity over that at the 10 ug/100 g dose level, although body weight was decreased by 19% (126).

7. Biochemical and Histochemical Changes

Hrdina et al. (127) found that treatment of rats for 45 days ip with cadmium chloride at levels that did not cause any apparent neurotoxic signs produced significant changes in regional levels of various brain biogenic amines. Acetylcholine (ACh) levels were significantly depressed to 84% and 76% by doses of 0.25 mg/kg/day and 1.0 mg/kg/day, respectively. Serotonin (5-HT) levels were likewise significantly decreased by treatment with CdCl₂. The amount of dopamine in the striatum was markedly increased at both dose levels. Neither dosage produced any apparent changes in the concentration of brain-stem norepinephrine (NE) or in cerebrocortical acetylcholine esterase (AChE) activity over the 45-day period. The decrease in brain-stem 5HT persisted even after withdrawal of the higher dose (1.0 mg/kg/day) for 28 days. Cadmium is known to cause CNS damage, and the authors suggested that the observed changes in brain biogenic amine levels may represent early and subtle signs of deleterious effects on brain function, which can be detected even before the behavioral or histopathological signs of toxicity appear.

Cadmium chloride administered to newborn Sprague-Dawley rats at a dosage of 10 ug/100 g by intubation daily for 30 days significantly increased striatal tyrosine hydroxylase and mid-brain tryptophan hydroxylase. In the mid-brain region, 5-hydroxyindoleacetic acid was elevated also.

A dosage of 100 ug/100 g cadmium chloride by the same route daily for 30 days decreased (12%) activity of catechol-O-methyl transferase. Dopamine and norepinephrine levels in several brain regions examined were enhanced. A significant increase in the 5-hydroxyindoleacetic acid level was noted also.

The authors stated that the data suggest that cadmium acts at some point in the sequence of intracellular events that lead to increased synthesis and presumably turnover of brain catecholamines and serotonin (126).

Administration of 0.25 mg/kg CdCl₂ per day for 21 days to rats significantly enhanced the basal form of the hepatic cyclic AMP (cAMP)-synthesizing enzyme by 56%. When given for 45 days, the fluoride-activated adenylate cyclase (AC) as well as the basal form were elevated significantly above control values for the liver. A dose of 1.0 mg CdCl₂ per day for 21 days stimulated hepatic AC activity; increased effects were observed in animals treated for a longer period (45 days). The authors attributed the elevation in endogenous levels of cAMP to the enhanced AC activity (76).

Exposure of rats to "Cd" (dosage of 2 mg/kg per day for 7 days, not clearly indicated whether cadmium metal, cadmium ion, or a compound of cadmium) induced hyperglycemia with below normal insulin values (76).

Daily ip injections of cadmium chloride (0.25 or 1.0 mg/kg) for 21 or 45 days also caused a significant stimulation in the activities of hepatic pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (F-D-Pase) and glucose-6-phosphatase (G-6-Pase). The authors pointed out that these enzymes are the four key enzymes involved in the synthesis of glucose from non-carbohydrate precursors. Blood glucose and urea were also increased; liver glycogen was lowered. The metabolic changes were dose-related, being greater at the higher dose for 45 days than at the lower dose for 21 days (76).

Singhal et al. (76) also pointed out that exposure to cadmium results in:

- o Stimulation of cyclic AMP synthesis
- o Enhanced catecholamine synthesis
- o Abnormal insulin secretory response
- o Alteration in the cyclic AMP protein kinase systems of testes and prostate
- o Alteration in brain biogenic amines.

A summary of the biochemical toxicology of cadmium is presented by the authors in the table on the following page.

Singhal et al. (76) concluded from the evidence available that cadmium has a broad toxicologic potential and that there is little doubt that exposure can result in biological changes not only in the central nervous system but also in the organs involved in reproduction, excretion, and general body metabolism.

Tissue examined	Direction of change	Parameters examined
Liver	↑	Gluconeogenesis, glycolysis, AC, cAMP Glycogen, cAMP-binding, PK (+cAMP) PDE, PK (-cAMP)
	↓	
Kidney	↑	Gluconeogenesis cAMP, PK (-cAMP), PK (+cAMP)
	↓	
Serum	↑	Urea, glucose, PGF IRI
	↓	
Testes	↑	PGF, AC, PDE PK (+cAMP), PK (-cAMP) cAMP, cAMP-binding
	↓	
	0	
Prostate	↑	PK (+cAMP), cAMP-binding AC, cAMP PDE, PK (-cAMP)
	↓	
	0	
Adrenals	↑	TH, norepinephrine, epinephrine
	↑	
Brain	↓	Dopamine ACh, 5-HT AChE, norepinephrine
	↓	
	0	

0 = no change; ↑ = increase; ↓ = decrease.

Abbreviations: AC, adenylate cyclase; ACh, acetylcholine; AChE, acetylcholine esterase; cAMP, cyclic AMP; FD-Pase, fructose-1,6-diphosphatase; G-6-Pase, glucose-6-phosphatase; 5-HT, serotonin; IRI, immunoreactive insulin; PC, pyruvate carboxylase; PDE, phosphodiesterase; PEPCK, phosphoenolpyruvate carboxykinase; PGF, prostaglandin F; PK, protein kinase; and TH, tyrosine hydroxylase (76).

CHRONIC TOXICITY (Long-term tests, 2 years, or lifetime treatment)

5. Behavioral Effects

Highly significant differences were found between learning disabled (LD) and normal children in the amounts of cadmium, cobalt, lead, and manganese in the hair (P less than 0.001) (128). F ratios were 84.52, 35.00, 28.32, and 15.21, respectively. There was also a positive correlation between lead and cadmium levels ($r = + 0.53$, P less than 0.001). The hair has been shown to be one of the avenues of excretion of cadmium from the body (2). The authors regarded the elevated lead and cadmium levels in the learning disabled groups as being of particular importance.

8. Effects on Tissues and Organs (Humans)

Nose

Chronic exposure to cadmium may result in damage to the olfactory apparatus of sufficient severity to cause hyposmia or total anosmia. The extent of damage appears to be dependent on duration and concentration of the exposure. In one study, olfactory damage was found in 53-65% of workers exposed for 10-29 years to cadmium and in 91% of those exposed for more than 30 years. In another survey, olfactory impairment was found in 37% of the workers studied. A positive correlation between anosmia and proteinuria has also been found in workers exposed to cadmium (1).

Teeth

A yellow ring at the neck of the tooth has been observed in workers exposed to cadmium and was at one time proposed as a warning sign of chronic cadmium intoxication. Mechanisms postulated include surface absorption of cadmium, reaction with sulfur-containing substances in the saliva, or metabolic conditions (1).

Bone

Itai-Itai disease found in certain sections of Japan has been attributed to pollution of water and crops by industrial cadmium wastes. The condition appears to be an osteomalacia involving painful joints and bones, particularly in the back and legs. A calcium and protein dietary deficiency and possibly low estrogen levels may also be etiologic factors in the disease.

Several studies of industrial workers exposed to cadmium have revealed bone changes similar or identical to those found in osteomalacia (1).

Lungs

There is considerable evidence that prolonged exposure of industrial workers to cadmium compounds may result in chronic obstructive pulmonary disease (emphysema). In one group of workers exposed to cadmium oxide fumes, severity of the obstructive lung disorder required hospitalization for shortness of breath. The mechanism of cadmium-induced emphysema may be related to cadmium's capacity to inhibit antitrypsin activity. Damage to nerve tissue has also been suggested as a contributing factor in the pathogenesis of cadmium-induced emphysema in man (1).

Kidneys

Proteinuria. Kidney dysfunction, as evidenced by proteinuria, is the most common abnormality found in workers chronically exposed to cadmium. The incidence of proteinuria in workers exposed to cadmium for 20 years in the alkaline storage battery industry was 81%. One theory of the pathogenesis of the proteinuria in cadmium intoxication is decreased reabsorption of low molecular weight proteins by renal tubules. It has also been suggested that protein in the urine may result from interference by tubular-bound cadmium with the kidney's ability to catabolize proteins (1).

Renal Stones. Kidney stones were found in 44% of a group of cadmium workers in Sweden who had been exposed to cadmium dust for more than 15 years. The stones were composed mainly of calcium phosphate. Disturbed excretion of calcium and phosphorus has been suggested as a possible mechanism of the stone formation (1).

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Cadmium Toxicity - Matrix

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
1. Hematologic	<ul style="list-style-type: none"> o Hemolysis (4). o Cytopathic effects in RBC and platelets (108). 	<ul style="list-style-type: none"> o Anemia (mainly hypochromic & microcytic) (64). 	<ul style="list-style-type: none"> o Anemia (low hemoglobin & RBC count) (86,93). o Increased reticulocytes and eosinophils (87). 	<ul style="list-style-type: none"> a. b.
2. Bone Marrow	<ul style="list-style-type: none"> o Inhibition of hemoglobin synthesis (109). 	<ul style="list-style-type: none"> o No pathologic changes (humans) (66). 	<ul style="list-style-type: none"> o Hyperplasia in rats (87). o No pathologic changes (humans) (66). 	<ul style="list-style-type: none"> a.
3. Immunologic Effect	<ul style="list-style-type: none"> o No effects reported. 	<ul style="list-style-type: none"> o Decreased viral antibody titer (67). o Adverse effect on B lymphocytes (125). 	<ul style="list-style-type: none"> o Decreased number antibody-producing cells (spleen), primary, and secondary immune responses (88). 	<ul style="list-style-type: none"> a.
4. Central Nervous System (CNS)	<ul style="list-style-type: none"> o Hemorrhagic lesions in cerebrum, cerebellum, Gasserian and sensory spinal ganglia (5,6). o Degeneration in dorsal root ganglia mitochondria and unmyelinated axons; glycogen deposits in neuronal perikarya (<u>in vitro</u>) (7). o Degeneration in cerebellar cells <u>in vitro</u> (110). 	<ul style="list-style-type: none"> o No effects reported. 	<ul style="list-style-type: none"> o Sensory, optical, and motor nerve chronaxy changes proportional to exposure (humans) (89). o No studies in experimental animals reported (2). 	

Cadmium Toxicity - Matrix

Acute Treatment Short term tests, (days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
a (mainly chronic & cytotoxic) (64).	<ul style="list-style-type: none"> o Anemia (low hemoglobin & RBC count) (86,93). o Increased reticulocytes and eosinophils (87). 	<ul style="list-style-type: none"> a. Hemolysis b. Anemia (Subchronic) 	a. <u>In vivo</u> hematologic tests
Pathologic effects (humans)	<ul style="list-style-type: none"> o Hyperplasia in rats (87). o No pathologic changes (humans) (66). 	a. Inhibition of hemo- globin synthesis	<ul style="list-style-type: none"> a. <u>In vitro</u> cytotoxicity test b. Bone marrow differential (turn over and cycle rates)
Decreased viral antibody titer and effect on lymphocytes (125).	<ul style="list-style-type: none"> o Decreased number antibody-producing cells (spleen), primary, and secondary immune responses (88). 	a. Decreased viral anti- body titer (subchronic)	a. Immunological tests
Effects reported.	<ul style="list-style-type: none"> o Sensory, optical, and motor nerve conduction changes proportional to exposure (humans) (89). o No studies in experimental animals reported (2). 	None	None

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pred Endp
5. Behavioral Effects	o None reported.	o Significantly increased spontaneous locomotor activity (126).	o None reported.	a. b.
6. Cardiovascular Effects	o Hypertension (rat) after 1 month (8).	o Hypertension (rabbit); decreased response to angiotensin (68). o Constriction of smaller, and dilation of larger, renal arteries; fibrosis of peritubular capillaries in rats (70). o Desensitization of vasculature to vasoconstrictors and dilators in rats; no hypertension (69).	o Hypertension results in rats inconclusive (90,91).	a.
7. Biochemical and Histochemical Effects	o <u>Kidney.</u> <u>Decreased</u> alkaline phosphatase activity (18). <u>Inhibited</u> glucogenesis <u>in vitro</u> (16).	o <u>Kidney.</u> <u>Increased</u> glucose-synthesizing enzyme activity (71) <u>Decreased</u> leucinaminopeptidase activity (72). <u>Decreased</u> glucose formation by cortex slices <u>in vitro</u> (16).	o <u>Kidney.</u> None reported.	a. b.

Cadmium Toxicity - Matrix (Cont.)

Acute Treatment (short term tests, 30 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Significantly decreased spontaneous locomotor activity (67).	o None reported.	a. Signifi- cantly increased spontaneous locomotor activity b. See No. 11 below	a. Observation routine
Hypertension (68); decreased response to angiotensin (68). Constriction of arteries, and narrowing of larger, elastic arteries; degeneration of glomerular capillaries in rats (70). Sensitization of arteries to constrictors and hypertension in rats; no hypertension (69).	o Hypertension results in rats inconclusive (90,91).	a. Hypertension	a. Cuff systolic pressure b. Cardiac sensitization (EPI) test c. Tachycardia- bradycardia
Decreased parathyroid hormone-synthesizing enzyme activity (71) Decreased aminopeptidase activity (72). Decreased glucose oxidation by cortex in vitro	o <u>Kidney</u> . None reported.	a. Decreased calcium serum level (blood) b. Serum calcium- phosphorus ratio	a. Biochemical tests b. Histochemical tests

2

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predict Endpo:
7. Biochemical and Histochemical Effects (cont'd)	<p><u>Decreased</u> glucose-6-phosphate, adenosinetriphosphate, and glycogen levels (17).</p> <p>o <u>Prostate.</u> <u>Decreased</u> alkaline phosphatase activity (18). <u>Inhibited</u> succinoxidase activity (15).</p> <p>o <u>Lung.</u> <u>Inhibited</u> phosphorylation and respiration in alveolar macrophage mitochondria (14).</p> <p>o <u>Blood.</u> <u>Increased</u> alpha- and beta-globulins (20). <u>Increased</u> glucose (76). <u>Increased</u> serum urea (76). <u>Decreased</u> serum albumin (20).</p>	<p><u>Decreased</u> cyclic AMP-dependent and AMP-independent protein kinase activity (73).</p> <p>o <u>Prostate.</u> o <u>Increased</u> cyclic AMP binding to prostatic protein and increased cyclic AMP-dependent protein kinase activity (73). <u>Decreased</u> adenyl cyclase activity and cyclic AMP (73).</p> <p>o <u>Lung.</u> None reported.</p> <p>o <u>Blood.</u> <u>Increased</u> glucose level (71, 76). <u>Decreased</u> calcium level (79). <u>Decreased</u> insulin values (76).</p>	<p>o <u>Prostate.</u> None reported.</p> <p>o <u>Lung.</u> None reported.</p> <p>o <u>Blood.</u> None reported.</p>	

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (cont'd)	<ul style="list-style-type: none"> o <u>Dental Pulp (in vitro)</u>. <u>Increased</u> amount ¹⁴C-activity in TCA- soluble pool (19). <u>Reduced</u> incorporation ¹⁴C-activity into total protein and collagen; reduced proportions ¹⁴C-labeled protein and collagen and reduced proportion of total protein-bound ¹⁴C-activity and ¹⁴C-hydroxy-proline in medium (19). o <u>Inhibited</u> succinoxidase activity (15). o <u>Induction</u> of thionein (9). 	<ul style="list-style-type: none"> o <u>Dental Pulp</u>. None reported. o <u>Brain</u> <u>Increased</u> dopamine (127). <u>Increased</u> tyrosine hydroxylase (126). <u>Increased</u> tryptophan hydroxylase (126). <u>Increased</u> 5-hydroxyindoleacetic acid (126). <u>Enhanced</u> norepinephrine levels (126). <u>Decreased</u> acetylcholine (127). <u>Decreased</u> serotonin (127). <u>Decreased</u> catechol-o-methyl transferase (126). 	<ul style="list-style-type: none"> o <u>Dental Pulp</u>. None Reported.
8. Effect on Organs and Tissues	<ul style="list-style-type: none"> o <u>Body weight</u>. None reported. o <u>Teeth</u>. None reported. 	<ul style="list-style-type: none"> o <u>Body weight</u>. Decrease (73). o <u>Teeth</u>. None reported. 	<ul style="list-style-type: none"> o <u>Body weight</u>. Growth retardation (95). o <u>Nose</u>. Damage to olfactory system (human) (1). o <u>Teeth</u>. Yellow ring at neck of tooth (human) (1).

... Toxicity - Matrix (Cont.)

Treatment Tests,	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None	o <u>Dental Pulp.</u> None Reported.		
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...opamine

...tyrosine
(126).
...ryptophan
(126).

...adoleacetic

...ine
(126).

...ine

...erotonin

...methyl
(126).

... (126).	<ul style="list-style-type: none"> o <u>Body weight.</u> Growth retardation (95). o <u>Nose.</u> Damage to olfactory system (human) (1). o <u>Teeth.</u> Yellow ring at neck of tooth (human) (1). 	<ul style="list-style-type: none"> a. Decreased mineral content (bone) (subchronic) (correlation with biochemical) 	<ul style="list-style-type: none"> a. <u>In vitro</u> cell chemistry (liver)
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Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
8. Effect on Organs and Tissues (cont'd)	<ul style="list-style-type: none"> o <u>Bone</u>. None reported. o <u>Thyroid</u>. None reported. o <u>Lung</u>. Edema, proliferative interstitial pneumonitis, perivascular and peribronchial fibrosis (25). Reduced viability of alveolar macrophages accompanied by reduced acid phosphatase activity and cell surface changes (27). 	<ul style="list-style-type: none"> o <u>Bone</u>. <u>Increased</u> collagen solubility and soluble/insoluble collagen ratio (75). <u>Decreased</u> total collagen (75). <u>Decreased</u> mineral content (79). <u>Inhibited</u> bone formation; osteoporotic/changed (121). o <u>Thyroid</u>. Adverse changes in thyroxine secretion rate (TSR) and histology of gland (122). o <u>Lung</u>. None reported. 	<ul style="list-style-type: none"> o <u>Bone</u>. <u>Increase</u> in ratio of calcium to hydroxyproline (95). <u>Decrease</u> in weight of femur, greater in females than in males (95). Abnormal proportion of water and calcium (95). Osteomalacia disorders (human) (1). o <u>Thyroid</u>. None reported. o <u>Lung</u>. Emphysema (human) (1). 	<ul style="list-style-type: none"> b. c. d. e.

1

Cadmium Toxicity - Matrix (Cont.)

Acute Treatment (short term tests, 30 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<p>o <u>Increased</u> collagen solubility and soluble/insoluble collagen ratio (75). o <u>Increased</u> total collagen (75). o <u>Increased</u> mineral content (79). o <u>Thinned</u> bone density; osteoporotic/changed architecture).</p>	<p>o <u>Bone</u>. <u>Increase</u> in ratio of calcium to hydroxyproline (95). <u>Decrease</u> in weight of femur, greater in females than in males (95). Abnormal proportion of water and calcium (95). Osteomalacia disorders (human) (1).</p>	<p>b. Peribron- chial fibrosis (lung) c. Decreased insulin (pancreas) d. Fatty in- filtration (subchronic) (liver) e. Kidney necrosis</p>	<p>b. Zinc/cadmium relationship (pancreas) c. Microscopic examination (lung and kidney) d. Protein synthesis (RNA/DNA) e. Kidney/liver function tests</p>
<p>o <u>Altered</u>. Adverse effects in thyroxine secretion rate (TSR) and histology of thyroid (122).</p>	<p>o <u>Thyroid</u>. None reported.</p>		
<p>o None reported.</p>	<p>o <u>Lung</u>. Emphysema (human) (1).</p>		

2

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predi Endpo
8. Effect on Organs and Tissues (cont'd)	<ul style="list-style-type: none"> o <u>Pancreas.</u> Inhibition of insulin secretion (26). o <u>Liver.</u> Growth of cell cultures inhibited <u>in vitro</u> (114). o <u>Kidney.</u> Bilateral hemorrhagic necrosis (21). Tubular lesions of nephrotic type (22). Fat deposition in tubular epithelium (24). o <u>Adrenals.</u> None reported. o <u>Gonads.</u> See Reproductive & Teratogenic Effects. 	<ul style="list-style-type: none"> o <u>Pancreas.</u> Decreased insulin secretion and immunoreactive insulin release (77). o <u>Liver.</u> Fatty infiltration (24). o <u>Kidney.</u> None reported. o <u>Adrenals.</u> Increased adrenal weight. Increases in norepinephrine and epinephrine levels as well as increased adrenal tyrosine hydroxylase activity. (76) o <u>Gonads.</u> None reported. 	<ul style="list-style-type: none"> o <u>Pancreas.</u> None reported. o <u>Liver.</u> None reported. o <u>Kidney.</u> Tubular damage in proximal segment (87,92). Microscopic changes in glomeruli (92). Cadmium deposition in renal cortex (92). Proteinuria and increased amino acid and cadmium excretion (92). Kidney stones (human) (1). o <u>Adrenals.</u> None reported. o <u>Gonads.</u> See Reproductive and Teratogenic Effects. 	

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predi Endpo
9. Cytologic and Cytogenetic Effects	<ul style="list-style-type: none"> <li data-bbox="448 516 756 747">o Chromatid and isochromatid breaks, translocations, and dicentric chromosomes in human leucocytes <u>in vitro</u> (115). <li data-bbox="448 779 756 1010">o Chromatid breaks and other chromosomal abnormalities in peripheral leucocytes from Itai-Itai disease patients (116). <li data-bbox="448 1041 756 1304">o Reduced mitotic index, stickiness and pycnosis of chromosomes, stathmokinetic effect, structural chromosome aberrations <u>in vitro</u> (117). <li data-bbox="448 1335 756 1598">o Destruction of ribosomes and mitochondria. Severe adverse effects on endoplasmic reticulum and nucleus <u>in vitro</u> (118). <li data-bbox="448 1629 756 1736">o No increase in dominant lethal mutations in mice (28). 	o None reported.	o None reported.	a. C b

Cadmium Toxicity - Matrix (Cont.)

Acute Treatment term tests, (0 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None reported.	o None reported.	a. Chromatid breaks	a. Sister chromatid exchange

2

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
9. Cytologic and Cytogenetic Effects (cont'd)	<ul style="list-style-type: none"> o No chromosomal rearrangements in dividing mouse spermatocytes in male parent or offspring (28). 			
10. Molecular Effects	<ul style="list-style-type: none"> o <u>RNA synthesis</u>, both enhanced and inhibited in rats and in isolated rat liver nuclei, depending on dose and time (30). o <u>DNA synthesis</u>, decreased in partially hepatectomized rats (30). o <u>RNA Synthesis, DNA Synthesis, and RNA/DNA ratio</u>, decreased in rat testes (120). o <u>Cytoplasmic Protein Synthesis</u>, inhibited in the rat (29). o <u>Base mispairing</u>, between synthetic polyribonucleotides <u>in vitro</u> (119). 	<ul style="list-style-type: none"> o None reported. 	<ul style="list-style-type: none"> o None reported. 	a.

Toxicity - Matrix (Cont.)

Treatment Tests,	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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d.	o None reported.	a. Decreased RNA, DNA, protein synthesis (correlation with liver changes)	a. RNA-ASE test b. Cytochrome P450 changes
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Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predicted Endpoints
11. Reproductive and Teratogenic Effects	o <u>Testes</u> . Vascular damage (34). Capillary stasis, ischemic necrosis of seminiferous tubules followed by complete (10,23,31,32). Replacement of testicular tissue by eosinophilic material; sterility (33).	o <u>Testes</u> . Decrease in weight (73).	o <u>Testes</u> . Slight changes (10).	a. Testes
		o <u>Prostate</u> . Decrease in weight (73).	o <u>Prostate</u> . None reported.	b. Prostate
	o <u>Ovaries</u> . Reduced weight (38). Follicular atresia (36). Sterility (36-38). Massive hemorrhage and necrosis (37).	o <u>Ovaries</u> . None found.	o <u>Ovaries</u> . None found.	c. Ovaries
	o <u>Estrous cycle</u> . Prolongation (81).	o <u>Estrous cycle</u> . Prolongation (81).	o <u>Estrous cycle</u> . None found.	
o <u>Behavior</u> . Temporary loss of copulatory activity (39).	o <u>Behavior</u> . None reported.	o <u>Behavior</u> . None reported.		
o <u>Performance</u> . Reduced pregnancy rate in matings with treated males (35).	o <u>Performance</u> . None found.	o <u>Performance</u> . Slight deficit in weight gain and a slight increase in fertility. Longevity decreased (high dosage) or increased (low dosage) (98). Loss of strain in two generations. High death rate among newborn. Growth retardation in progeny (124).		

mium Toxicity - Matrix (Cont.)

Treatment tests, (ys)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Decrease in (73).	o <u>Testes</u> . Slight changes (10).	a. Testicular damage	a. Histological examination (testes)
. Decrease at	o <u>Prostate</u> . None reported.	b. Behavior (temporary loss of copulatory activity) c. Teratogenic effects	b. Reproduction assessment screening (Nardone-Wilson)
None found.	o <u>Ovaries</u> . None found.		
<u>cycle</u> . tion	o <u>Estrous cycle</u> . None found.		
. None	o <u>Behavior</u> . None reported.		
<u>ance</u> . None	o <u>Performance</u> . Slight deficit in weight gain and a slight increase in fertility. Longevity decreased (high dosage) or increased (low dosage) (98). Loss of strain in two generations. High death rate among newborn. Growth retardation in progeny (124).		

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Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predicted Endpoint
11. Reproductive and Teratogenic Effects (cont'd)	<ul style="list-style-type: none"> o <u>Teratogenesis.</u> Anophthalmia or microphthalmia, dysplastic or absent ears, hydrocephaly (40). Exencephaly, absence of tail, vaginal atresia, skeletal malformations; abortion, hemorrhagic, amniotic fluid (41). Abnormalities in cartilage formation, delayed ossification, cleft lips and palate (42). 	<ul style="list-style-type: none"> o <u>Teratogenesis.</u> Decreased fetal weight and increase in fetal death rate (80). Decreases in size and weight of neonates (81). Fetal anomalies - micrognathia, cleft palate, clubfoot, and small lungs (80). 	<ul style="list-style-type: none"> o <u>Teratogenesis.</u> Sharp angulation of distal third of tail (124). 	
12. Metabolism	<ul style="list-style-type: none"> o <u>Absorption.</u> <u>Skin.</u> Only 1-2% aqueous cadmium chloride absorbed over period of 5 hours (44). <u>Respiratory tract.</u> Estimated to be 10-40% of amount inhaled (2). <u>Gastrointestinal tract.</u> Approximately 1-2% absorbed after 24 hours (46). 	<ul style="list-style-type: none"> o <u>Absorption.</u> <u>Skin.</u> None reported. <u>Respiratory tract.</u> None reported. <u>Gastrointestinal tract.</u> An estimated 2% of cadmium ingested is absorbed (2). 	<ul style="list-style-type: none"> o <u>Absorption.</u> None reported. 	<ul style="list-style-type: none"> a. <u>Body</u> (liv kidn b. <u>Excr</u> (uri rac

toxicity - Matrix (Cont.)

nt	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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ease
rate
s in
of
-
left
t,
(80).

o Teratogenesis. Sharp angulation of distal third of tail (124).

o Absorption. None reported.

a. Body burden (liver/kidney)
b. Excretion (urine)

a. Pharmacokinetic studies (absorption, distribution, excretion, body burden)

ct.

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Cadmium Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pred Endp
12. Metabolism (continued)	<ul style="list-style-type: none"> o <u>Transport.</u> Cadmium enters blood within a very short time after exposure via air, gastro-intestinal tract or injection (2). Most of the injected dosage disappears from plasma within 30-80 minutes (50,51). In man, blood contains only about 0.1% of total body burden of cadmium (53). o <u>Tissue Distribution and Retention.</u> Liver and kidney store 50-75% of body burden, prior to kidney damage. Pancreas and spleen also store relatively large amounts. (2) 	<ul style="list-style-type: none"> o <u>Transport.</u> Blood levels increase steadily to around 1750 ng/g and plateau at the 10th week during daily subcutaneous administration (64). Thionein involved in transporting cadmium as cadmium-thionein complex (123). o <u>Tissue Distribution and Retention.</u> Liver, kidney, pancreas, and spleen levels of 1480, 1000, 193, and 180 ug/g, respectively, were found after 10 weeks of daily subcutaneous injections (83). 	<ul style="list-style-type: none"> o <u>Transport.</u> Mean blood levels after 21 weeks of daily subcutaneous administration were found to be approximately 48 ng/g (100). o <u>Tissue Distribution and Retention.</u> Mean cadmium concentrations in liver, kidney, pancreas, and spleen were 188, 170, 29, and 10 ug/g, respectively, after administration in the drinking water for 6 months (101). 	

Cadmium Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predi Endpo
12. Metabolism (continued)	<ul style="list-style-type: none"> o <u>Transport.</u> Cadmium enters blood within a very short time after exposure via air, gastrointestinal tract or injection (2). Most of the injected dosage disappears from plasma within 30-80 minutes (50,51). In man, blood contains only about 0.1% of total body burden of cadmium (53). 	<ul style="list-style-type: none"> o <u>Transport.</u> Blood levels increase steadily to around 1750 ng/g and plateau at the 10th week during daily subcutaneous administration (64). Thionein involved in transporting cadmium as cadmium-thionein complex (123). 	<ul style="list-style-type: none"> o <u>Transport.</u> Mean blood levels after 21 weeks of daily subcutaneous administration were found to be approximately 48 ng/g (100). 	

Cadmium Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints
12. Metabolism (Continued)	<ul style="list-style-type: none"> o <u>Tissue Distribution and Retention.</u> Liver and kidney store 50-75% of body burden, prior to kidney damage. Pancreas and spleen also store relatively large amounts. (2) o <u>Placental transfer.</u> Large doses of cadmium may destroy the placental barrier and enter fetus (2,57,58). o <u>Excretion.</u> Cadmium is excreted via urine, feces, bile, hair, and saliva (2). o <u>Biological Half-Time.</u> Approximately 200 days (rodents) to 1.5 years (monkey) (2). 	<ul style="list-style-type: none"> o <u>Tissue Distribution and Retention.</u> Liver, kidney, pancreas, and spleen levels of 1480, 1000, 193, and 180 ug/g, respectively, were found after 10 weeks of daily subcutaneous injections (83). o <u>Placental transfer.</u> Fetal liver concentration was more than twice that of controls, in dams exposed from day of conception (81, 84). o <u>Excretion.</u> Daily urinary excretion of subcutaneous cadmium was less than 1% of daily dose up to 8 weeks when the excretion rate rose sharply and reached a level approximately 100 times that of first weeks (83). o <u>Biological Half-Time.</u> None reported. 	<ul style="list-style-type: none"> o <u>Tissue Distribution and Retention.</u> Mean cadmium concentrations in liver, kidney, pancreas, and spleen were 188, 170, 29, and 10 ug/g, respectively, after administration in the drinking water for 6 months (101). o <u>Placental transfer.</u> None reported. o <u>Excretion.</u> Urinary excretion was insignificant during first four months of subcutaneous administration, a sudden increase then occurred, reaching a level exceeding the daily dose in some instances. Fecal excretion was slightly higher but corresponded to only 6.6% of the daily dose after 29 weeks. (92) o <u>Biological Half-Time.</u> Two (2) years in mice after daily injection for 25 weeks (2). 	

Cadmium Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in</u> <u>vitro</u> tests)	Subchronic Treatment (short term tests, up to 90 days)	Chronic Treatment (long term test, up to 2 years or lifetime)	Pre End
13. Carcinogenesis	o None reported.	o None reported.	<ul style="list-style-type: none"> o Injection-site sarcomas have been produced in rats by a number of workers (102-105). Metastatic tumors in regional lymph nodes and lungs were found in one study (104). o Interstitial cell tumors of the testes have also been induced in rats and mice by subcutaneous injections of cadmium chloride (106). o Teratomas or Sertoli-cell adenomas induced by subcutaneous, intramuscular, or intratesticular injection of cadmium (112). o Oral administration of cadmium acetate to rats in the drinking water (5 ppm) from weaning until death (4 years) did not cause a significant increase in tumors over the untreated control incidence (107). 	a.

Chronic Toxicity - Matrix (Cont.)

Treatment Tests,	Chronic Treatment (long term test, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
ted.	<ul style="list-style-type: none"> o Injection-site sarcomas have been produced in rats by a number of workers (102-105). Metastatic tumors in regional lymph nodes and lungs were found in one study (104). o Interstitial cell tumors of the testes have also been induced in rats and mice by subcutaneous injections of cadmium chloride (106). o Teratomas or Sertoli-cell adenomas induced by subcutaneous, intramuscular, or intratesticular injection of cadmium (112). o Oral administration of cadmium acetate to rats in the drinking water (5 ppm) from weaning until death (4 years) did not cause a significant increase in tumors over the untreated control incidence (107). 	a. None	<ul style="list-style-type: none"> a. Sister chromatid exchange b. <u>In vivo</u> and <u>in vitro</u> tests (Ames, Drosophila, etc.)

Tracor Jitco

EXPERIMENTAL PHOSPHORUS INTOXICATION

Backup Report to Phosphorus Toxicity Matrix

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7. Biochemical and Histochemical Effects

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4. Central Nervous System (CNS) Effects
5. Behavioral Effects
6. Cardiovascular Effects
7. Biochemical and Histochemical Effects

Blood

8. Effects on body weight, organs and tissues

Body weight

Liver

Osseous system

9. Cytologic and Cytogenetic Effects
10. The Molecular Effects
11. Embryonic and Teratogenic Effects
12. Metabolism
13. Carcinogenicity

IV. PHYSICAL AND CHEMICAL PROPERTIES.

EXPERIMENTAL PHOSPHORUS TOXICITY

INTRODUCTION

Phosphorus exists in several allotropic forms of which the red and white varieties are the most common. Both forms are employed in combination with butyl rubber to produce smoke screens and signaling smokes for the military. On combustion both the red and the white forms produce a dense white smoke of phosphorus pentoxide with the density of the smoke being qualitatively greater for white phosphorus.

Red Phosphorus. Although red phosphorus is generally considered as having low toxicity, experimental data to support this claim are scarce. No report was found in the literature regarding its carcinogenic and mutagenic or teratogenic potential either in man or animals. Furthermore, information on its chronic toxicity in humans under occupational exposure conditions are of doubtful significance because workers were exposed to white phosphorus as well during working hours. The experimental data on its metabolism remain unconfirmed. No occupational safety standards have as yet been established for red phosphorus.

White Phosphorus. White phosphorus is highly toxic and produces a strong system effect. The toxicity data on this element are certainly more extensive and more informative than those on red phosphorus, both in the experimental and occupational spheres. The occupational safety standard for this element has been established. The current permissible human exposure limits for white phosphorus is $0.1\text{mg}/\text{m}^3$ (1). The acute fatal dose of white phosphorus for humans is 60 mg but as little as 15 mg have resulted in untoward symptoms (2).

Acute phosphorus poisoning usually occurs as a result of accidental or suicidal ingestion. However, experimental studies have demonstrated that acute systemic toxicity may occur as a result of burns.

Acute phosphorus poisoning symptoms appear in two stages. During the first 24 hours there is severe gastrointestinal irritation and the victim sometimes dies of cardiovascular failure as early as 12 hours after ingestion of a fatal dose. This stage may be followed by a latent period which may last from a few hours to a few days depending on the amount ingested. The systemic stage is characterized by abdominal pains, nausea, vomiting, hematemesis and other hemorrhagic manifestations. Jaundice, hepatomegaly, oliguria toxic psychosis, coma and shock also occur. There is severe damage to the liver and kidney. Death may occur at any time. Cirrhosis of the liver has been reported after recovery from acute state. Abnormal electrocardiographs, urinary and serum levels of calcium and phosphate, proteinuria and aminoaciduria as well as elevation of serum glutamic - pyruvic transaminase (SGPT) are all indicative signs of phosphorus intoxication (3, 4, 5, 6).

Chronic phosphorus poisoning is a result of continued absorption of small amounts of white phosphorus over long periods. Chronic intoxication is characterized by periostitis with suppuration, necrosis, ulceration and deformity of the jaw bone. Polymorphic leucopenia and susceptibility to bone fractures are secondary clinical signs (3, 4, 5).

The oral LD₅₀ for male and female rats was reported as 3.76 and 3.03 mg/kg, respectively (7). In mice, the oral LD₅₀ was found to be 4.82 mg/kg for males and 4.85 mg/kg for females (7). In both species the symptoms of intoxication included anorexia, jaundice and enlarged livers, followed by death which occurred over a period of several days (7). The lowest lethal concentration through the inhalation route was reported to be 500 mg/m³ in mice with animals dying within 10 minutes of exposure to this concentration (8). The oral LD₅₀ in other animals has not been reported.

Since red and white phosphorus differ so greatly in their toxicity to humans and experimental animals, their effects will be discussed under separate divisions, A. Red phosphorus and B. White phosphorus. Their toxicity will be discussed under subtitles I. Acute Toxicity, II. Subchronic Toxicity, and III. Chronic Toxicity. Section IV. will describe some of the pertinent physical and chemical properties relating to their biological effects.

Acute toxicity is defined as that resulting from a single exposure, injection, administration or application (skin). Under acute effects will also be included in vitro observations. Subchronic toxicity is defined as that resulting from repeated (continuous or intermittent) treatment during a 90-day experimental period or less (short term tests); chronic toxicity is defined as that resulting from continuous treatment over a period of 2 years or more (long term tests or lifetime tests).

Typical examples will be selected to illustrate toxic end points.

Information Sources. The information contained in the body of this document is derived from the following sources.

- o Problem Definition Study on Occupational Health and Safety Aspects of Phosphorus Smoke Agents. Document prepared by Franklin Institute Research Laboratory under Contract No. DAMD -17-77-C-7020 for U.S. Army Medical Research and Development Command, Washington, D.C. 1977.
- o Mammalian Toxicology and Toxicity to Aquatic Organisms of Four Important Types of Waterborne Munitions Pollutants. NTIS AD-778 725, 1974.
- o Citations identified through a Tracor Jitco Literature Search.
- o Occupational Diseases, a Guide to their Recognition. Revised Edition. 1977. DuEW, Ed. M. M. Key, A.F. Henschel, J. Butler, R.N. Ligo, I.R. Tabershaw.

Matrix. The pertinent effects of Red and White phosphorus contained in this document will be summarized (see appended matrix), where experimental animal data were not included in both the text and the matrix.

A. RED PHOSPHORUS

1. ACUTE TOXICITY (including in vitro tests)

1. Hematologic Effects

Leucopenia. Single doses of 100 mg/kg of red phosphorus given iv to rabbits resulted in decreased leucocyte and erythrocyte counts within hours of treatment. Both parameters declined progressively with time with all treated animals succumbing between the 6th - 8th post-treatment day (9).

No information was found on humans.

2. Bone Marrow Changes

No information was available in experimental animals or humans.

3. Immunologic Effects

No information was available in experimental animals or humans.

4. Central Nervous System (CNS) Effects

Rabbits given single injections of 100 mg/kg of red phosphorus into the jugular vein showed a loss of appetite by the 3rd day and died by day 6-8 of the experiment (9).

5. Behavioral Effects

See CNS effects for experimental animals. No information was found regarding behavior in humans.

6. Cardiovascular Effects

No information was found on experimental animals or humans regarding the effect of red phosphorus on the cardiac muscles, pericardium, blood pressure etc.

7. Biochemical and Histochemical Changes

No information was found regarding enzymatic changes induced in either experimental animals or humans by red phosphorus.

8. Effects on Body Weight, Tissues and Organs

Body Weight: None Reported.

Liver: Extensive fatty degeneration was observed in rabbits following iv injection of 100 mg/kg (9).

Kidney: Fatty degenerative changes were found in the kidney following iv injection (100 mg/kg) of red phosphorus into rabbits (9).

Spleen: Hyperplasia in splenic tissue has been reported as a result of acute red phosphorus injections (100 mg/kg) into rabbits (9).

Genital: Hyperplastic changes were observed in the testes and ovaries of rabbits subjected to acute iv injections of red phosphorus (100 mg/kg) (9).

Brain: Nerve cell degeneration was observed in rabbits given 100 mg/kg iv dose of red phosphorus (9).

There is no information on the acute effects of red phosphorus on human tissues and organs.

9. Cytologic and Cytogenetic Effects

There is no information available in the literature on the acute toxic effects of red phosphorus on the chromosomes of experimental animals or humans.

10. The Molecular Effects

No information was found regarding the effects of red phosphorus on the DNA, RNA and protein synthesis in vivo or in vitro testing in experimental animals or in humans.

11. Embryonic and Teratogenic Effects

No studies have been reported in the literature on the possible embryo-lethal and teratogenic effects of red phosphorus in experimental animals. Nor are there any reports on fetal deaths or abnormalities in humans due to acute accidental (suicidal) phosphorus exposure.

12. Metabolism

Dalhamn and Holma (1) studied the distribution and excretion of ^{32}P -labeled red phosphorus aerosol (0.46 microns) in 15 mice (sex, age not given). The mice were exposed to 5 mg/m^3 concentrations in an inhalation chamber for one hour and sacrificed at time intervals varying from 0 to 10 days following exposure. Autoradiographic measurements of whole sectioned mice revealed highest radioactivity in the respiratory and digestive tracts immediately after exposure (0 time). No activity was detected in the upper respiratory tract 20 minutes after exposure and after 2 days the lung was the only organ which showed some radioactivity. The metabolism of phosphorus was not discussed.

There is no information in the current literature on absorption, distribution and excretion of red phosphorus in humans.

13. Carcinogenicity

No animal or human data are available on the carcinogenic potential of acute exposure to red phosphorus.

II. SUBCHRONIC TOXICITY (Short term tests)

No information was found regarding subchronic studies on red phosphorus on laboratory animals in any of the categories listed under the acute studies. Nor were any data found in the literature related to subchronic toxicity of red phosphorus in humans.

III. CHRONIC TOXICITY (Long term tests)

Only one animal study relating to chronic toxicity of red phosphorus was found in the literature (9). The results of this study indicated that animals exposed to red phosphorus showed acute parenchymatous or interstitial nephritis, alopecia and desquamation of the skin. However the study could not be properly evaluated because the dose, species, and duration of exposure were not given.

Another report based on chronic exposure of a 40-year-old man in a plant which converted white phosphorus to red phosphorus is questionable because of the circumstances under which the patient worked (11). Osteomyelitis of the mandible diagnosed in this patient was attributed to the presence of unconverted white phosphorus during the manufacture of red phosphorus.

IV. PHYSICAL AND CHEMICAL PROPERTIES

Red phosphorus is the other class of solid allotropes of phosphorus, after white phosphorus, having some commercial importance. It is prepared commercially by heating white phosphorus at about 400°C for several hours. Iodine, sulfur or sodium may be used as a catalyst. Red phosphorus as prepared by commercial processes is almost entirely amorphous. Red phosphorus prepared by various methods exhibits different properties. The melting point is reported to range from 585-600°C; and measured densities have been found to vary between 2.0 and 2.4 g/cm³; the color of red phosphorus is found to vary from a deep scarlet to brown, and to violet, depending on the method of preparation. As many as six modifications of red phosphorus may exist but not all of them are structurally characterized. (100,101)

The physical properties of red phosphorus are given in Table 1.

Table 1

PHYSICAL PROPERTIES OF RED PHOSPHORUS (100)

Appearance:	Reddish-brown amorphous powder (commercial)
Melting point (triple point):	590°C at 43.1 atmosphere
Boiling point:	280°C
Ignition temperature:	260°C
Density:	2.31 g/cm ³
Heat of sublimation:	19.7 - 28.8 Kcal/mole P ₄
Heat of combustion (amorphous):	703.2 = 0.5 Kcal/mole P ₄
(crystalline):	697.7 = 0.4 Kcal/mole P ₄
Solubility:	Very slightly soluble in cold water, insoluble in organic solvents, soluble in phosphorus tribromide.

Red phosphorus is relatively less reactive than white phosphorus. It is stable in air and ignites only when heated in air at 260°C . At normal temperatures and humidities, red phosphorus reacts slowly with the water vapor and oxygen in air to form phosphine and a mixture of oxyacids of phosphorus. This slow oxidation is exothermic and is accelerated by an increase in temperature. Traces of copper, iron, silver, nickel and bismuth catalyze the oxidation of red phosphorus by moist air. Red phosphorus reacts with atmospheric oxygen to give phosphorus pentoxide as the major product which is then converted by the moisture in air to phosphoric acid. (100,101)

The reactions of red and white phosphorus are similar. Red phosphorus reacts with aqueous alkali to give phosphine, combines with halogens to give tri- or pentahalides and with sulfur to give sulfides. Phosphides are produced by reaction with metals and non-metals. (100)

B. WHITE PHOSPHORUS

I. ACUTE TOXICITY (including in vitro observations)

1. Hematologic Effects

Leucopenia - Neutropenia (Humans). Buckanan et al. (12) injected 3 dogs (breed not indicated) with 0.4 mg/kg of white phosphorus in peanut oil subcutaneously (sc). Three days later the dogs showed hematemesis and died by the 6th day post treatment. Examination of the blood during intoxication did not reveal any leucopenia. In one case of human poisoning where a 21-year-old man ingested 21.4 mg/kg of white phosphorus (60 g of rat poison containing 2.5% of white phosphorus), marked leucopenia and neutropenia were observed (13). This observation was later confirmed by others (2).

Leucocytosis (Humans). Hizenshstaut et al. (14) studied the acute toxicity of phosphorus vapors in industrial workers exposed to 0.035 mg/l of white phosphorus and 0.22 mg/l of phosphorus pentoxide for 2-6 hours at 7.0 hour intervals. Six to 20 hours later, workers complained of headache, vertigo and chest pains. Blood examination revealed an increase in leucocytes (9,350-14,500) and neutrophils (69-80%). In other instances there was an increase in lymphocytes and monocytes (2).

Erythrocytosis (Human). Diaz-Riveral et al. (2) observed an increase in red blood cell number in humans exposed acutely to white phosphorus. The increase appeared earlier during intoxication and persisted at times for as long as 14 days even in the presence of sufficient hydration.

Most workers believe that changes in the blood picture (such as described above), with the exception of the blood-chemistry aberrations of hyperphosphatemia and hypocalcemia, are too general and uncharacteristic and may not necessarily indicate phosphorus poisoning (2,4).

Fibrinogen. Whipple et al. (15) reported on a case where a fox terrier (male) was given a single injection of 10 mg of white phosphorus subcutaneously. Seven days later a blood sample was taken and the plasma was found to clot normally but the clot was decidedly flabby, indicating a drop in fibrinogen. The animal was very sick and was sacrificed. Autopsy was performed at once. Again the blood clotted but the clots formed soft jelly like masses, indicating very little fibrinogen.

Massive Hemolysis (Human). Massive hemolysis was reported in one patient who suffered white phosphorus burns over 29% of his body when a grenade containing white phosphorus exploded at his feet (16). Seventy-two hours after the injury the patient's hematocrit dropped from 18 to 11%, and the hemoglobin declined from 7.9 to 4.1 g/100 ml. The plasma hemoglobin was 4 g/100 ml and the urine supernatant hemoglobin was 4.7 g/100 ml.

The hemorrhagic manifestations of acute phosphorus toxicity are described in Section 8. They all have their origin, apparently, in the fatty changes which occur in the coats of the arterioles and are not due to blood changes.

2. Bone Marrow Changes

No reports were found on the acute effects of white phosphorus on the bone marrow in experimental animals or humans.

3. Immunologic Effects

No reports were found on the acute effects of white phosphorus on the immunologic system in experimental animals or humans.

4. Central Nervous System (CNS) Effects

Bowen et al. (17) induced white phosphorus burns on shaved backs of rabbits anesthetized with ether (130 animals) using the Standard White

Phosphorus Burn (SWPB) procedure. The burn time was limited to 1 minute and the amount of white phosphorus used to produce the burn was 10 mg. Death occurred in 65% of the animals with 18 hours post-burn. Death was preceded by shivering, twitching, loss of appetite, poor response to stimulation and depression. These changes were usually evident within 4 hours of treatment. No pathologic observations were reported.

Takeya-Siko (18) administered phosphorated oil containing 8% white phosphorus to dogs (8 animals) and studied the effect on the brain structures. The animals showed strong hyperemia and congestion of the meninges, hemorrhage in the midbrain and hindbrain, damage to ganglion and glial cells and extensive subarachnoidal hemorrhage.

Wertham (19) reviewed the literature of histopathological investigations in clinical cases relating to acute phosphorus poisoning and its effect on the CNS. A range of effects such as parasthenia, delirious states, acute excitement, psychosis and coma were noted. Pathologic findings included hemorrhage and softening of the subcortical gray matter, paralysis of facial nerve, pronounced fatty degeneration of the brain cortex, destruction of ganglion cells, severe damage to the inferior olives (incrustations of the outer Golgi nets of ganglion cells) and hyperemia.

Winek et al. (20) noted depressive neurosis in a 45-year-old man who ingested rat paste containing 3% white phosphorus.

5. Behavioral Effects

See CNS Effects.

6. Cardiovascular Effects

In humans regional cerebral arterial hypotension was reported in 4 of 5 patients following exposure to white phosphorus vapors (0.035 mg/l) for 2-6 hours at 7-hour intervals. The brachial arterial pressure was 120/60 - 140/90 mm Hg and the maximal recorded in the temporal arteries was 15-30 mm Hg in all four patients (14).

Bowen et al. (7) studied the effect of white phosphorus burn on the cardiovascular system of 130 rabbits. The burn was produced by the standard method (see CNS effects). Electrocardiographic tracings were made hourly for the first 12 hours postburn under light sedation. Abnormalities were observed in 70% of the animals within 1 hour postburn, and these consisted of prolonged Q-T interval, S-T segment depression, T-wave changes, bradycardia and low voltage of the QRS complex. Ventricular arrhythmias were not observed.

Winek et al. (20) reported on two patients who had ingested 3% white phosphorus in rat paste and later died of cardiopulmonary failure. Newburger et al. (13) found a similar reaction in one patient who had ingested 60 g of rat paste containing 2% of white phosphorus.

Talley et al. (21) reported a case of poisoning in which 15.7 mg/kg of white phosphorus ingested accidentally produced a pulse rate of 110/min with a systolic pressure of 60 mm Hg. The electrocardiogram revealed arterial fibrillation with wide slurred QRS complexes. Chest X-rays showed diffuse cardiac enlargement. Death due to cardiac arrest occurred 22 hours after ingestion of poison. Other abnormalities observed in instances where 2.5 - 8% white phosphorus was ingested include abnormal P-waves, prolonged P-R intervals, delayed intraventricular conduction, nodal rhythms, prolonged Q-T intervals, abnormal S-T segments, T-wave changes and ventricular fibrillation (22, 23). Death in all instances was usually due either to peripheral vascular collapse or cardiac standstill (2).

7. Biochemical and Histochemical Effects

Serum. Rabbits receiving the SWPB (17) showed a decrease in serum calcium and increase in serum phosphorus. In similar studies in rats where the SWPB was induced with 10 and 50 mg of white phosphorus, Ben-Hur et al. (24), Ben-Hur and Appelbaum (25) and Appelbaum et al. (26) observed a rise in serum phosphorus within 2 hours postburn which reached more than twice the normal level by 24 hours postburn (normal level 4.5

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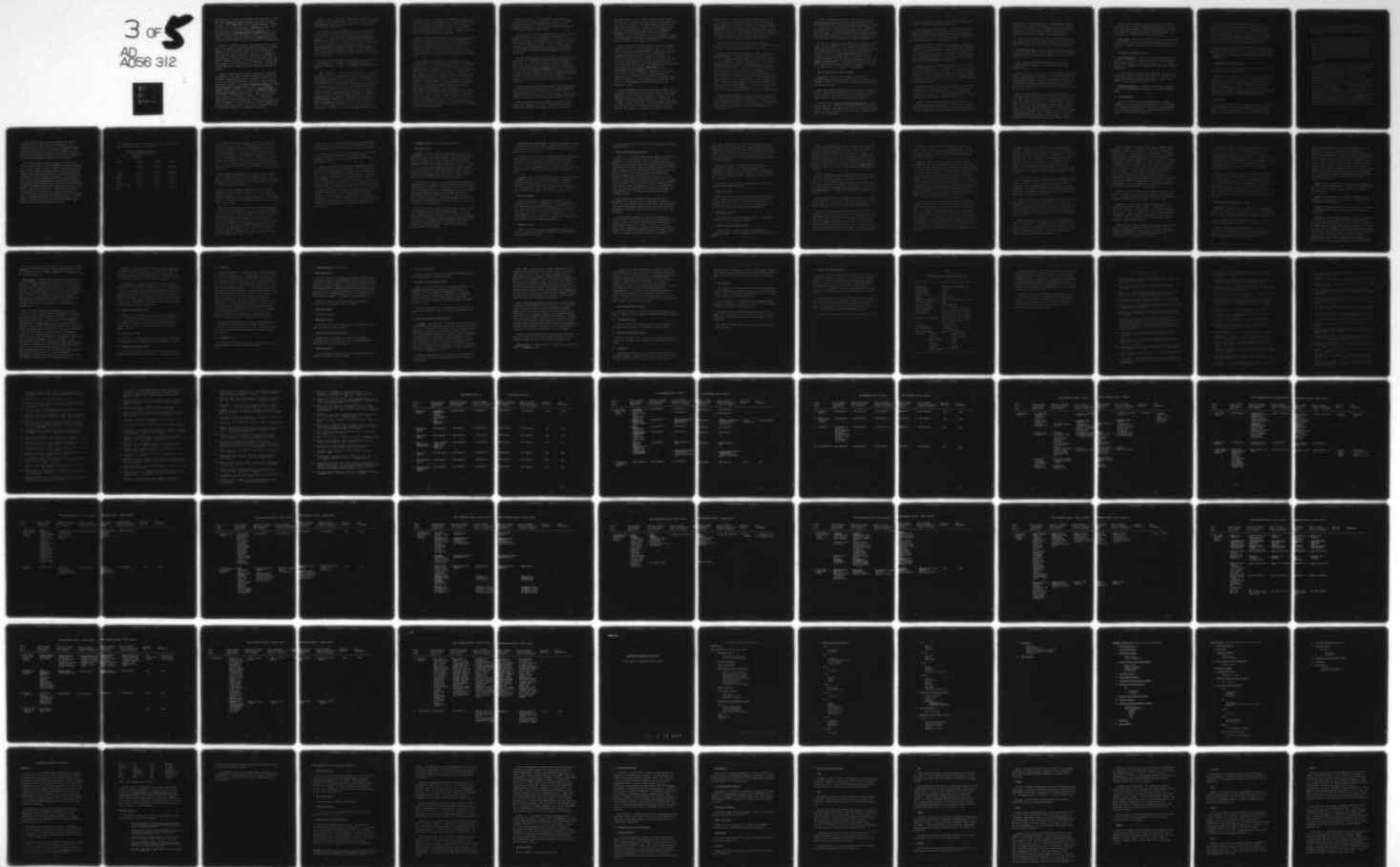
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mg %; burned level 11 mg %). More than 66% of the burned rats (10 of 15) had elevated plasma urea nitrogen (PUN) values above 100 mg % (normal, 10-12 mg %) and low sodium value (125-130 milliequivalents/l) as compared to controls (135-143 milliequivalents/l). Potassium values were also in excess of control levels (8 milliequivalents/l vs normal 4-5 milliequivalents/l). Serum glutamic-pyruvic transaminase (SGPT) activity was significantly elevated (100 units/ml vs normal 10 units/ml) in all animals. A transitory decrease was observed in creatinine blood levels at 72 hours but this gradually returned to normal by the 5th postburn day.

Bodansky (27) studied the effect of white phosphorus on the glucose levels in the blood of a female dog which had been injected sc with 0.38 mg/kg of the element dissolved in olive oil. The blood sugar level assessed prior to treatment was 0.096%. Three days after treatment it was 0.108%. The animal was then given a second dose of 0.38 mg/kg of white phosphorus. Three days after the injection the glucose level was 0.068%. Although no statistical evaluations were made, it appeared that white phosphorus did not influence the level of glucose in the blood of the dog significantly. A small increase was also seen in creatine and creatinine.

Huraya (28) injected rabbits sc with 0.5 mg/kg of white phosphorus and determined total nitrogen, residual nitrogen, total fatty acid and cholesterol, and polypeptide nitrogen in the blood at various times (8, 16, 24, 32, 40 and 48 hours) after the injection. The most significant change was the increase in the amount of non-protein nitrogen. The fatty acid, cholesterol and lecithin remained normal, while the ethereal sulfate increased considerably. The amount of polypeptide nitrogen and total residual nitrogen remained normal for 48 hours and then decreased as the damage to the kidney became apparent. These changes in blood chemistry reveal that the products of autolysis in liver cells are flooded into the blood stream, while the fat content remains normal. The fatty infiltration in the liver and kidney which always accompanies white phosphorus intoxication probably results from the inability of the cells of these organs to manipulate the incoming fatty substance.

In dogs a single sc injection of 10 mg of white phosphorus in oil was fatal within 6 days of administration. During the period of intoxication plasma lipolytic activity was greatly increased (15). Similar results were observed with rabbits (29).

In humans white phosphorus intoxication produced a blood chemistry picture similar to that seen in animals (13, 30). The evidence for significant acute liver involvement included increased blood protein nitrogen level, concomittantly with a low normal blood urea nitrogen level (13). This abnormal ratio returned to normal in a relatively short time. Bile pigment metabolism remained intact throughout. The cephalin-cholesterol flocculation test (Hanger test) was abnormal when first done and remained so during a 4-month period. The iv hippuric acid test for Quick became progressively abnormal but finally returned normal after 4 months (13).

Aijenshtadt et al. (14) observed that in humans exposed to 0.035 mg/l of white phosphorus for 2-6 hours, the erythrocyte cholinesterase activity was reduced by 17%, while in the plasma its activity was reduced by 35%.

Liver. Truhaut et al. (31) studied the acute toxicity of 10 mg/kg of white phosphorus in 33 five-and-one-half-month-old male Wistar rats. The phosphorus was dissolved in olive oil and injected sc. The number of rats comprising the controls was not indicated. Two of the 33 rats died in less than 24 hours, 7 died between 24-48, hours and 1 died between 48 and 72 hours after the injection. Surviving rats were killed at 18, 24, 36, 48, 72 and 96 hours post-treatment. Up to 18 hours there was no significant change in total lipid content of the liver as compared with controls. Thereafter, the value increased significantly. The total triglycerides showed a significant increase from 18 hours onwards. The total cholesterol increases were not remarkable (p less than 0.02). Significant phospholipid increases were seen at 18 hours and after 48 hours (p less than 0.001). Variations at 24 and 36 hours were not significant. Thirty-six hours post-treatment appeared to be the time at

which "pure steatosis" was optimal since complications appeared later. The authors concluded that phosphorus-induced steatosis was most likely not due to an increase of intrahepatic biosynthesis of triglycerides, since no increase was observed in the activity of the two enzymes, glucose-6-phosphate dehydrogenase and fructose-1-6-diphosphate aldolase, involved in the biogenesis of these compounds.

Studies on radioactive incorporation of tritiated palmitate following phosphorus intoxication in rats (10 mg/kg, sc) confirmed the above results. A significant decrease in total triglyceride production at 15 hours after phosphorus administration was observed and became progressively greater with time (significant at 24 hours). This reflected a progressive effect on the esterification-process leading to the more rapid increase of "free triglycerides" and to hepatic steatosis (32).

Acute phosphorus toxicity induced by fatty infiltration and/or degeneration of liver of rats was studied by Pani et al. (33). Female rats (numbers not specified) received 10 mg/kg of white phosphorus by stomach intubation. The animals were killed 12 hours after treatment and the hepatic diene conjugation in microsomal lipids as well as the polyribosomal pattern and total lipid was studied. The results showed that the triglycerides begin to accumulate in the liver 4 hours post-treatment with the accumulation reaching a maximum by 12 hours post-treatment. To ascertain whether lipid peroxidative decomposition was involved in the mechanism of phosphorus intoxication, rats were pretreated (ip) with phenobarbital (PB) (80 mg/kg) or *N-N'*-diphenyl-*p*-phenylenediamine (DPDD) (500 mg/kg) prior to phosphorus intoxication. Hepatic triglycerides and ultraviolet absorption spectra of microsomal lipids were studied. DPDD failed to effect the damage produced by phosphorus and PB did not cause a further increase in triglyceride accumulation in the liver. There was no evidence of lipid peroxidation in either of the pretreated groups of animals as determined by diene conjugation absorption.

Hurwitz (34) came to a similar conclusion in rats and mice intoxicated with 2-7.5 mg/kg of phosphorus. However, if the animals received phetharbital (50-75 mg/kg/day) soon (12-18 hours) after phosphorus intoxication, the rise in sulfobromo-phthalein (BSP) induced by the toxicant returned to normal rapidly.

Jobling et al. (35) studied esterase activity in the liver of one dog poisoned sc with 3 milliliters of phosphorus oil (amount of white phosphorus not given). The animal was killed 3 hours after the injection and the liver assayed for its esterase activity. Esterase activity was also determined in untreated controls. The liver of the phosphorus treated dog showed marked fatty degeneration and decreased esterase activity. Similar observations were made by Whipple et al. (15) and Califano (36).

One hundred and six male Wistar rats were given by gastric intubation 10 microcurie of ^{32}P mixed with 7.5 mg/kg of unlabeled phosphorus in mineral oil. The control received equivalent amounts of mineral oil alone. The rats were killed at 4, 12 and 24 hours after treatment and the livers assayed for glucose-6-phosphatase activity in the microsomes. At 4 hours there was no significant difference in enzyme activity between the control and treated animals, but at 12 and 24 hours post-treatment a significant increase (p less than 0.025 and p less than 0.01, respectively) in activity was noted in the treated versus the control animals (37).

Bueding and Ladewig (38) studied the synthesis of glucuronic acid by liver slices excised from guinea pigs poisoned with white phosphorus. Intoxication was produced by sc injection of 7.5 mg/kg of white phosphorus dissolved in mineral oil. The results showed a considerable reduction in the synthesis of glucuronic acid by poisoned liver slices.

Huruya (28) injected male rabbits (number not given) sc with 0.5 mg/kg of white phosphorus in olive oil. The animals were killed at varying time intervals after treatment (8-48 hours), and the livers

removed and analyzed for water content, total and residual nitrogen, total fatty acids, polypeptide nitrogen, lecithin and cholesterol (total, free and ester cholesterol). The results showed that water content, total nitrogen, fatty acids and cholesterol increased significantly, while the lecithin showed only a small increase. The polypeptide nitrogens and residual nitrogens increased with the degree of intoxication. All of these changes in the liver biochemistry indicated that white phosphorus interfered with the fat metabolism by inhibiting the transformation of the fat molecule in the liver.

Seakins and Robinson (39) administered 1.5 mg of white phosphorus dissolved in 0.3 ml of olive oil (test group) or 0.3 ml of olive oil alone (control group) by stomach intubation to female rats. Two hours later 20 microcuries of DL(I-¹⁴C) leucine, 6 microcuries of (³²P) orthophosphate and 20 microcuries of sodium (I-¹⁴C) acetate were injected iv into the tail vein, the animals were killed 1.5, 3.0 hours, or at various times after injection of the radioactive precursors, respectively, and the livers assayed for incorporation of the precursors into protein (DL- (I-¹⁴C) leucine), phospholipids ((³²P) orthophosphate) and free cholesterol (sodium (I-¹⁴C) acetate). The results showed a rise in the amount of esterified fatty acid in the liver. Two hours after administration of white phosphorus, the incorporation of DL-(I-¹⁴C) leucine into the liver was markedly reduced while the incorporation of (³²P) orthophosphate and of sodium (I-¹⁴C) acetate into liver phospholipid and free cholesterol were not significantly changed.

Studies on glycogen levels in livers from rabbits poisoned with 7.8 and 34 mg of white phosphorus showed that there was a decrease in liver glucogen at all three doses (40). The glycogen content of the rabbit livers poisoned with 4.5 mg/kg of phosphorus (sc injection) was determined before and after the Modified Dextrose Tolerance Test (41). The number of animals treated was 7. Two of the rabbits died by 36 hours post-injection, two more were killed for observations on the hepatic glycogen. Three rabbits were subjected to the sugar tolerance and

epinephrine tests 15 hours after injection of white phosphorus. The results showed that the initial blood sugar in all three rabbits was markedly lowered. In two animals the 3-hour curve showed a remarkable hyperglycemia; in the third there was practically no elevation of blood sugar after dextrose, water and insulin had been given and the average blood sugar was very low. After epinephrine the blood sugar in the first two animals rose sharply (2.5-fold), while in the third it fell to a very low level (32 mg) and remained low without causing convulsions.

Similar results were reported earlier by other investigators (42-45). In most instances a progressive decrease in hepatic glucose was accompanied by an increase in urinary levels of lactic acid.

Califano (48) studied oxygen consumption in rabbit liver poisoned with white phosphorus. The animals were given 1% phosphorated oil at doses of 1.5 mg/kg/day via gastric intubation, and were killed on the 8th day (the day of maximal fatty degeneration) and the liver assayed for glycolytic activity. A manometric determination showed that oxygen consumption of the hepatic tissue from treated animals was higher than that of normal liver. The CO₂ production was also greater, but since the increase was not parallel to that of oxygen consumption the respiratory quotient was diminished. Anaerobic glycolysis was similar to that of normal liver. The observed increase in oxygen consumption was probably due to oxidation of the double bonds of the unsaturated fatty acids. Dephenilamine, which inhibits autooxidation of the double bonds, does not modify the oxygen consumption of the treated livers as compared with control tissue. The dehydrogenating activity of the higher and lower fatty acids was considerably reduced in livers from treated animals.

Increased oxygen consumption and increased rate of ketogenesis was also observed in guinea pig livers following oral administration of 1.0 mg of white phosphorus dissolved in olive oil (47). The ability of the liver to oxidize added substrate was not impaired, and no association was found between the metabolic behavior and fatty changes found in the treated livers.

The effect of phosphorus intoxication on the level of microsomal cytochrome b-5 and P-450 in rat liver was analyzed by Borone et al. (48). White phosphorus was administered by gastric intubation at a dose of 7.5 mg/kg and the animals were killed after 24 hours. Intoxication with phosphorus did not cause significant changes in the amount of microsomal pigment. The data indicated that concentrations of phosphorus which cause fatty degeneration in the liver, do not influence the level of the microsomal respiratory pigments, cytochrome b-5 and P-450.

In the rat the increase in oxygen consumption by poisoned liver and kidney slices was accompanied (in the mitochondria) by inhibition of oxidative phosphorylation, which was not specific to phosphorus poisoning (49). Animals poisoned with sc injections of 0.5% phosphorated oil exhibited a marked fall in hepatic pyridine nucleotides, which was accompanied by a decrease in the ratio of the oxidized to reduced pyridine nucleotides, a decrease in hepatic cytochrome-C and a decline in adenosine triphosphate (ATP). On the basis of these observations, the author thinks that uncoupling of oxidative phosphorylation is the result of the insufficiency of the formation of ATP from ADA+P.

8. Effects on Body Weight, Organs and Tissues.

Body Weight. No effects were reported on body weights of experimental animals poisoned with white phosphorus, and reports dealing with human exposures did not describe the effect of accidental phosphorus poisoning on body weight.

Skin. White phosphorus was applied as a 0.1% solution in peanut oil (volume of application not given) to the skin of rabbits. There was no primary irritation in the treated animals at this concentration (7). A dermal sensitivity test in guinea pigs has not been reported.

An unusual case of extensive, subcutaneous hemorrhage was reported by Hann and Veale (50) in a patient who had ingested rat poison containing 4% white phosphorus. This manifestation of acute toxicity has its origin apparently in fatty changes which occur in the coats of arterioles and are not due to blood changes.

Liver. Single sc injections of 10 mg/kg of white phosphorus to rats produced a significant increase in liver weight (31).

A SWPB was produced in 96 rats with 25 or 50 mg of white phosphorus. The burning time was 4 minutes. Following the burn, the wound was sutured, the animals were killed and the livers examined histologically 2-72 hours post-burn. Extensive degeneration of hepatic cells with microthrombi in the portal veins was observed (24-26).

Rabbits injected with 5 mg/kg of white phosphorus in olive oil were killed from 8-48 hours after injection and the livers were removed and examined grossly for abnormalities. As intoxication proceeded, increase in size and weight of the liver was noted. Fatty infiltration and necrosis was also observed (28). These observations confirmed earlier reports by Neubauer and Porges (40) in rabbits which had been given 2.5 and 5.0 mg/kg of white phosphorus subcutaneously.

Pathological changes caused by white phosphorus in dog liver were studied by Buchanan et al. (12). The dogs received single doses of 0.4 mg/kg of white phosphorus via the sc route. Apart from the zone around the central vein which showed fatty metamorphosis, necrosis encompassed the whole of the hepatic parenchymal tissue. At 0.2 mg/kg the liver was hemorrhagic and consolidated with fatty vacuolation, especially along the periphery of the lobules.

Accidental ingestion of large amounts of rat paste or firecrackers containing white phosphorus (amount not given) was shown to produce eosinophilic necrosis in the liver cells, cellular infiltrations (granulocytes and lymphocytes) into portal spaces and inside of lobules, proliferation of bile ducts and fibrosis in humans (51).

Kidney. Ben-Hur and coworkers (24-26) observed swelling of cells, desquamation and perinuclear vacuolization and necrosis in the kidney tubules with white debris in their lumen, 72 hours after rats had received the SWPB (10-50 mg of white phosphorus). The inability of the

burned animals to concentrate urine and the serum phosphate (sodium and potassium) levels 72 hours post-treatment corresponded to the changes seen in acute renal failure. These biochemical alterations, which were probably the result of glomerular and tubular necrosis seen histologically, could lead to cardiac arrest from hyperkalemia.

In humans, fatty degeneration of the renal cortex was observed in large number of patients (77%) poisoned by ingestion of large doses (rat paste, firecrackers, etc., amount not given) of white phosphorus (51).

Gastrointestinal Tract. In a report in which a 45-year-old man had ingested rat paste containing 3% white phosphorus, the cause of death was listed as "hemorrhage into the gastrointestinal tract and soft tissues of the body" (20).

Lung. Hemorrhagic bronchopneumonia was found in one patient following accidental ingestion of rat poison containing 3% white phosphorus (20).

Heart. Talley et al. (21) reported a case of poisoning in which 15.7 mg/kg of white phosphorus were ingested. The patient showed an abnormal electrocardiogram and died 22 hours following ingestion of poison. An autopsy revealed pathologic changes in the myocardial cells (vacuolated cytoplasm) and interstitial edema without cellular infiltration. In another study fatty degeneration of the myocardium was reported in 29% of the fatal cases (51).

Eye. White phosphorus applied as a 0.1% solution in peanut oil (volume not specified) to the rabbit eye did not produce any irritation (7). However Scherling and Blondis (52) reported a case in which a worker was accidentally exposed to a spill of white phosphorus. Twenty minutes after the accident, smoke began to emanate from his conjunctival sacs. On examination, the eyes showed particles of white phosphorus embedded in the bulbar and tarsal conjunctivae. The eyes were irrigated and the particles removed. Shortly thereafter the patient developed slight blepharospasm which disappeared after 30 minutes. A case of ophthalmoplegia due to phosphorus poisoning has described by Medea (53).

Curreri et al. (54) reviewed the clinical records of 111 patients, mostly with burns due to white phosphorus, who were admitted to the U.S. Institute of Surgical Research. They noted a high incidence of residual ocular damage and suggested requirements for successful systemic and local wound care. No toxicological data were evaluated.

Osseous System. Schautz (55) reported a case of bone necrosis of the feet of a 38-year-old woman who suffered acute phosphorus burns while attempting to extinguish a fire after an air raid in Germany during World War II.

9. Cytologic and Cytogenetic Effects

Nuclear Aberrations. In livers of guinea pigs poisoned with white phosphorus (0.75 mg given sc) Bueding and Ladewig (38) observed fatty infiltration, swelling of the nuclei, vacuolation and, in the terminal stages of the poisoning, diffuse nuclear destructions with no evidence of regeneration.

In livers of human who had died after ingesting a large dose of rat paste, some firecrackers, heads of match sticks, etc., the hepatic cells showed irregular nuclear membranes, hyperchromasia, shrunken nuclei, pyknosis, karyorrhexis and delayed mitosis (51).

Chromosomal Abberations. No reports were found on the cytogenetic effects of acute phosphorus for intoxication in experimental animals or humans.

10. Molecular Effects

Talley et al. (21) studied the biochemical effect of elemental phosphorus on rat myocardium (no other data given) by evaluating amino acid incorporation into soluble myocardial proteins and actinomyosins. Their data indicated that phosphorus significantly depresses protein synthesis in the myocardium of treated rats.

White phosphorus (6.0 mg/kg) dissolved in mineral oil was administered by stomach tube to male rats. Two hours later radiolabeled valine 2,3-T was injected intravenously (iv). The animals were killed 1.0 hour later and blood collected by cardiac puncture. The liver, pancreas and duodenum were removed and the proteins isolated, dried and counted for radioactivity. Phosphorus significantly decreased the rate of incorporation of radioactive valine into TCA-insoluble liver pancreatic and duodenal proteins and into serum albumin (50).

Female Sprague rats (number not specified) received 10 mg/kg of white phosphorus by stomach intubation. The animals were killed 12 hours later and the polyribosomes from treated livers assayed for protein synthesis. A disaggregation of the polysomes was observed and protein synthesis was inhibited (33).

11. Embryonic and Teratogenic Effects

There are no reports on experimental animals regarding the effects of white phosphorus on the developing embryo.

Salfelder et al. (51) reviewed 45 cases of fatal phosphorus poisoning from 3 institutes in Venezuela. In most cases large amounts of poison were ingested (rat paste, firecrackers, etc). Ten women were, or had recently been pregnant. The motive of poisoning was generally suicide. In three intoxicated pregnant women, the livers of the fetus were examined and no lesions were found. The lack of liver lesions in these specimens indicated that white phosphorus ingested by the mother had not passed through the placenta. No other information was given.

12. Metabolism

The absorption and distribution of white phosphorus was determined in animals through the use of radioactive phosphorus ^{32}P . Cameron and Patrick (57) studied the distribution of white phosphorus after oral administration of phosphorus ^{32}P in mice, rats and rabbits at single

doses of 0.5, 3.5 and 20.0 mg/animal, respectively. The radioactivity per dose was 0.1, 0.35 and 1.0 millicuries (Mc), respectively. The animals were killed after 48 hours and the organs and tissues assayed for radioactivity.

The radioactivity varied greatly among different tissues and organs, but was uniform for the three species of animals investigated. The highest activity was found in the blood, liver, kidney, spleen, lung, muscle, brain and bone in that order. Further analysis of the liver, kidney, heart and brain showed that in the case of the first three organs, most of the radioactivity was associated with the dry residue but in the brain it was associated with the extractable fractions. This would suggest that the phosphorus may be bound to tissue protein or protein complexes.

In female rats (number not specified) given single oral doses of 0.3 mg/kg of phosphorus, about 60-65% of the given dose of labeled phosphorus was absorbed within the first 24 hours. The highest concentration was found in the liver (16.1% of the dose). Five days later the concentration in this organ was only 6.3% of the given dose. Radioactivity in the blood represented 6.1, 4.1 and 1.7% of the administered dose at the end of 4 hours and 1 and 5 days, respectively. The amount of radioactivity in the muscle averaged 4.0, 5.5 and 6.0% at 4 hours, 1 and 5 days, respectively. The amount of radioactivity remaining in the gastrointestinal tract was 57, 15.3 and 1.7% at 4 hours, 1 and 5 days, respectively. The ratio of radioactivity of the blood to various organs at 4 hours was in the following order: liver-kidney-lung-spleen bone-muscle-brain. The radioactivity in the blood decreased slowly and inconsistently, whereas the radioactivity in the organs and tissues remained high or decreased only slightly. This resulted in large increases in the tissue to plasma radioactivity ratios in all tissues (7).

Another group of female rats (number not given) was dosed with radioactive ^{32}P for 5 consecutive days (0.3 mg/kg/day). The radioactivity in the tissues 24 hours after the last dose was compared with the activity in the tissue 24 hours after a single dose. The tissues of the rats which had received 5 doses contained 4.1 to 10.5 times higher radioactivity than those which had received a single dose, indicating an accumulation of radioactivity in all tissues(7).

The intestinal absorption and distribution of ^{32}P in different tissues of rats intoxicated with white phosphorus was studied by Ghoshal et al. (37). The animals received 7.5 mg/kg of the toxicant mixed with 10 μCi of ^{32}P , by gastric intubation, and were killed at various times ranging from 0.5-10 hours post-treatment. The results indicated that phosphorus is rapidly absorbed and is principally incorporated into the liver where it reaches a maximum (69-73% of the given dose) within 2-3 hours. At the time of maximum hepatic incorporation the percentages of the given dose recovered from the blood (12%), kidney (4%) spleen (0.4%), pancreas (0.4%) and brain (0.39%) were significantly lower than the liver (65%) (p 0.001 for all comparisons). At 3 hours the percentages of incorporation in all these organs were similar to those found in 2 hours. The subcellular incorporation determined at the point of maximum hepatic incorporation showed that 54% of the total hepatic isotope was present in the supernatant fraction and the rest was almost equally distributed between the other subcellular fractions.

The tissue/plasma ratios of radioactivity in rats receiving a single dose of ^{32}P white phosphorus are as follows (58).

Tissue/Plasma Radioactivity Ratio

(1 g wet weight of tissue to
1 ml plasma)

<u>Tissue</u>	<u>4 hours</u>	<u>24 hours</u>	<u>5 days</u>
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Liver	18.7 \pm 2.5	51.4 \pm 3.9	103.2 \pm 10.0
Kidneys	4.2 \pm 1.0	14.4 \pm 1.2	33.5 \pm 4.1
Spleen	1.8 \pm 0.4	6.4 \pm 2.6	18.6 \pm 2.6
Brain	0.3 \pm 0.0	0.7 \pm 0.0	3.6 \pm 0.4
Lungs	2.6 \pm 0.1	5.8 \pm 0.5	16.5 \pm 1.0
Skeletal Muscle	0.4 \pm 0.0	1.8 \pm 0.1	8.7 \pm 0.5
Bone	1.7 \pm 0.7	12.7 \pm 0.1	66.9 \pm 17.2

The uptake of radioactive phosphorus ^{32}P by the rabbit skin was studied by Whiteley et al. (59). The phosphorus was injected iv (75 microcurie/g of animal) and radioactivity examined at 1, 24, 48 and 72 hours post-treatment. The results showed that skin areas with active hair growth took up more of the isotope than did areas in which hair follicles were quiescent. The factors which contributed to the greater activity in the growing zone were the extracellular space per unit area (shortly after injection), the large amount of acid soluble phosphorus per unit area (1 hour after injection) and the greater concentration and higher specific activity of the nucleic acid phosphorus (24 hours after injection).

White phosphorus is metabolized in the body of animals. Urinary excretion includes organic and inorganic phosphates (7, 60). It is generally accepted that phosphorus in the organs is rapidly oxidized into hypophosphorus and phosphoric acid (61, 62). However, the site or sites of oxidation have not been determined.

Lee et al. (7) gave single oral doses of 0.3 mg/kg of ^{32}P white phosphorus to rats and found that 4 hours later 54% of the dose was excreted in the urine, 34.5% at 1 day and 46.7% at 5 days. During this time some radioactivity was also discovered in the feces in the following amounts - 2.0, 16.6 and 33% of the administered dose at 4 hours and 1 and 5 days - respectively.

Huruya (28) injected rabbits sc with 5 mg/kg of white phosphorus and collected the 24 hour urine for analysis. It was found that the amount of urine excreted after injection of phosphorus decreased with time. The alkalinity increased in later stages. The excretion of nitrogenous material into the urine increased during mild intoxication but as intoxication proceeded it decreased. The ratio of urea to total nitrogen in the urine decreased and that of ammonia increased with time. The ratio of non-urea and ammonia nitrogen to total nitrogen excreted was over 30% in intoxicated animals. There was an increase in sulfur excretion during mild intoxication, while under severe intoxication the excretion of sulfur was very close to normal.

These changes in urine composition were correlated with pathologic findings which were observed in the liver and kidney and were the result of the autolytic process in the liver and the disability of the kidney.

In dogs given 1% phosphorus in oil subcutaneously, increased urinary nitrogen and ammonia were observed at 18 hours post-treatment, accompanied by albuminuria and severe kidney damage and death by day 2 post-treatment (63).

Intravenous administration of phenoltetra-chlorophthalein (PTCP) can give important evidence concerning the amount of liver injury and the degree of functional impairment. It was found that after acute liver injury cause by sc administration of 10 mg of phosphorus to dogs, there was an immediate drop in liver phthalein output in the feces and this drop corresponded very closely to the degree of injury, falling to zero with fatal intoxication. It was observed that PTCT, which does not come through the urine of a normal dog, will appear very promptly in the urine after the liver injury is established. The reason for this could be that the phthalein is modified by contact with the injured liver cell and it can pass the kidney filter which is impermeable to the unchanged phthalein. As the injury to the liver was repaired, the output of phthalein in the feces rose to normal while the urine output diminished to zero (15). Large increases (3 to 8X normal) in lactic acid in urine of rabbits with liver damage due to phosphorus poisoning was noted by Mizuno (64).

11. SUBCHRONIC TOXICITY (less than 90 days; short term)

1. Hematologic Effects

Monocytes. There are a number of observations, both clinical and experimental, which indicate that there may be some obscure relationship between injury to the liver and the number of circulating monocytes. Lawrence and Huffman (65) injected 13 guinea pigs sc and 6 were given phosphorus by mouth. The injections were given at intervals of 3-5 days at a dose of 0.5 mg. The smallest total dose was 3.5 and the largest, 5.5 mg of white phosphorus. Total and differential counts of white cells were made daily for several days throughout the injection period. The animals receiving phosphorus orally were given 1-2 mg daily for 6 days. The animals were killed at the end of the treatment period.

The animals that received sc injections of white phosphorus showed an appreciable rise in the number of monocytes. The maximal average increase was 87.9% and this occurred after the 2nd and 3rd injection. The animals treated orally did not exhibit the same response. Four were essentially normal, one showed marked terminal increase in monocytes (5615), associated with marked leucocytosis (31,200), and the other showed a moderate increase in monocytes. Histological examination of the liver did not indicate any direct relationship between injury to the liver and the number of circulating monocytes.

An increase in monocyte counts was also perceived in rabbits exposed to white phosphorus vapors of 150-160 mg/m³ continuously for 60 days (66). The white blood cells (WBC) increased at first, then decreased. On the WBC differential counts, acidophils decreased and even disappeared in cases of severe poisoning. Pseudo-eosinophils increased rapidly right after exposure to white phosphorus and maintained the high level throughout the experimental period. Lymphocytes decreased, while monocyte basophils increased (66).

Erythrocytes (RBC) and Hemoglobin. Both RBC and hemoglobin decreased in rabbits exposed to vapors of white phosphorus (150-160 mg/m³) for 60 days (66) and in dogs poisoned with repeated injection of 0.1 mg/kg of white phosphorus for 56 days (12).

A similar effect was observed in rabbits which received 1.0 mg/kg of white phosphorus for 30 days. The erythrocyte counts and amount of hemoglobin were decreased and there was a decline in cell fragility as well (67). However Prader (68) gave 0.5% solution of phosphorus to rabbits and found that hemoglobin values and erythrocyte counts increased during the first 2 weeks of treatment but returned to normal after 3 weeks.

Fibrinogen. One of the most noticeable changes observed in the blood of phosphorus-poisoned dogs was the marked decrease in fibrinogen. Marshall and Rowntree (69) gave 2-6 sc injections of 5-20 mg of phosphorus to 3 dogs and noted jaundice and failure of the blood to clot. All three animals died of fatal poisoning. Similar observations were reported earlier by Whipple et al. (15) in the same species.

2. Bone Marrow Changes

Heiman (11) stated that the morphological changes observed in circulating blood in chronic poisoning may be caused by damage to the bone marrow produced by the encroachment upon the medullary canal which contains the major elements of the hematopoietic system or by direct action upon the bone marrow, described as being hyperemic at first and later, degenerative (70). It could also be effected secondarily to the septic absorption from jaw bone necrosis. Anemia of the "secondary" type has been reported from the industrial environment (11).

3. Immunologic Effects

Tanaka (67) observed a decrease in antibody and the agglutination activity of serum and the red cells in the blood of rabbits poisoned with white phosphorus (oil-2.0 mg/kg).

No data were found on the acute effects of white phosphorus on human immunologic system.

4. Central Nervous System (CNS) Effects

Ferraro et al. (71) described pathological changes which occurred in the brains of 12 rabbits which received iv injections of 1% white phosphorus in oil twice and thrice a week. The animals were killed at different times (3-10 weeks) during the experiment and the brain examined for pathologic alterations. Significant decrease was observed in the number of ganglion cells in the cerebellum and the inferior olives. The nerve cells in the striate areas appeared to have undergone more pronounced changes than in the cortex, and the nerve cells of the inferior olive showed more severe effects than the cells of any other part of the brain. A gradual necrobiosis of the nerve cells was observed in the gray matter. Vascular disturbances (swollen endothelial cells of blood vessels, occlusion, inflammation etc.) were seen throughout the brain. Occasionally perivascular infiltration was also observed. The glia showed the feature of hyperplasia generally coexisting with hypertrophic changes.

Ferraro noted that in the lesions of the nerve cells due to phosphorus, fatty degeneration does not seem to play an important role. In fact, his study showed that fatty substances were detected rarely and only in small amounts within the nerve cells, whereas they infiltrated abundantly the choroid plexi and the cells of the viscera (liver, lung, and kidney).

This dissociation of behavior, as far as fatty metamorphosis is concerned, between nervous tissue and the other parenchymata remains a singular feature. The results of histologic studies on the CNS in experimental poisoning are not uniform. In the rabbit there is an initial stage of proliferation of the small blood vessels (specially the striatum) and a second stage of diffused encephalitic changes. These changes were regarded as secondary to the effect of phosphorus on the

liver. These observations were confirmed in the dog in which severe degenerative processes were found in the nerve parenchyma without fatty degeneration of the ganglion cells such as had occurred in acute yellow atrophy of the liver. There was a diffuse decrease of nerve cells in the cortex and other parts of the gray matter, a regressive change in the glia, and accumulation of fat in the blood vessel walls and the swelling of the endothelium (19).

Marcovitz and Alpers (72) noted a range of abnormalities in microglial cells from brains of 17 rabbits treated with fresh phosphorated oil in doses of 0.25-0.35 milliliters injected usually iv and only twice subdurally, 2-3 times a week up to 10 weeks. The authors stated that these changes were chiefly regressive but may have been in part reparative.

No reports were found on the effect of phosphorus on the CNS of humans following subchronic exposure.

5. Behavioral Effects

Krasnov (73) has reported on the effect of white phosphorus on the motor-food conditioned reflex of white rats which received 0.015 mg of the pure element daily for 30 days. The study showed that white phosphorus given at this dose to rats produces an intensification of the excitatory process and an increase in cortical excitability.

6. Cardiovascular Effects

No cardiovascular effects of white phosphorus were reported during subchronic exposure in experimental animals.

7. Biochemical and Histochemical Effects

Phosphorus-induced biochemical changes have been studied in experimental animals. The most important changes occur in the liver and blood.

Blood. To establish whether changes in serum protein were caused by an inflow from tissues of preformed "reserve protein" or by formation of new serum proteins in the liver or bone marrow, experiments were performed using white phosphorus as the intoxicant (74). Rabbits were given repeated doses of 0.5% of the element (number of animals not given) through stomach tube until they died. Blood samples taken at different times during the treatment were analyzed electrophoretically. Blood from phosphorus treated animals showed a marked increase in gamma-globulin as compared to controls. The effect of white phosphorus on glucosamine was transient.

Changes in plasma proteins due to phosphorus poisoning was studied by Land and Frenreisz (75) in dogs (7 males, 1 female). The animals were given 0.4-1.4 g oleum phosphoratum by stomach tube for 30 days. Periodically blood samples were taken from treated animals and analyzed for albumin, globulin and fibrinogen. By the end of the test period a decrease was observed in plasma albumin and fibrinogen and an increase in the plasma globulins. No attempts were made to statistically analyze the results.

Tanaka (67) administered 0.1-2.0 mg/kg of white phosphorus to rabbits (30 days) and observed a significant decrease in total protein in the blood and increase in non-protein nitrogen, amino acid and uric acid. A small increase was also seen in the creatine and creatinine levels.

Alterations in serum esterase activity following sc injection of 1.0 milliliter of phosphorus oil daily for 3 days was studied in 5 dogs. The blood samples taken for analysis were drawn from the inferior vena cava, hepatic vein, and the portal vein. It was found that in animals with severe liver damage, the serum esterase activity was highest from the portal vein and least active from the hepatic vein. The low activity of the serum esterase from the hepatic vein suggests the possibility of the esterase being adsorbed or destroyed as it passes through the liver (35).

Simmonds (76) also assayed serum esterase activity of dogs (4 animals) given sc doses of 15-27.5 mg of phosphorus over a 3-10 day period and found it to be moderately increased. The feeding of sugar did not prevent the increase in esterase in the blood serum of animals poisoned with phosphorus.

Among the characteristic effects brought about by phosphorus poisoning are fatty infiltrations of the liver and other changes which indicate a serious disturbance in the fat metabolism and in the mechanism of fat deposition (77). Ingested fat is predominantly transported to the liver as chylomicrons (78). It is believed that the study of blood lipids during phosphorus poisoning using the chylomicron counting technique might be helpful in clarifying some of the aspects of this disease. Fleming and Collings (79) used the method to study the effect of white phosphorus in rats. Twenty-seven animals were injected sc with 1.1 mg/kg/day of white phosphorus 3 times a week throughout the duration of the experiment (45 days), and the chylomicron counts made once a week. Twelve animals served as controls. Although elevated counts were obtained in the blood of treated animals, the differences between these values and those obtained in controls were not significant.

The effect of phosphorus exposure on blood levels of guanidine was studied in dogs by Cutler (80). Six dogs were given a total of 3 and 4 mg/kg of white phosphorus in oil orally in divided doses (2 mg on the first day, 1 mg on the third day, and 1 mg on the fifth day). All of these doses were sufficient to produce severe and, in a majority of cases, fatal intoxication to the dogs which were kept on a diet of lean meat without bones. Blood samples were taken after dosing at intervals which varied with the course of intoxication. Phosphorus was found to produce general tissue destruction and an increase in guanidine levels in the blood which were reflective of liver damage. Hypoglycemia and nervous symptoms were also observed which could be alleviated by calcium medication.

Liver. Truhaut et al. (81) injected 2.5 and 5.0 mg/kg of white phosphorus sc into 30 rats (15 rats at each dose). The element was dissolved in olive oil and the injection given every 2 or 3 days, until the animals got a total dose of 15 mg/kg (5 mg/kg group) or 24 mg/kg (2.5 mg/kg group). At the 2.5 mg/kg dose 3 animals died after the ninth injection, while at the 5 mg/kg dose 4 rats died after the third injection. Treatment of 20 control rats was not described in the report. All surviving rats were killed two days after the last injection, i.e., on day 28 (2.5 mg/kg group) and day 8 (5.0 mg/kg group) of the experiment, and their livers were removed and assayed for enzyme activity.

The 2.5 mg/kg dose caused a significant increase in triglycerides and decrease in phospholipids (p less than 0.01) as compared with the controls. However the increase in total lipids and cholesterol was insignificant. At 5.0 mg/kg the increase in triglycerides was highly significant (p less than 0.001) whereas the total lipid elevation was not significant (p less than 0.2). White phosphorus did not significantly modify total cholesterol (p less than 0.05).

The effects of both doses of white phosphorus on enzymatic activity were comparable. The fructose 1,6-diphosphate aldolase (F16D) was significantly inhibited as compared to controls (p less than 0.001). The decrease in malic dehydrogenase (MDH) activity was not significant (p less than 0.02) at 2.5 mg/kg, nor was there any appreciable change in the activity of this enzyme at 5.0 mg/kg. The activities of G6PD and LDH were not effected.

Eight dogs received capsules of cod liver oil containing 0.05 mg of phosphorus until they died. Each animal received 2 capsules/day (0.10 mg/d). The length of the experimental period was not stated, thus the total dose could not be calculated. At the end of the experiment 3 dogs showed marked terminal hypoglycemia. The livers of all animals were normal, but 6 of them had hyperuric acidemia (45).

Rabbits (8 animals) receiving small daily doses of phosphorus (0.75 mg/kg) were subjected to a modification of the dextrose tolerance test followed by an injection of epinephrine. Blood sugar curves following these procedures and determination of liver glycogen before and after the modified dextrose tolerance test were made. The first functional deficiency of the liver to appear was a failure on the part of this organ to maintain the normal blood sugar level. Later a progressive decrease in sugar tolerance was manifested, which could in part be explained by lack of glycogen deposition in the liver after dextrose administration, water and insulin. In the last stages of poisoning (after modified dextrose tolerance test), blood sugar curves usually ended in hyperglycemia. Epinephrine hyperglycemia was first increased, then decreased and finally abolished altogether in these animals. Under these circumstances, the observed hypoglycemia was explained by failure of glycogenolysis to check the downward trend of the blood sugar initiated by the stimulus of the ingestion of dextrose (41).

Jobling et al. (35) determined the liver esterase activity in 5 dogs following sc injections of 1.0 milliliter of phosphorus oil daily for 3 days. Liver tissues showing fatty degeneration obtained from phosphorus poisoned animals showed decreased amounts of esterase activity.

Simmonds (76) also studied the esterase activity in the liver of phosphorus poisoned dogs. Doses of 15-27.5 mg of phosphorus oil were given sc to 4 dogs over a 3-10 days period. The amount of esterase in the liver did not vary to any great extent from the normal in phosphorus treated animals. However, feeding of large amounts of sugar, which increases the amount of glycogen, did increase the esterase activity in the hepatic tissue of poisoned animals.

Rabbits with fatty livers caused by white phosphorus injections (0.5% twice a week), given subcutaneously for 5-36 days, showed increased glycogen and cytochrome-C (3-fold increase over controls) levels after 3 weeks of intoxication (68). Milder poisoning resulted in less cytochrome-C and was accompanied by a lag period.

Dianzani (48,82) studied the adenosine polyphosphates in fatty livers of rats and guinea pigs. The animals received from 2-6 injections (0.2 ml to rats; 0.4% to guinea pigs) of 0.-0.5% of phosphorated oil subcutaneously. The animals were killed and livers and kidneys removed and the mitochondrial fraction assayed for phosphorylating activity. The observed decrease in ATP which occurred in the livers of treated animals before the beginning of fatty infiltration suggested that the phosphorated oil does not possess the ability to uncouple oxidative phosphorylation in vitro.

Octanoate oxidation by mitochondria was studied with both normal and fatty livers of rats (83). Fatty livers were produced by injecting sc white phosphorus (0.1 ml of a 0.5% olive oil solution) each day for 2 days. The animals were fed on a semi-synthetic diet and killed 10-12 hours after the last meal, and their livers measured for oxygen uptake in the presence of octanoate. In the case of mitochondria from fatty livers, oxygen uptake was significantly decreased as compared with normal values and the acetoacetate blood levels were significantly decreased (475.3 \pm 199.4 normal vs treated 168.9 \pm 65.8 micrograms acetoacetate/100 ml blood).

8. Effect on Body Weight, Organs and Tissues

Body Weight. Rabbits and rats given 0.6 mg and 0.01% phosphorus in cod liver oil, respectively, for 13 days (rabbit) or 22-57 days (6 of the rats received an additional 0.04% during the last week of the experiment) showed an overall reduction in weight gain as compared to controls (84).

Liver. Livers of rabbits intoxicated with phosphorus (2.5 mg/kg) showed a significant increase in weight (p less than 0.001).

Livers of dogs poisoned with white phosphorus (0.1 mg/kg, sc injections) for 56 days showed fine deposits of fat and slight pathologic changes, but there was no difference in the dry weight, glycogen and total fat between the livers of treated and control animals (12).

Rabbits receiving repeated doses of 0.5% white phosphorus by stomach intubation (until they died) showed liver damage with fatty degeneration. Hemorrhage and cellular necrosis were also observed (74). Histopathologic abnormalities in liver parenchyma and mitochondria were observed in rabbits dosed orally with 1 or 3% of white phosphorus (in oil) for 50 days. The overall picture of fatty infiltration was much less severe than that observed with acute doses. Increase in glycogen content was also recorded in animals intoxicated with phosphorus. Rabbits receiving repeated (3 injections) doses (2.5-6.5 mg/kg) of white phosphorus showed fatty infiltration and lacked cellular epinephrine, while others receiving repeated (3 injections) doses of higher amounts (2.5-8.3 mg/kg) showed no fatty infiltration and were devoid of chromaffin reaction (40).

Kidney. Kidneys from dogs given 0.1 mg/kg of white phosphorus for 56 days showed hydropic degeneration (12). A higher dose (1.0 mg/day) for 8 days (sc or per os) resulted in fatty infiltration and tubular degeneration (85).

Spleen. Enlarged spleens and large amount of hemosiderin were observed in dogs poisoned over a period of 56 days with 0.1 mg/kg of white phosphorus injected sc (12).

Adrenal. Subcutaneous injections of 1.6-10 mg/kg of white phosphorus (in oil) administered 2 or 3 times induced adrenal insufficiency in rabbits (40).

Pancreas. The effect of white phosphorus on pancreatic mitochondria in mice was studied by Scott (86). Phosphorus solutions in concentrations of 0.0125-0.05% (vol 0.1-0.2 ml) were injected into white mice. The dosage schedule lacked uniformity, and doses were given at intervals of a day or more (for a longer or shorter time) depending on the severity of the reaction. When sufficient toxicity was observed, the animals were sacrificed, the pancreata were removed, stained, and examined microscopically. The findings were those of gradual to gross morphological deformity of the mitochondria, and no explanation was given as to the factors responsible for each level of deformation.

Ear. In one study in which 12 rabbits were injected iv with 1% white phosphorus (in oil) 2-3 times a week, 2 animals developed necrosis of the ear after 4 and 7 weeks of treatment, respectively. The authors did not give any other detail (73).

Osseous System. Involvement of the osseous system is characteristic of chronic phosphorus poisoning. Most typical of this involvement is the jaw necrosis. However, other changes such as those of the periosteum, and those produced by deposition and resorption of calcium salts leading eventually to bone atrophy, have also been described. Most experimental studies have paid special attention to the ossification of the growing centers of the bone and the appearance in these bones of widened epiphyseal areas. Chemical analysis of these areas of thickening have also been made, and an abnormal relationship in the quantity of phosphorus and calcium have been described. Three such studies are described below.

Rabbits (17 animals) and rats (14 animals) were given an 0.6 mg coated pill of white phosphorus (rabbits) or phosphorized cod liver oil containing 0.01 mg % of white phosphorus (rats) daily, for 13-117 days (rabbit) or 22-57 days (rat). In the diet of 6 rats, white phosphorus was increased 4-fold (0.04%) during the last week of the experiment (50th-57th days). Seventeen rabbits and 14 rats served as controls. A roentogram of the tibia taken after treatment showed a zone of increased density (the phosphorus band), and there was a reduction in the process of tubulating of the shaft. These effects were due to diminished resorption of the cartilaginous matrix and bone, which was probably generalized but most readily evident only in the metaphyses, where active bone resorption was a prominent feature of the process of normal endochondrial bone growth. No roentogenographic changes were observed in the teeth of the treated animals, but in the skull phosphorus bands were present on both sides of the basispheno-occipital and basis phenosphenoidal junctions. The rats that received that additional 0.04% phosphorus daily during the last week of the experiment had a calciotraumatic line in the labial dentition which was absent in the group which did not receive this extra dose of white phosphorus (84).

Inuzuka (87) examined 20 male and female rats which were exposed to 150-160 mg/m³ white phosphorus vapors for 30 minutes daily for 60 days and found the presence of wide metaphyseal lines, irregular cell configuration and remarkable trabeculation associated with insufficient ossification and disordered axile development of long bones.

The pathogenesis of abnormal remodeling of bone caused by white phosphorus toxicity was studied by Whalens et al. (88) in 16 female rats (23 days old). The rats were fed 1.3 mg/kg/day of white phosphorus for 8 or 16 days and showed widespread metaphyseal trabeculae of proximal tibia which gradually returned to normal after the treatment was suspended. Inhibition of osteocytic osteolysis and chondrolysis was observed in both treated groups. This inhibition resulted in widening of the trabeculae and retention of the chondroid core. The quantitative difference between the two treatment groups was not mentioned.

9. Cytologic and Cytogenetic Effects

Mitosis. Rabbits (134 animals, sex not specified) were given either 1 or 3% of white phosphorus in oil orally (3-14 drops daily) for 50 days. The animals were killed and their livers examined histologically for abnormalities. In both treatment groups there was an increased mitotic activity in the fat string cells of the sinusoid walls and Kupffer cells (89).

10. The Molecular Effects

There were no reports on the effect of white phosphorus on DNA, RNA and protein synthesis in experimental animals during subchronic exposure.

11. Embryonic and Teratogenic Effects

No reports were found relating to the effects of white phosphorus on reproduction, embryogenesis and teratogenesis in experimentals or humans.

12. Metabolism

Marshall and Rowntree (69) studied the effect of white phosphorus on liver and kidney functions of dogs. Fifteen animals were given repeated sc injections of varying amounts of white phosphorus (5-20 mg) over different time periods (3-21 days). The blood, and urine were examined for changes in total non-protein nitrogen, urea and amino acids. All three substances showed a definite and sometimes marked increase in the serum following phosphorus poisoning. The urinary nitrogen partition between the urea, ammonia and amino acids was not always disturbed. The most important change observed was an increase in amino nitrogen. Sugar tolerance was also tested and found to be markedly decreased. In phosphorus poisoning, liver functional changes occurred without concomittant renal changes, and renal insufficiency usually occurred at a terminal event. Increased nitrogenous products in the blood (total non-protein nitrogen, urea and amino nitrogen) were associated with a corresponding increase of these substances in the urine. Consequently, an increased protein catabolism, as well as renal insufficiency, is necessary to explain this accumulation. A terminal acidosis, as evidenced by increased hydrogen ion concentraton was also observed.

Dogs poisoned subcutaneously with 0.1 mg/kg of white phosphorus for 56 days showed a progressive increase in creatine and changes in the creatine to creatinine ratios in the urine. These changes were presumed to be caused by tissue breakdown as evidenced by the loss in weight of the animal (12).

13. Carcinogenicity

No reports were found on the carcinogenic effect of subchronic white phosphorus poisoning in experimental animals or humans.

III. CHRONIC TOXICITY (Long Term Tests)

1. Hematologic Effects

No reports were found on hematologic effects due to chronic exposure of experimental animals to white phosphorus. However, Hughes et al. (90) investigated 46 healthy men working in a phosphorus plant with 28 healthy controls not exposed to phosphorus. Exposure varied between 1-17 years in the phosphorus group. Hemoglobin and total leucocyte counts were made and no statistically significant differences were found between the two groups except for the polymorphonuclear leucocytes to lymphocyte ratio (30:61%) which was abnormal. No explanation was offered for this.

Heiman (11) observed leucopenia and anemia and the absence of methemoglobin in some instances of chronic exposure.

2. Bone Marrow Changes

See subchronic studies.

3. Immunologic Effects

No data were found on the chronic effects of white phosphorus on the immunologic system in animals or humans.

4. Central Nervous System (CNS) Effects

Although there is involvement of the CNS in acute phosphorus intoxication there is no evidence that chronic phosphorus exposure in humans produces effects on this system (11).

5. Behavioral Effects

No data were found on the chronic effects of white phosphorus on the behavior of experimental animals or humans.

6. Cardiovascular Effects

No data were found on the chronic effects of white phosphorus on the cardiovascular system in experimental animals.

7. Biochemical and Histochemical Effects

Blood. Hughes et al. (90) investigated 15 healthy men working in a phosphorus plant and 5 healthy controls who were not exposed to phosphorus. Duration of exposure varied between 1-17 years in the phosphorus group. Determinations were made on inorganic phosphorus, alkaline phosphorus, calcium and magnesium levels in the plasma. No difference was observed in any of these parameters between the exposed and control groups.

In another study, the levels of potassium and chloride were high in groups exposed to white phosphorus in comparison to non-exposed workers (11).

8. Effect on Body Weight, Organs and Tissues

Body Weight. Thirty-two rats (22 young female and mature males) were administered 0.003-0.072 mg/kg/day of white phosphorus in their food over a period of 5-6 months (91). The highest dose (0.072 mg) produced marked and progressive deficiency of growth so that the treated animals, at the end of the experiment, weighed only half as much as controls. Withdrawal of poison from food did not result in recovery but merely checked the rate of weight loss. At lower doses (0.018 and 0.0032 mg) the weight loss was somewhat less (about 15%) and apparent only after 15 weeks. These rats not only recovered on withdrawal of the toxicant (0.0018 mg), but become considerably heavier than controls. The author suggested that this could be a cumulative toxic effect.

In another study rabbits given 0.6 mg/kg/day of white phosphorus for 117 days showed an overall reduction in weight gain as compared with controls (84).

Liver. Rabbits and guinea pigs (85 animals) administered 0.6-1.0 mg/kg/day of white phosphorus in sweet almond oil orally for 3 months showed definite liver cirrhosis. The induced changes were often complicated by ascites and jaundice. Phosphoral induced injury was directed to stromal fibroblasts, especially around the portal vessel and parenchymal cells throughout the liver. Destruction of the fibroblast was followed by regeneration, as evidenced by presence of occasional mitotic figures and by fibrosis which was periportal initially, but soon extended irregularly into the lobes. At the higher dose (1.0 mg/kg/day), the processes of destruction and regeneration were accelerated, while at the lower dose (0.33 mg/kg/day) they were retarded. The shortest time required to produce these changes was 4 months (92).

Hepatic parenchymal destruction was observed in 51 guinea pigs given 0.75 mg/kg of white phosphorus orally, 4 days a week or 1.5 mg/kg, twice a week for a total of 35 weeks. Two to four animals were killed at regular intervals during the experiment, and the liver examined for abnormalities. After 9 weeks of treatment, all liver samples examined showed focal loss of parenchymal substance which extended to surrounding lobes (pre-cirrhosis stage) as the period of testing advanced. Failure to produce clear-cut portal cirrhosis seemed to be due to inconsistent and minimal degree of periportal cirrhosis (93, 48). Other abnormalities included increased amounts of collagens, fibrosis, fatty degeneration, proliferation of bile ducts, and hyalinization of cell cytoplasm.

Although the typical damage in acute phosphorus poisoning is severe damage to the liver with its attendant fatty infiltration, in chronic poisoning in humans, no definite liver disease has been described.

Osseous System. For effects on growth of long bones in experimental animals see Subchronic effects.

In humans the most typical involvement of the osseous system is the "phossy jaws" (11), or necrosis of the jaw seen among workers in match factories. The first change in the bones is a generalized hyperostosis. The process starts with the deposition of calcium followed by its resorption leading eventually to bone atrophy. As a result of these changes, the bones become brittle and fracture easily (chronic osteomyelitis). There is ossification of the growing centers of the bone and the appearance in these bones of a widened epiphyseal and subepiphyseal areas. The effect of chronic phosphorus exposure on the jaw bones producing necrosis is well documented in humans (4, 90, 94-96). The onset of the necrosis after first exposure is said to be an average of 5 years. However this is questionable, since to establish the date of "onset" is itself usually difficult.

9. Cytologic and Cytogenetic Effects

No data were found on the chronic effects of white phosphorus on in vivo or in vitro nuclei and chromosomes of experimental animals or humans.

10. The Molecular Effects

No data were found on the long term effects of white phosphorus on DNA, RNA and protein synthesis in experimental animals or humans.

11. Embryonic and Teratogenic Effects

No reports were found on the chronic effects of white phosphorus on reproduction, embryogenesis and teratogenesis in experimental animals or humans.

12. Metabolism

In humans white phosphorus is excreted essentially as organic and inorganic phosphates. Insignificant amounts may be exhaled in the breath and sweat or excreted in the feces (97). Urinary changes include

elevation of ammonia nitrogen at the expense of urea nitrogen, increased oxidized sulfur content and albuminuria (11). Other compounds which may appear in the urine are acetone, diacetic acid, leucine, and tyrosine (98).

13. Carcinogenicity

White phosphorus does not appear to be a carcinogen in experimental animals. Sollman (91) exposed 32 rats (both sexes) to 0.0027-0.072 mg/kg daily for 4 months. The toxicant was mixed with the food. At 22 weeks 23 animals were alive and no tumors were found.

Similarly Felming et al. (99) administered 1.6 mg/kg/day of white phosphorus in peanut oil to rats in their diet for 512 days and observed no tumors at the end of the experimental period.

Subcutaneous injection of white phosphorus into rats at 0.05-3.2 mg/kg and into guinea pigs at 0.2-1.6 mg/kg in olive oil or peanut oil 2 times weekly for more than 610 days did not produce tumors in these animals (99).

No reports were found on human carcinogenicity relating to chronic phosphorus exposure.

IV. PHYSICAL AND CHEMICAL PROPERTIES

The best-known and most common variety of solid elemental phosphorus is alpha-white phosphorus. It is also the form of greatest commercial importance. The solid is obtained by the condensation of phosphorus vapor to a liquid and then allowing the later to solidify under water. White phosphorus, when pure, is a colorless waxy solid melting at 44.1°C to a clear liquid. As a solid, it darkens when exposed to light and glows in the dark (phosphoresces), emitting a pale greenish light. (100, 101)

Commercial white phosphorus is 99.9% pure, with the major impurities being arsenic and traces of hydrocarbons. Commercial white phosphorus has a slight yellow color and melts to a straw-colored liquid. The yellow color is presumably due to traces of red phosphorus, which is the other allotropic form having commercial importance. (100, 101)

The physical properties of 2-white phosphorus are summarized in Table 2.

Table 1

PHYSICAL PROPERTIES OF α -WHITE PHOSPHORUS (100)

Appearance:	colorless to yellow, waxy solid
Melting point:	44.1°C
Boiling point:	280.5°C
Crystal structure:	cubic
Density:	1.828 g/cm ³
Autoignition temperature:	30°C in moist air, higher in dry air
Critical temperatures:	695°C
Critical pressure:	82.2 atmospheres
Index of refraction:	1.8244 for D line at 29.2°C
Heat of Fusion:	600 \pm 3 cal/mole P ₄ at 317.26°K
Heat capacity:	at 25°C = 22.18 cal/mole/degree at 44.1°C = 22.73 cal/mole/degree
Sublimation pressure (mm):	0.025 (at 20°C), 0.043 (at 25°C), 0.072 (at 30°C), 0.089 (at 35°C), 0.122 (at 40°C)
Vapor pressure:	1 mm Hg at 76.6°C
Heat of sublimation:	13.4 Kcal/mole P ₄
Heat of combustion:	710.2 \pm 1.0 Kcal/mole P ₄
Solubility:	
Cold water	: Almost insoluble
Hot water	: Slightly soluble
Absolute alcohol	: 2.5 g/l
Ether	: 10 g/l
Chloroform	: 25 g/l
Benzene	: 28.5 g/l
Carbon disulfide	: 1250 g/l

White phosphorus is by far the most reactive form of phosphorus. The most important reaction of elemental phosphorus is oxidation. White phosphorus must be stored under water to protect it from air where it inflames spontaneously. Combustion in air produces a dense white smoke of phosphorus pentoxide (P_4O_{10}) which in the presence of moisture is then converted to phosphoric acid. White phosphorus has a high heat of combustion (710 Kcal/mole), and the smoke produced by it has a tendency to rise in a pillar-like mass. (100)

Phosphorus reacts with aqueous alkali to give phosphine which can also be formed by hydrolysis of the metal phosphides and by strong reduction of most phosphorus compounds. Halogens, sulfur, and the oxidizing acids react with phosphorus to give halides, sulfides and oxyacids, respectively. Phosphorus also reacts with metals and non-metals to produce phosphides. (100, 101)

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RED PHOSPHORUS TOXICITY

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
1. Hematologic Effects	<ul style="list-style-type: none"> o <u>Leucopenia</u> (rabbit) (9). Decreased erythrocyte counts (rabbit) (9). o <u>Human</u> None Reported. 	o None Reported.	o None Reported.
2. Bone Marrow Changes	o None Reported.	o None Reported.	o None Reported.
3. Immunologic Effects	o None Reported.	o None Reported.	o None Reported.
4. Central Nervous System (CNS) Effects	<ul style="list-style-type: none"> o Loss of appetite (rabbit) (9). o <u>Human</u> None Reported. 	o None Reported.	o None Reported.
5. Behavioral Effects	o See CNS effects.	o None Reported.	o None Reported.
6. Cardiovascular Effects	o None Reported.	o None Reported.	o None Reported.
7. Biochemical and Histochemical Effects	o None Reported.	o None Reported.	o None Reported.

RED PHOSPHORUS TOXICITY

Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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RED PHOSPHORUS TOXICITY (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
8. Body Weight, Tissues and Organs	<ul style="list-style-type: none"> o <u>Body Weight</u> No effect (9). o <u>Liver</u> extensive fatty degeneration (rabbit) (9). o <u>Kidney</u> fatty Degenerative changes (rabbit) (9). o <u>Spleen</u> hyperplasia (rabbit) (9). o <u>Genital Organs</u> hyperplastic changes in ovaries and testes (rabbit) (9). o <u>Skin</u> None Reported. o <u>Brain</u> nerve cell degeneration (rabbit) (9). o <u>Osseous System</u> None Reported. 	<ul style="list-style-type: none"> o None Reported. o None Reported. o None Reported. o None Reported. o None Reported. 	<ul style="list-style-type: none"> o None Reported. o None Reported. <u>Kidney</u> parenchymatous and interstitial nephritis (9). o None Reported. o None Reported. o <u>Skin</u> desquamation (9). o <u>Osseous System</u> (human) Osteomyelitis of mandible questionable (11).
9. Cytologic and Cytogenetic Effects	o None Reported.	o None Reported.	o None Reported.

RED PHOSPHORUS TOXICITY (CONT'D)

Chronic Treatment (short term tests; 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None Reported.	<ul style="list-style-type: none"> o None Reported. o None Reported. 		
None Reported.	<ul style="list-style-type: none"> o <u>Kidney</u> parenchymatous and interstitial nephritis (9). 	<ul style="list-style-type: none"> 1. Kidney degeneration 	<ul style="list-style-type: none"> 1. Histology-kidney
None Reported.	<ul style="list-style-type: none"> o None Reported. 		
None Reported.	<ul style="list-style-type: none"> o None Reported. 		
None Reported.	<ul style="list-style-type: none"> o <u>Skin</u> desquamation (9). o <u>Osseous System</u> (human) Osteomyelitis of mandible questionable (11). 		
None Reported.	<ul style="list-style-type: none"> o None Reported. 	None	None

RED PHOSPHORUS TOXICITY (CONT'D)

System or Effect	Acute Treatment (including <u>in</u> <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
10. Molecular Effects	o None Reported.	o None Reported.	o None Reported.
11. Embryonic and Teratogenic Effects	o None Reported.	o None Reported.	o None Reported.
12. Metabolism	o Red phosphorus vapors are rapidly absorbed through the respiratory and digestive tracts. No other information was given. (10)	o None Reported.	o None Reported.
13. Carcinogenicity	o None Reported.	o None Reported.	o None Reported.

1

RED PHOSPHORUS TOXICITY (CONT'D)

Acute Treatment short term tests; (0 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Reported.	o None Reported.	None	None
Reported.	o None Reported.	None	None
Reported.	o None Reported.	None	None
Reported.	o None Reported.	None	None

2

WHITE PHOSPHORUS TOXICITY - MATRIX

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
1. Hematologic Effects	<ul style="list-style-type: none"> o Leucopenia and neutropenia (human) (2, 12,13). o Leucocytosis (human) (2,14). o Increased monocytes (14). o Erythrocytosis (human) (2). o Decreased fibrinogen (uncoagulable blood) (15). o Hemolysis (human) (16). 	<ul style="list-style-type: none"> o Increased monocytes (65, 66). o Decreased erythrocytes (dog) (12, 66). Tests in rabbit inconclusive (67, 68). o Decreased fragility of erythrocytes (68). o Decreased hemoglobin (dogs) (12, 66). Tests in rabbits inconclusive (67, 68). o Decreased fibrinogen (uncoagulable blood) (15,69). o Agglutination (rabbit) (67). 	<ul style="list-style-type: none"> o Leucopenia and anemia (human) (11). o <u>Leucocyte count.</u> (human), insignificant difference between exposed (1-17 years) and nonexposed workers, but polynuclear to lymphocyte ratio was affected (90). o Absence of methemoglobin (11).

WHITE PHOSPHORUS TOXICITY - MATRIX

Acute Treatment (short term tests; days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Increased monocytes (66).	<ul style="list-style-type: none"> o Leucopenia and anemia (human) (11). o <u>Leucocyte count.</u> (human), insignificant difference between exposed (1-17 years) and nonexposed workers, but polynuclear to lymphocyte ratio was affected (90). 	1. Leucopenia	<ul style="list-style-type: none"> 1. <u>In vitro</u> cytotoxicity tests 2. Complete RBC, differential, hematocrit
Increased eosinophils (dog) (66). Increased in rabbit (68). Increased fragility of erythrocytes	<ul style="list-style-type: none"> o Absence of methemoglobin (11). 		
Increased hemoglobin (12, 66). Increased in rabbits (67),			
Increased fibrinogen (clotting time) (69).			
Termination (67).			

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in</u> <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
2. Bone Marrow Changes	o None Reported.	o Changes affected <u>directly</u> by action on bone marrow elements causing hyperemia and degeneration (70) and <u>indirectly</u> by encroachment upon medullary canal containing the hematopoietic system (11) or through septic absorption from jaw bone necrosis (11).	o Same as subchronic.
3. Immunologic Effects	o None Reported.	o Decreased antibody production (67).	o None Reported.
4. Central Nervous System (CNS) Effects	o The Standard Phosphorus Burn (SWPB) White produced shivering, twitching, loss of appetite, poor response to stimulation and depression (rabbit) (17).	o None Reported using SWPB.	o None Observed (11).

1

PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Subchronic Treatment (short term tests; 30 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<p>organs affected directly by action bone marrow elements causing anemia and degene- ration (70) and directly by encroachment upon salivary canal maintaining the hematopoietic system or through gastric absorption in jaw bone necrosis (11).</p>	<p>o Same as subchronic.</p>	<p>1. Jawbone necrosis</p>	<p>1. Histological study</p>
<p>decreased antibody production (67).</p>	<p>o None Reported.</p>		
<p>Reported using</p>	<p>o None Observed (11).</p>	<p>1. Neuronal damage 2. Glioma damage</p>	<p>1. Neuroblastoma cytotoxicity test 2. Glioma cytotoxicity test</p>

2

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
4. Central Nervous System (CNS) Effects (Cont'd)	<ul style="list-style-type: none"> o Damage to midbrain and hindbrain ganglia and glia cells, specifically inferior olives; extensive subarchnoid and subcortical hemorrhage, facial paralysis and hyperemia (rabbit, dog, human) (18-20). o Parasthesis, delerium, acute excitement, psycosis, coma (human) (19,20). 	<ul style="list-style-type: none"> o Same as acute studies (rabbit, dog) (17,19,71,72). None reported in humans. 	<ul style="list-style-type: none"> o None Observed (11).
5. Behavioral Effects	<ul style="list-style-type: none"> o See CNS effects 	<ul style="list-style-type: none"> o Intensified excitation and increased cortical excitability (rats) (73). 	<ul style="list-style-type: none"> o None Reported.

PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Acute Treatment Term tests; days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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as acute studies o None Observed (11).
it, dog)
9,71,72).
reported in
s.

nsified o None Reported. None None
tation and
eased cortical
tability (rats)

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up 2 years or lifetime)
6. Cardiovascular Effects	<ul style="list-style-type: none"> o Electrocardiograph measurements showed abnormal Q-Tintervals, S-T segment depression, T-wave changes, brachycardia, low voltage QRS complex, ventricular arrhythmia (rabbit, human) (2,14,17,20-23). o Arterial hypotension (human) (14). 	o None Reported.	o None Reported.
7. Biochemical and Histochemical Effects	<ul style="list-style-type: none"> o <u>Blood</u> (experimental animals) <u>Elevated</u> serum phosphorus (17,24-26), plasma urea nitrogen (24-26), plasma sodium and potassium (24-26), non-protein nitrogen (28) and ethereal sulfates (28). 	<ul style="list-style-type: none"> o <u>Blood</u> (experimental animals) <u>Elevated</u> a-globin (74,75), nonprotein nitrogen, amino acid and uric acid (67), guanidine (80). 	<ul style="list-style-type: none"> o <u>Blood</u> (experimental animals). None Reported.

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Chronic Treatment Short term tests; to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None Reported.

o None Reported.

None

None

Blood (experimental
animals)

o Blood (experimental
animals).
None Reported.

None

None

Elevated a-globin
(74,75), nonprotein
nitrogen, amino acid
and uric acid (67),
guanidine (80).

2

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (Cont'd)	<p>Serum SGPT (24-26). Lipase (15,29). <u>Depressed</u> serum calcium (24-26), serum creatinine (24-26), polypeptide nitrogen (28), and total residual nitrogen (28).</p> <p><u>No change</u> glucose (27), fatty acids (28), cholesterol (28), lecithin (28).</p> <p>o <u>Blood</u> (human) blood chemistry picture similar to that seen in experimental animals (14,34). <u>Elevated</u> protein nitrogen (13,30). <u>Depressed</u> erythrocyte cholinesterase (14). <u>No change</u> urea nitrogen (13,30). bile pigment metabolism (13).</p>	<p>Serum esterase (35,76). <u>Depressed</u> plasma albumin and fibrinogen (75), total proteins (67).</p> <p><u>No change</u> chylomicron counts (77-79).</p> <p>o <u>Blood</u> (human) None Reported.</p>	<p>o <u>Blood</u> (human). <u>Elevated</u> blood potassium and chloride (11). <u>No change</u> inorganic phosphorus, alkaline phosphorus, calcium and magnesium (90).</p>

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Chronic Treatment Short term tests; to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Serum esterase
(35,76).

Depressed plasma
albumin and
fibrinogen (75),
total proteins (67).

No change chylomicron
counts (77-79).

Blood (human) None
Reported. o Blood (human).

Elevated blood
potassium and
chloride (11).

No change inorganic
phosphorus, alkaline
phosphorus, calcium
and magnesium (90).

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Pr E
7. Biochemical and Histochemical Effects (Cont'd)	<ul style="list-style-type: none"> o <u>Liver</u> <u>Elevated</u> triglycerides (31-34), total lipids (31,32), phospholipids (31), total nitrogen (28), fatty acids (28,39), cholesterol (28), poly- peptide nitrogen (28), residual nitrogen (28), lecithin (28), glucose-6- phosphatase activity (37). 	<ul style="list-style-type: none"> o <u>Liver</u> <u>Elevated</u> triglycerides (81), total lipids and cholesterol (insignificant) (81), glycogen(40). Cytochrome-C (68). 	<ul style="list-style-type: none"> o <u>Liver</u> None reported. 	1.

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Chronic Treatment (short term tests; 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<p><u>Liver</u> <u>elevated</u> triglycerides (81), total lipids and cholesterol insignificant) (81), glycogen(40).</p>	<p>o <u>Liver</u> None reported.</p>	<p>1. Liver cirrhosis</p>	<p>1. Histologic tests 2. Biochemical tests</p>

Urobilinogen-C (68).

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (Cont'd)	<p><u>Depressed</u> glucuronic acid (38), glycogen levels (40-47).</p> <p>Esterase activity (15,35,36).</p> <p>Mitochondrial ATP⁴⁹, <u>No change</u> cytochrome b-5, P-450 (48).</p>	<p><u>Depressed</u> phospholipids (81). hypoglycemia (41,45), octanoate oxidation (83).</p> <p>Esterase activity (35,76), malic dehydrogenase (MDH) (insignificant) (81). Fructose, 1, 6-diphosphate aldolase (F16D) activities (81). Mitochondrial ATP (48,82). <u>No change</u> lactic dehydrogenase and glucose 6-phosphate dehydrogenase(81).</p>	
8. Body Weight, Organs and Tissues	<p><u>Body Weight</u> None Reported. <u>Skin</u> non-irritant (7), produced subcutaneous hemorrhage (50).</p>	<p><u>Body Weight</u> Decreased (84). <u>Skin</u> None Reported.</p>	<p><u>Body Weight</u> Decreased (84,91). <u>Skin</u> None Reported.</p>

TE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Acute Treatment term tests; 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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pressed
 spholipids (81).
 oglycemia (41,45),
 anoate oxidation
).
 erase activity
 ,76), malic
 ydrogenase (MDH)
 significant) (81).
 ctose, l,
 iphosphate
 olase (F16D)
 ivities (81).
 ochondrial ATP
 ,82).
 change lactic
 ydrogenase and
 cose 6-phosphate
 ydrogenase(81).

<u>Body Weight</u> reased (84). n None Reported.	<u>Body Weight</u> Decreased (84,91). <u>Skin</u> None Reported.	None	None
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2

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
8. Body Weight, Organs, and Tissues (Cont'd)	<ul style="list-style-type: none"> o <u>Liver</u> increased weight (31), extensive degeneration of hepatic cells with micro-thrombi in portal vein (18,24-26), fatty infiltration, necrosis and hemorrhage (12,18,40). Eosinophilic necrosis of hepatic cells, cellular infiltration (granulocytes, lymphocytes) into portal spaces, proliferation of bile ducts and fibrosis (humans) (51). 	<ul style="list-style-type: none"> o <u>Liver</u> decreased weight (81), fatty degeneration, hemorrhage and cellular necrosis (74), lack of chromoffin reaction (40). 	<ul style="list-style-type: none"> o <u>Liver</u> cirrhosis, destruction of fibroblast and parenchymal cells, fibrosis (periportal) (92, 93).
	<ul style="list-style-type: none"> o <u>Kidney</u> glomerular and tubular necrosis, desquamation and perinuclear vacuolization of cells (24-26). Renal cortical degeneration (humans) (51). 	<ul style="list-style-type: none"> o <u>Kidney</u> Hydropic degeneration (12), tubular degeneration (85). 	<ul style="list-style-type: none"> o <u>Kidney</u>. None Reported.

SPHORUS TOXICITY - MATRIX (CONT'D)

Treatment tests; s)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Increased (1), fatty infiltration, necrosis and a reaction	o <u>Liver</u> cirrhosis, destruction of fibroblast and parenchymal cells, fibrosis (periportal) (92, 93).		(See No. 7 above)

Tropic (12), regeneration	o <u>Kidney</u> . None Reported.		
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WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
8. Body Weight, Organs, and Tissues (Cont'd)	<ul style="list-style-type: none"> o <u>Spleen</u> None Reported. o <u>Gastrointestinal Tract</u> massive hemorrhage (20). o <u>Endocrine Organs</u> None Reported. o <u>Lung</u> hemorrhagic broncho-pneumonia (20). o <u>Pancreas</u> None Reported. o <u>Heart</u> pathologic changes in onyocardial cells and interstitial edema (21), fatty infiltration (51). o <u>Eye</u> non-irritant (rabbit) (7) but in humans it produced burning sensation, blepharospasm and ophthalmophegia (52-54). o <u>Ear</u> None Reported. 	<ul style="list-style-type: none"> o <u>Spleen</u> enlarged with large amounts of hemosiderin (12). o <u>Gastrointestinal Tract</u> None Reported. o <u>Endocrine Organs</u> adrenal insufficiency (40). o <u>Lung</u> None Reported. o <u>Pancreas</u> mitochondrial deformities (86). o <u>Heart</u> None Reported. o <u>Eye</u> None Reported o <u>Ear</u> necrosis of the ear (no other detail given) (71). 	<ul style="list-style-type: none"> o <u>Spleen</u> None Reported. o <u>Gastrointestinal Tract</u> None Reported. o <u>Endocrine Organs</u> None Reported. o <u>Lung</u> None Reported. o <u>Pancreas</u> None Reported. o <u>Heart</u> None Reported. o <u>Eye</u> None Reported. o <u>Ear</u> None Reported.

PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Treatment Term tests; Days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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enlarged with amounts of Merlin (12).	o <u>Spleen</u> None Reported.		
Intestinal None d.	o <u>Gastrointestinal Tract</u> None Reported.		
Line Organs	o <u>Endocrine Organs</u> None Reported.		
iciency (40). None Reported.	o <u>Lung</u> None Reported.		
as ndrial ities (86).	o <u>Pancreas</u> None Reported.		
None Reported.	o <u>Heart</u> None Reported.		
ne Reported	o <u>Eye</u> None Reported.		
rosis of the other detail (71).	o <u>Ear</u> None Reported.		

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
8. Body Weight, Organs, and Tissues (Cont'd)	o <u>Osseous Skeleton</u> bone necrosis of feet (55).	o <u>Osseous Skeleton</u> , jaw necrosis, periosteal changes and calcium resorption from bone leading to bone atrophy (84,87,88).	o <u>Osseous Skeleton</u> jaw necrosis, periosteal changes and calcium resorption from bone leading to bone atrophy (4,11,90, 94-96).
9. Cytologic and Cytogenetic Effects	o <u>Nuclear aberrations</u> swelling of nuclei, vacuolation, membrane destruction, hypochromasia, pyknosis, karyorrhexis, delayed mitosis (38,51).	o <u>Mitotic arrest</u> (Kuffer cells) (89).	o None Reported.
10. Molecular Effects	o Inhibition of protein syntheses in rat myocardium (21), liver (33,56), duodenum and pancreas (56).	o None Reported.	o None Reported.
11. Embryonic and Teratogenic Effects	o No effect on fetus (human) (51).		

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Chronic Treatment (short term tests; 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<p><u>Osseous Skeleton,</u> bone necrosis, periosteal changes and calcium resorption from bone leading to bone atrophy (84,87,88).</p>	<p>o <u>Osseous Skeleton</u> jaw necrosis, periosteal changes and calcium resorption from bone leading to bone atrophy (4,11,90, 94-96).</p>	<p>1. Bone necrosis and atrophy</p>	<p>1. Bone fracture strength test 2. Calcium balance</p>
<p><u>Myeloid arrest</u> (leukocytes) (89).</p>	<p>o None Reported.</p>	<p>None</p>	<p>None</p>
<p>None Reported.</p>	<p>o None Reported.</p>	<p>None</p>	<p>None</p>
		<p>None</p>	<p>None</p>

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Pred: Endp
12. Metabolism	<ul style="list-style-type: none"> <li data-bbox="456 583 753 1314">o Phosphorus is rapidly absorbed from the gut and is principally incorporated into the liver where it reaches a maximum (60-73% of dose) within 2-3 hours. The order of concentration in other organs is as follows: blood (12%), kidney (4.2%), spleen (1.8%), bone (1.7%) and brain (0.3%). Concentration in skin greatest in growing areas compared to quiescent areas (7,28,37,58-65). <li data-bbox="456 1318 753 1520">o Phosphorus is rapidly oxidized to hypophosphorus and phosphoric acid in tissues and organs (61, 62). 	<ul style="list-style-type: none"> <li data-bbox="781 583 1068 638">o Same as in acute studies. <li data-bbox="781 1318 1068 1373">o Same as in acute studies. 	<ul style="list-style-type: none"> <li data-bbox="1159 583 1438 638">o Same as in acute studies. <li data-bbox="1159 1318 1438 1373">o Same as in acute studies. 	

TE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Acute Treatment (short term tests; 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Same as in acute studies.

o Same as in acute studies.

None

None

Same as in acute studies.

o Same as in acute studies.

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short term tests; up to 90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)
12. Metabolism (Cont'd)	<ul style="list-style-type: none"> o Major route of excretion is the urine (54% of dose in 4 hrs) with lesser amounts in feces (2.0% of dose in 4 hrs) (7,28). Urine alkalinity is increased as is the ratio of urea to total nitrogen and lactic acid. The ratio of non urea and ammonia nitrogen to total nitrogen excreted was 30% in intoxicated animals (28), albuminaria occurs under severe intoxication (63,64). 	<ul style="list-style-type: none"> o Major route of excretion is the urine. The urinary nitrogen partition between the urea, ammonia and amino acids was not always disturbed. Most important change was increased in amino nitrogen. Sugar tolerance test was markedly decreased. Terminal acidosis was also observed (dogs) (69). Decrease in urinary creatine and changes in creatine to creatinine ratios in the urine was also seen (dogs) (12). 	<ul style="list-style-type: none"> o In humans white phosphorus is excreted essentially as organic and inorganic phosphates; insignificant amounts are exhaled in the breath, sweat and feces; urinary changes include elevation of ammonia nitrogen at the expense of urea nitrogen, increased oxidized sulfur content and albuminaria. Other compounds which may appear in the urine are acetone, diacetic acid, leucine and tyrosine (11,12, 97,98).
13. Carcinogenicity	None Reported.	None Reported.	<ul style="list-style-type: none"> o Phosphorus did not produce tumors in rats and guinea pigs (91,99). There are no reports on carcinogenicity of phosphorus in humans.

WHITE PHOSPHORUS TOXICITY - MATRIX (CONT'D)

Chronic Treatment Short term tests; (90 days)	Chronic Treatment (long term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Major route of excretion is the urine. The urinary nitrogen partition between the urea, ammonia and amino acids was not always disturbed. Most important change was increased in amino nitrogen. Sugar tolerance test was markedly decreased. Terminal acidosis was also observed (dogs) (69). Increase in urinary creatinine and changes in creatinine to urea ratios in the urine was also observed (dogs) (12).

o In humans white phosphorus is excreted essentially as organic and inorganic phosphates; insignificant amounts are exhaled in the breath, sweat and feces; urinary changes include elevation of ammonia nitrogen at the expense of urea nitrogen, increased oxidized sulfur content and albuminuria. Other compounds which may appear in the urine are acetone, diacetic acid, leucine and tyrosine (11,12, 97,98).

None Reported.

o Phosphorus did not produce tumors in rats and guinea pigs (91,99). There are no reports on carcinogenicity of phosphorus in humans.

None

None

Tracor Jitco

EXPERIMENTAL FORMALDEHYDE INTOXICATION

Backup Report to Formaldehyde Toxicity Matrix

78 07 11 052

Experimental Formaldehyde Intoxication

INTRODUCTION

ACUTE TOXICITY (Including in vitro studies)

1. Hematologic Effects

Oxygen combining capacity
Selective permeability RBC

2. Bone Marrow Changes

3. Immunologic Response

4. Central Nervous System (CNS) Effects

Optical chronaxie changes
Cerebral electrical activity
Central nervous system damage
Nasopalatine nerve response
Depression olfactory structures
Stress rhythm brain
Cortical medial nucleus
Neuroblastoma cells in vitro

5. Behavioral Effects

6. Cardiovascular Effects

Hypotension
Circulatory collapse
Respiration inhibition

7. Biochemical and Histochemical Changes

Enzymes

Alkaline phosphatase
Glutamic dehydrogenase
Lactic dehydrogenase
Nicotinamide-Adenine coenzymes

Proteins

Heme
Hyperglycemia
Acidosis

78 07 11 055

8. Effects on Tissues and Organs

Skin

Irritation
Anesthesia

Eyes

Irritation
Intraocular hypertension
Protein leakage
Corneal injury

Nose

Irritation

Larynx

Irritation
Edema
Spasm

Trachea

Irritation
Cilia inhibition

Bronchi

Bronchitis

Lungs

Edema
Hemorrhage
Alveolar damage
Other lesions
Airflow resistance
Respiratory rate
Tidal volume

Stomach

Inflammation
Ulceration
Hematemesis
Strictures

Intestines

Peristalsis

Liver

Hyperemia
Edema
Necrosis

Kidneys

Hyperemia
Edema
Other damage

Uterus

Edema
Hemorrhage
Necrosis
Polynuclear infiltration

Bladder

Edema
Inflammation
Necrosis
Adhesions
Ulceration
Hemorrhage
Leucocytic infiltration

9. Cytologic and Cytogenetic Effects

Protein coagulation
Caryorhexis
Pyknosis
Mutagenicity
Escherichia coli
Drosophila melanogaster

10. Molecular Effects

DNA cross-linking

11. Reproductive and Teratogenic Effects

Testis

Inhibition spermatogenesis
Tubular degeneration
Calcification tubules
Atrophy
Spermatocidal effect

12. Metabolism

Absorption

Biotransformation

Formic acid (in vivo and in vitro)

Methanol (in vivo and in vitro)

Excretion

13. Carcinogenesis

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

1. Hematologic Effects
2. Bone Marrow Changes
3. Immunologic Response
 - Sensitization
 - Allergic dermatitis
4. Central Nervous System (CNS) Effects
 - Optic cortex
 - Olfactory analyzer
 - Olfactory bulbs
 - Visual center
5. Behavioral Effects
6. Cardiovascular Effects
7. Biochemical and Histochemical Changes
8. Effects on Tissues and Organs
 - Eye
 - Lachrymation
 - Discharge
9. Cytologic and Cytogenetic Effects
10. Molecular Effects
11. Reproductive and Teratogenetic Effects
 - Gestation time
 - Embryo organ weights
 - Adrenals
 - Kidneys
 - Thymus
 - Lung
 - Liver
12. Metabolism
13. Carcinogenesis

CHRONIC TOXICITY (Long-term tests, up to 2 years or lifetime)

1. Hematologic Effects
2. Bone Marrow
3. Immunologic Response
 - Sensitization
 - Allergic dermatitis
4. Central Nervous System (CNS) Effects
 - Cerebral cortex
5. Behavioral Changes
6. Cardiovascular Effects
 - Inflammation of heart
7. Biochemical and Histochemical Changes
 - Liver glycogen
8. Effects on Tissues and Organs
 - Skin
 - Fingernails
 - Inflammation
 - Suppuration
 - Lungs
 - Inflammation
 - Proliferation lymphohistiocytic elements
 - Hyperemia
 - Stomach
 - Irritation
 - Liver
 - Cellular changes
 - Focal hyperplasia
 - Kidneys
 - Focal inflammatory changes
 - Reticuloendothelial system
 - Activation of liver elements

9. Cytologic and Cytogenetic Effects
10. Molecular Effects
 - RNA granules
 - Enlargement
 - Rarefaction
11. Reproductive and Teratogenic Effects
12. Metabolism
13. Carcinogenesis
 - Injection site sarcomas
 - Spindle cell sarcomas

EXPERIMENTAL FORMALDEHYDE INTOXICATION

INTRODUCTION

Toxic effects of formaldehyde ingested accidentally or suicidally were reported as early as 1904. During the investigation (in 1909) of the possible value of formaldehyde as a preservative for milk, the U.S. Department of Agriculture found that formaldehyde is capable of inducing a systemic allergic sensitization and skin reaction. The severe effects of formaldehyde vapors on the respiratory tract were also reported early in the history of the compound. Human exposure results from its use as a fumigant, disinfectant, germicide, and fungicide, as well as its wide application in industry. Formaldehyde is used in the manufacture of phenolic resins, artificial silk, dyes, explosives and organic chemicals. It is also used for coagulating rubber latex, tanning and preserving hides, waterproofing fabrics, hardening gelatin plates, and for other purposes in photography.

The present federal standard for formaldehyde in the workplace specifies an 8-hour time weighted average (TWA) limit of 3 ppm formaldehyde. The acceptable ceiling concentration is 5 ppm formaldehyde, with an acceptable maximum peak above the acceptable ceiling concentration of 10 ppm for a total of no more than 30 minutes during an 8-hour shift (1).

Formaldehyde vapors are strongly irritating to mucous membranes of the eyes and respiratory tract. Skin contact may result in an irritant or allergic dermatitis. Ingestion may cause severe gastrointestinal pain, hematemesis, hematuria, proteinuria, anuria, as well as acidosis, vertigo, coma, and death (2).

Formaldehyde intoxication can be induced in common laboratory animals (rats, mice, rabbits, guinea pigs, dogs, cats, and monkeys) via inhalation, oral administration, parenteral injection or by direct contact with the skin. Acute toxic doses of formaldehyde for various experimental animals are given in the following table:

Rat	Oral	LD ₅₀	800 mg/kg
Guinea pig	Oral	LD ₅₀	260 mg/kg
Rat	Inhalation	*LCLo	250 ppm/4 hr
Mouse	Inhalation	LCLo	900 mg/m ³ /2 hr
Cat	Inhalation	LCLo	820 mg/m ³ /8 hr
Rabbit	Skin	LD ₅₀	270 mg/kg

*LCLo - lowest published lethal dose (3).

Specific effects of formaldehyde on the various organs and body systems in experimental animals will be discussed in the following sections on: I. Acute Toxicity, II. Subchronic Toxicity, and III. Chronic Toxicity studies. Acute toxicity in this report is limited to toxic effects following administration of a single dose. Subchronic toxicity refers to toxicity observed in short-term tests of less than 90 days. Chronic toxicity includes toxic effects occurring in long-term tests, two years or lifetime treatment.

Information Sources

The information contained in the body of this document was derived mainly from such critical current reviews as:

- o Occupational Exposure to Formaldehyde, DHEW (NIOSH) Publ. No. 77-126, National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1976.
- o Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health, U.S. Government Printing Office, 1976.
- o Industrial Hygiene and Toxicology, Vol. II, Patty, F.A. (ed.), Interscience Publishers, New York, N.Y., 1963.
- o Clinical Toxicology of Commercial Products, 2nd edition, Gleason, M.N., R.E. Gosselin, and H.C. Hodge, Williams and Wilkins Co., Baltimore, Maryland, 1963.
- o The Merck Index, 9th edition, Windholz, M. (ed.), Merck and Co., Inc., Rahway, N.J. 1976.

In addition, some pertinent research reports identified through a Tracor Jitco literature search were included.

The information contained in the backup document is summarized in the appended matrix. The term "none reported," which appears in the matrix, indicates that no literature reference related to that particular item was identified.

ACUTE TOXICITY (Including in vitro observations)

1. Hematologic Effects

Red blood cells subjected to 0.2% formaldehyde in solution lost the capability of taking up oxygen but remained permeable to ammonium chloride and impermeable to sodium chloride. This normal selective permeability was lost, however, when the cells were treated with a 4% solution of the aldehyde. The authors postulated that the effects resulted from crosslinking of protein chains and opening pores in the envelope of the erythrocytes (1,4).

2. Bone Marrow Changes

No data reported in literature surveyed to date.

3. Immunologic Response

A direct sensitization to formaldehyde in the vapor phase appears to be relatively rare (5).

4. Central Nervous System (CNS) Effects

Melekhina (6) determined optical chronaxie* effects and odor threshold in human subjects exposed to formaldehyde gas. Concentrations as low as 0.084 mg/m^3 (0.07 ppm) inhaled for a period of 9 minutes decreased the chronaxie in two individuals and increased it in a third. Changes were more severe at concentrations of 0.2 and 1.59 mg/m^3 (0.16 and 1.29 ppm) but still showed individual variations, i.e., two subjects showed decreases and a third had an increase in the chronaxie. Concentrations of 0.068 to 0.075 mg/m^3 had no effect on rheobase or

*Chronaxie or chronaxy - the minimum time an electric current must flow at a voltage twice the rheobase (minimum potential of electric current necessary to produce stimulation) to cause a muscle to contract.

chronaxie. At 0.084 mg/m^3 , the electrical chronaxie was decreased from $0.06\text{--}0.23 \text{ uF}$ in two subjects. Two individuals had decreases of 0.10 and 0.22 uF at 0.2 mg/m^3 , and of 0.08 and 0.23 uF at 1.59 mg/m^3 , whereas a third had increases of 0.09 and 0.39 uF at these two formaldehyde concentrations.

Fel'dman and Bonashevskaya (7) found the olfactory threshold for formaldehyde in human subjects (7 of 15) to be 0.073 mg/m^3 . All 15 individuals were able to detect odor of the gas at a concentration of 0.09 mg/m^3 . None of the subjects were able to detect 0.054 mg/m^3 of formaldehyde by odor. These results agree closely with those obtained in a study by Melekhina (6) where the odor perception threshold for formaldehyde gas was found to be 0.07 mg/m^3 for all subjects tested.

Statistically significant changes in cerebral electrical activity ($p \pm 0.05$) were detected by EEG determinations in five human subjects exposed to formaldehyde gas at a concentration of 0.053 mg/m^3 . The 'no effect' level was found to be 0.04 mg/m^3 . The individuals tested in the study were the five most sensitive subjects found in the olfactory threshold tests conducted by the authors in the study described above (7).

Gleason et al. (8) stated that central nervous system depression may occur as a result of exposure to formaldehyde but that the condition is rare. The ingestion of formalin, however, has caused central nervous system damage as well as circulatory collapse and kidney damage. The mean lethal dose for adults has been estimated to be about 60 ml (9).

Kulle and Cooper (10) studied the effects of formaldehyde on the electrical activity of the nasopalatine nerve in anesthetized rats. Exposure to the gas at levels of $0.5\text{--}2.0 \text{ ppm}$ for one hour caused a decrease in the nerve response to amyl alcohol that varied directly with the concentration of formaldehyde diffused. A partial recovery of the neural response to amyl alcohol was observed when the nasal cavities were perfused with air for one hour following the 2 ppm , one-hour formaldehyde exposure.

Effects of formaldehyde on various parts of the cerebral cortex of rabbits were determined by Bokina et al. (11) by means of functional electroencephalography (analysis of the readjustment reaction to a rhythmic light stimulus, evoked potentials of the cerebral cortex, and determination of the photometrazol thresholds). Exposure to formaldehyde for 10 seconds produced a nonspecific reaction of orientation and exploration at 3.7 mg/m^3 consisting of individual flashes of activity in the neocortex and quickened respiration without any change in the olfactory analyzer structures. Exposure to a concentration of 12.6 mg/m^3 caused a specific reaction represented by a depression of high frequency-induced activity in the olfactory structures. Changes in the electroencephalograms (EEG) after a 10-sec exposure were short with a quick return to initial brain functional state (1). After 20-minute exposures, there was a persistent stress rhythm in the cortex and limbic brain structures indicating a deleterious effect of the compound on the CNS. In an earlier study, Bokina and Eksler (12) found that formaldehyde gas at a concentration of 0.65 mg/m^3 had an adverse effect on the cortical medial nucleus in rabbits after exposure for 10-30 minutes.

Koerker et al. (13) followed the action of formaldehyde on cell growth, viability, adherence to culture flask, and morphology of a clone of mouse C1300 neuroblastoma cells in vitro. Molar concentrations of the aldehyde, ranging from 8.3×10^{-6} to 2.2×10^{-4} acting over a period of 24 hours, caused decreases in total cell number, viability of harvested and sloughing cells, and percentage of cells having neurites. Sloughing of cells from the flask surface into the medium was increased. The authors concluded that the neuroblastoma cell culture provides a convenient in vitro system for cytotoxicity tests as well as a model in which mechanisms of neurotoxicity can be studied, since the cell type is derived from nerve tissue.

5. Behavioral Effects

No data reported in literature surveyed to date.

6. Cardiovascular Effects

Gleason et al. (8) stated that circulatory collapse may occur on exposure to formaldehyde and may contribute to the rare instances of central nervous system depression reported. Akabane (14), in a review article on aldehydes and related compounds, also pointed out that formaldehyde causes a decrease in blood pressure as well as an irregularity in respiration at sufficient dosages. Depressor responses were induced in chloralose-anesthetized cats and dogs by iv injection of 2.5 to 5 mg/kg. Respiration changes were not observed at these dosages. With 10 mg/kg of formaldehyde, however, an initial drop in blood pressure, followed by a pressor response as well as marked inhibition of respiration, were observed. Blood pressure and respiratory responses of the same nature were observed in pentobarbital-anesthetized dogs. The action of formaldehyde on peripheral blood vessels, however, was found to be very weak as determined by perfusion experiments with the isolated rabbit ear.

Skog (15) detected slight hyperemia with small hemorrhages and edema around some vessels in the lungs in rats and mice that succumbed to sc injections of formaldehyde solution at levels of 300-640 mg/kg and 150-460 mg/kg, respectively.

7. Biochemical and Histochemical Changes

Alkaline Phosphatase

Liver alkaline phosphatase activity was found to be significantly higher in rats exposed to 35 ppm formaldehyde via the respiratory tract than in unexposed animals (16). The authors indicated that the increase may have been a non-specific effect, since several dissimilar chemicals induced the same response. An increase in activity of the enzyme was also observed in the testes of goats following a single intratesticular injection of formaldehyde (10 ml of a 4% soln.). The effect was particularly prominent in calcified areas of the tubules and interstitium (17).

Dehydrogenases

Estes and Pan (18) found formaldehyde to be a potent inhibitor of beef liver glutamic dehydrogenase in vitro at concentrations of 5 uM and 50 uM per milligram of enzyme protein. Lactic dehydrogenase (beef heart) was also inhibited but to a lesser extent.

Nicotinamide-Adenine Coenzymes

Formaldehyde, at a final concentration of 0.1%, lowered the oxidized and reduced nicotinamide-adenine coenzyme content (NAD^+ +NADH) of ram spermatozoa in vitro to 76% of untreated control values after incubation for 20 minutes at 25° C. Inhibition of spermatozoan motility was also noted (19).

Reaction with Proteins

Formaldehyde combines with methyl groups of proteins both in vivo and in vitro to form additional products (8).

Reaction with Heme

Akabane (14) cited a report by Guthe (1959) that formaldehyde establishes an irreversible heme-linked group with hemoglobin.

Hyperglycemia

Injection of starved rabbit with 10 mg/kg formaldehyde solution may result in hyperglycemia (14).

Acidosis

Windholz (2) stated that acidosis may result from the ingestion of aqueous formaldehyde.

8. Effects on Tissues and Organs

Skin

Explosure to vapor or solutions of formaldehyde may cause the skin to become white, rough, hard, and anesthetic. The mechanism, according to Gleason et al. (8), is a superficial coagulation necrosis. Dermatitis and hypersensitivity frequently develop after prolonged exposure.

Eyes

Formaldehyde vapors are intensely irritating to the mucous membranes of the eyes. Rats exposed to concentrations as low as 35 ppm for 18 hours showed definite evidence of acute eye irritation within 24 hours (16).

Humans unacclimated to formaldehyde vapors experience eye irritation at concentrations below 5-6 ppm. A vapor concentration of 10 ppm causes profuse lachrymation and can be tolerated for only a few minutes even by workers acclimated to lower levels. Experimental 30-minute exposures of human volunteers to 13.8 ppm formaldehyde caused considerable eye irritation although there was a decrease in lachrymation during exposure. Eye splashes are very serious and can result in coagulation of the corneal surface if prompt action is not taken (5,9,20).

Formaldehyde levels which averaged 0.4 ppm had no acute effects on workers' performance in tests of visual acuity, depth perception, peripheral vision, accomodation, fixation, and color vision in a study conducted in a large wood products plant (21).

Formaldehyde induces intraocular hypertension and protein leakage across the blood-aqueous barrier in the rabbit (22).

Nose

Inhaled formaldehyde vapors are very irritating to the nose as well as to the eyes. In the study described above by Murphy et al. (16), irritation of the nasal mucosa was observed in rats exposed to 35 ppm for 18 hours.

Human beings exposed to formaldehyde vapors usually experience a mild tingling sensation in the nose when the concentration reaches 2-3 ppm. Concentrations of 4-5 ppm can be tolerated fairly well for periods of 10-30 minutes by some people; longer exposures cause considerable discomfort. Concentrations of 10 ppm can be borne for only a few minutes. At levels of 10-20 ppm, formaldehyde causes severe burning of the nose and it becomes difficult to take a normal breath voluntarily. It is estimated that exposure for 5 to 10 minutes to concentrations of 50-100 ppm would probably lead to very serious injury (5).

Larynx

Mild throat irritation is experienced by most persons at formaldehyde vapor levels as low as 5 ppm. At 10-20 ppm, formaldehyde causes severe burning of the throat. High concentrations may produce edema or spasm of the larynx. It has been found that repeated exposures to formaldehyde concentrations below 10 ppm can lead to some degree of tolerance to the vapors (5,8).

No reports were found in the literature surveyed to date on effects of formaldehyde on the larynx in experimental animals.

Trachea

Formaldehyde concentrations of 10-20 ppm cause severe burning of the entire upper respiratory tract including the trachea (5).

Kensler and Battista (23) found ciliary activity of rabbit trachea exposed for 12 seconds in vitro to formaldehyde vapor, was inhibited almost 100% as compared with 10-15% inhibition in untreated control preparations.

Bronchi

Evidence of bronchitis was observed at autopsy in both rats and mice given single sc injections of formaldehyde solutions of 300-640 mg/kg and 150-460 mg/kg, respectively, in an acute toxicity study conducted by Skog (15). The LD₅₀ was found to be 420 mg/kg in rats and 300 mg/kg in mice.

Gleason et al. (8) stated that tracheobronchitis may develop in humans after inhalation of formaldehyde vapors.

Lungs

Slight pulmonary hyperemia with small hemorrhages and edema around some of the vessels was observed in rats and mice given single sc injections of formaldehyde solution at levels of 300-640 mg/kg for rats and 150-460 mg/kg for mice. Hemorrhages, as well as intra-alveolar and perivascular edema, were found in lungs of rats exposed to 0.6-1.7 mg/liter formaldehyde in air for 30 minutes (15). The LC₅₀ was found to be 1000 mg/m³ (810 ppm) (15).

In another study involving rabbits and guinea pigs as well as mice, exposure to formaldehyde (19 mg/m³) or to an aerosol of formalin (20 mg/m³) for up to 10 hours resulted in edematous and hemorrhagic lungs with distended alveoli. Most of the animals also had ruptured alveolar septa. Mortality rates were: mice - 96% (aerosol) and 34% (gas); guinea pigs - 5% (aerosol) and 40% (gas); rabbits - 20% (aerosol) and 60% (gas) (24). Lung lesions were also found in cats exposed to 820 mg/m³ (664 ppm) formaldehyde for 8-9 hours. Death occurred within 4-6 days following profuse salivation, marked dyspnea, and vomiting. At autopsy, pulmonary edema, hyperemia, and hemorrhages of the lungs with pus in the trachea and bronchi were found. Concentrations of up to 820 mg/m³ for 3.5 hours caused only temporary irritation of the mucous membranes (25).

Murphy and Ulrich (26) found a dose-related effect of formaldehyde on the respiratory rate, tidal volume, and resistance to airflow in guinea pigs exposed individually to the gas for a period of one hour. Concentrations of 3.9 and 12.5 ppm increased resistance to airflow by 69% and 81%, increased tidal volume by 29% and 36%, and decreased the respiratory rate by 27% and 37%, respectively.

In another study by Davis et al. (27), guinea pigs exposed to formaldehyde in air at levels of 50, 1000, and 6000 ppm showed increases in resistance, increases in tidal volume, and decreases in the respiration rate as well as a decrease in minute volume, and no change in compliance in intact animals. In tracheotomized animals, however, there were no changes in any of these areas due to formaldehyde exposure. The authors concluded, therefore, that the responses observed were nonspecific and due to receptors in the nasopharynx and larynx of the guinea pig which are stimulated by irritant and chemically inert substances in general.

Otte and Kropelin (28) detected peribronchial cell infiltration and interstitial edema in the bronchopulmonary system of guinea pigs following inhalation of formaldehyde.

Stomach

Ingestion of formalin or other solutions of formaldehyde may cause inflammation and ulceration of the gastrointestinal mucosa, severe abdominal pain, violent vomiting, hematemesis, and diarrhea. Corrosive damage and strictures commonly occur in the stomach. Hematuria, proteinuria, anuria, acidosis, vertigo, coma, and death may follow (2,8,29).

Intestines

Formaldehyde (5 to 10 mg/kg iv) caused an initial inhibition of intestinal movement in experimental animals, which was followed by an increase in tone and amplitude of contraction, according to a report by Akabane (14).

Liver

Exposure of rats to 0.6-1.7 mg/liter formaldehyde in air for 30 minutes caused hyperemia, perivascular edema, and necrosis in the liver. Hyperemia was also found in both rats and mice following single sc injections of formaldehyde solution at dosages of 300-640 mg/kg and 150-460 mg/kg, respectively (15).

Kidneys

In the study by Skog (15) described above, perivascular edema was found in the kidneys of rats exposed for 30 minutes to formaldehyde in air over a range of 0.6-1.7 mg/liter. Hyperemia was observed in the kidneys of both rats and mice following single sc injections of formaldehyde solution. The dosage for rats was 300-640 mg/kg and 150-460 mg/kg for mice. Iwanoff (25) observed hyperemia in the kidneys, as well as pulmonary changes in cats exposed to 820 mg/m^3 (664 ppm) formaldehyde in air for 8-9 hours. Gleason (8) reported that ingestion of formalin leads to kidney damage in humans also.

Uterus

Hemorrhage, edema, necrosis of endometrium, injury to myometrium, polynuclear infiltration, and finally, after 30 days, complete obliteration of the uterine cavity by connective tissue were found in mature virgin rabbits given a single intrauterine injection of 2 ml of 10% formaldehyde in saline (30).

Bladder

Formaldehyde causes severe adverse effects when injected into the bladder of experimental animals. In one study with rats, formalin concentrations of 1, 5, or 10% in amounts sufficient to fill the bladder (1-2 ml) caused edema, necrosis, and inflammation throughout all layers of the organ within 7 days. Macroscopic examination at autopsy revealed thickened walls and adhesions to other visceral structures. Microscopically, transmural inflammation, mucosal ulceration, and intense submucosal thickening and hemorrhage were observed in the 5% group. The authors stated that it was difficult to find an intact piece of bladder wall for sectioning in the animals given 10% formalin intravesically. Fifty percent of the animals in the 5% group died during the experiment (31).

Pust et al. (32) studied chemical stripping of the urinary bladder mucosa in cats with 5 to 25% formaldehyde solutions. The study was prompted by the possible value of a procedure that completely destroys the affected bladder mucosa in the management of low grade bladder tumors. Superficial coagulation necrosis with formaldehyde has already been used successfully to stop bleeding from inoperable cancer of the bladder and after radiation or chemotherapy. Clinical signs of systemic formaldehyde intoxication have not been seen in these emergency treatments (33).

In this study (32), complete stripping of the urinary bladder mucosa in the cat was achieved with a 20% solution of commercial aqueous formaldehyde (38% HCHO) and a contact time of one minute. Necrosis of the subepithelial layer did not occur and complete "reepithelialisation" and normal bladder function were seen 3-4 weeks after formaldehyde treatment. Clinical signs of systemic formaldehyde intoxication were not observed. Histopathologic effects detected 7 and 14 days after treatment included the following: 5% solution (5 min contact time), leucocytic infiltration and slight subepithelial edema; after 20 min,

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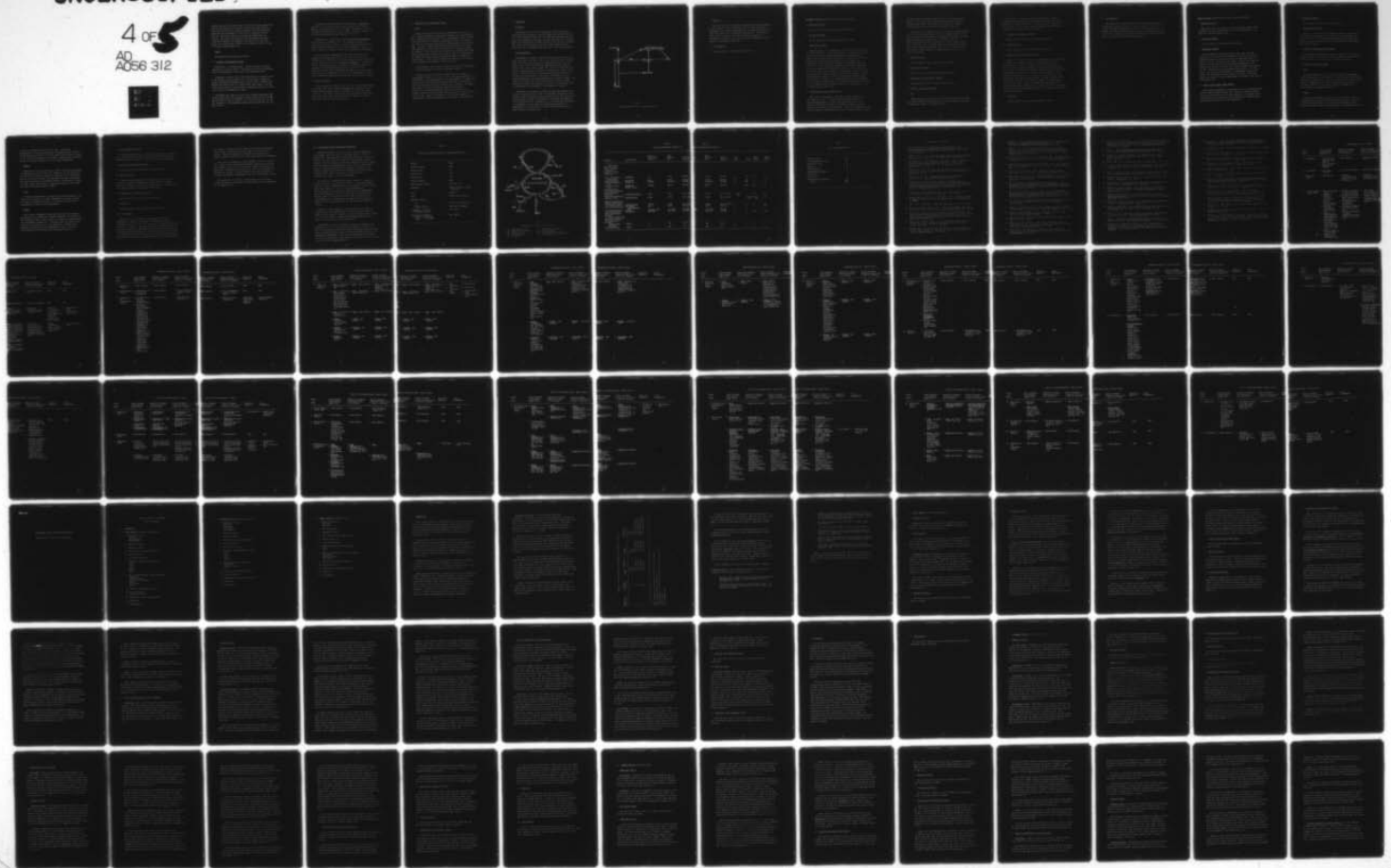
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complete necrosis of the epithelium; 7.5% solution (5 min), edema of the submucosa and loosening of connective tissue in the deeper muscular layers; 20% solution (1 min) complete destruction of the transitional epithelium with replacement by a thin layer of coagulated cellular proteins. The mucosal parts of the trigone and urethra were not affected. The authors attributed this to reflex contraction of the trigone when exposed to the compound. Cats were used in the study because the transepithelial structure of the bladder is similar to that of the human urinary bladder (32).

Gonads

See Reproductive and Teratogenic Effects.

9. Cytologic and Cytogenetic Effects

Formaldehyde is a hardening agent. Strong solutions bring about coagulation of cellular proteins. The effect on the skin of individuals exposed to formaldehyde is attributed to this mechanism (8,32).

Caryorhexis or pyknotic nuclei have been observed in epithelial cells of the bladders of cats instilled with a 7.5% solution of commercial aqueous formaldehyde (38% HCHO), after a contact time of 5 minutes (32). Schenker and Polishuk (30) also observed these same changes in uterine sections from rabbits 12-24 hours after intrauterine injection of 2 ml of a 10% solution of formaldehyde in saline.

Formaldehyde was found to be quite toxic to Ehrlich-Landschutz (ELD) diploid ascites tumor cells in vitro at concentrations as low as 50 ppm. The percentage of dead cells after 1, 2.5, and 5 hours was 8.5, 23, and 23%, respectively. Corresponding untreated control values were 2.6, 3.5, and 4.2% (34).

Formaldehyde induced both SM^F and Trp^+ mutations in Escherichia coli Hcr^- cells at levels as low as 0.04 M. With the Hcr^+ strain, however, no significant increase in the number of mutations occurred at formaldehyde concentrations as high as 0.64 M. The Hcr^- cells were also inactivated much more easily than Hcr^+ cells (35).

Formaldehyde is a strong mutagen for male Drosophila melanogaster larvae, according to Mahajani (36). The compound is capable of penetrating all germ cell stages of larvae and adults of both sexes but induces mutagenic changes only in the early spermatocytes of male larvae. Germ cells of female larvae are not influenced by the aldehyde.

In another study, Stumm-Tegethoff (37) found that neutralized commercial formaldehyde (pH 7.0) at a level of 7% in air or at a concentration of 0.025-0.05% in the larval medium, caused no significant increase in the mutation rate of Drosophila melanogaster. A significant increase was observed, however, when males or larvae were exposed to unneutralized formaldehyde (which contains formic acid) or to a combination of neutralized formaldehyde and formic acid, acetic acid, or hydrochloric acid. Mutagenic effects were also found, however, in the larval feeding experiment when the acids alone were added to the medium.

10. Molecular Effects

Wilkins and MacLeod (38) found that low doses of formaldehyde induced interstrand cross-links in DNA of Escherichia coli in vitro. Protein bridges between the DNA strands were involved in at least 50% of the cross-links. The authors called attention to the biological importance of the cross-links, since some repair-deficient mutants are unable to completely remove these abnormal structures.

11. Reproductive and Teratogenic Effects

Testis

A single intratesticular injection of formaldehyde (10 ml of a 4% soln.) caused enlargement and generalized atrophy of the organ within 20 days. Spermatogenesis was stopped at the spermatogonial stage at 10 days with degeneration of the tubules. Approximately 20-35% of the tubules were also calcified and at many places the spermatozoa were calcified in situ. The tunica albuginea was thickened and the Leydig cells showed mild degeneration. Blood vessels and capillaries in the interstitium were damaged. At 20 days, most of the tubules were atrophied and 20-80% showed calcification. In some of the tubules, however, repopulation of the seminiferous epithelium was noticeable with partial resumption of spermatogenesis. Mild to severe degenerative changes were also observed in the epididymis with mononuclear infiltration (17).

Paraformaldehyde, which dissolves with the evolution of formaldehyde, is an active ingredient in certain contraceptive creams (2).

Teratogenic effects were absent in Beagle dogs given commercial formaldehyde solution in the diet at levels of 125 ppm (equivalent to 3.1 mg/kg BW/day) or 375 ppm (equivalent to 9.4 mg/kg BW/day). Treatment was started 4 days after mating and continued until day 56. Duration of gestation and fecundity were not affected by the treatment. All animals were normal in behavior, appearance, motility, and muscular coordination. No malformations were found in any of the litters. Animals transferred to the breeding colony were observed over a period of nearly 2 years. None of these adults or their litters showed any physiological or skeletal abnormalities or reproductive disorders (39).

12. Metabolism

Absorption

Formaldehyde, by virtue of its high water solubility, appears to react quickly with the mucosa of the alimentary and respiratory tracts. In a study with mongrel dogs, Egle (40) found that both upper and lower parts of the respiratory tract were effective absorbers of the aldehyde. Upper tract absorption was 95-100%, and that of the lower tract exceeded 95%. The total-tract retention was nearly 100%, regardless of the ventilatory rate, formaldehyde concentration, or tidal volumes measured.

Biotransformation

Formaldehyde is oxidized to formic acid in various tissues of the body, especially in the liver. Some of the resultant formic acid may be found in the urine, but much of it is metabolized to CO₂ or to labile methyl groups. Part of the absorbed formaldehyde may also undergo metabolic reduction to methyl alcohol (8). As early as 1893, Pohl (41) reported an apparent minor oxidation of formaldehyde to formic acid in the dog. Verification of this finding was published by Malorny et al. (42) who also found evidence of the possible involvement of liver aldehyde dehydrogenase and nicotinamide adenine dinucleotide (NAD) in the formic acid oxidation and further esterification to methyl formate. Oxidation to formic acid, according to Williams (43) is the major route of formaldehyde biotransformation in the animal body (Figure 1).

Formaldehyde also undergoes oxidation in vitro when incubated with various tissue preparations. Pohl (41) found that formaldehyde could be oxidized slightly in vitro by liver preparations (horse and pig) but not by skeletal muscle (dog). Lutwak-Mann (44) and Kendal and Ramanathan (45) discovered that liver preparations can also bring about dismutation of formaldehyde to form formic acid and methanol in vitro (43). Formaldehyde was oxidized quickly to formic acid after absorption by human erythrocytes in vitro (42).

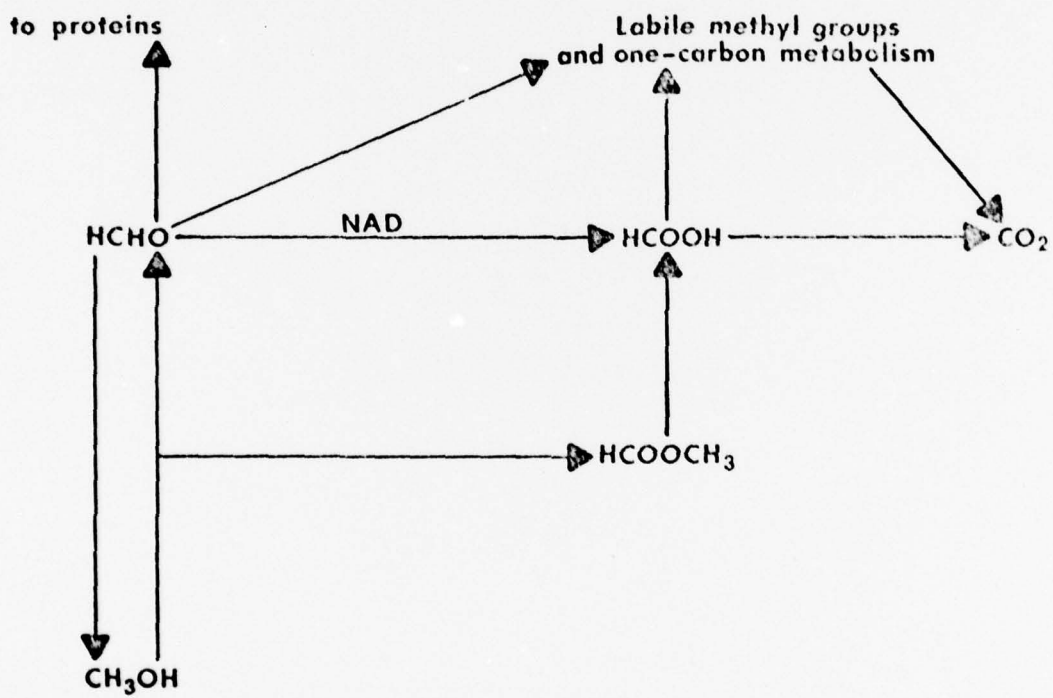


Figure 1
Proposed Metabolic Fate of Formaldehyde (43)

Excretion

Data on excretion of formaldehyde by experimental animals apparently is quite limited. Pohl (41) found that excess formate equivalent to approximately 2.4% of the formaldehyde dose as formate was excreted by a single dog injected with formaldehyde subcutaneously. As indicated above, some of the formic acid resulting from the oxidation of formaldehyde in animals is excreted in the urine, although most of the compound is metabolized further (8).

13. Carcinogenesis

No data reported in literature surveyed to date.

SUBCHRONIC TOXICITY (Short-term tests, less than 90 days)

1. Hematologic Effects

No data reported in the literature surveyed to date.

2. Bone Marrow Changes

No data reported in the literature surveyed to date.

3. Immunologic Response

Repeated exposure to formaldehyde may cause sensitization resulting in allergic dermatitis. Individual differences in susceptibility occur, however, and some individuals become adapted to formaldehyde with exposure, while others become increasingly more sensitive (1). Allergic dermatitis has been observed following direct skin contact with formaldehyde solutions (46), the handling of formaldehyde-containing textiles (47), skin contact with formaldehyde-containing resins (48), as well as with exposure to formaldehyde in the vapor state. After skin sensitization has occurred, exposure to vapor levels as low as 10.5 ppm have resulted in a definite allergic skin reaction. Allergic reactions to solutions have occurred in sensitized individuals when the fingers were immersed for 40 minutes in a formaldehyde solution of 1:5,000,000 (49). A definite systemic-induced skin reaction to formaldehyde occurred in 4 of 11 human volunteers who participated in a safety test on its use as a preservative in milk (50).

4. Central Nervous System (CNS) Effects

The effect of formaldehyde on the CNS of rabbits exposed to 0.1 mg/m³ HCHO in air for 1 1/2 months was determined by functional electroencephalography in a study conducted by Bokina et al. (11). Changes in parameters of primary and secondary responses of evoked potential of the optic cortex were found. A drop in the amplitude of the primary response, a slow negative wave, plus a delayed response as well as

a decrease in the slow negative wave near the end of exposure, were observed. A fall in overall EEG amplitude of all brain structures studied was also found. These changes, according to the authors, indicate a deterioration of the cortical inhibitory processes.

In an earlier study, Bokina and Eksler (12) found local epileptogenic foci in the olfactory analyzer, as well as paroxysmal responses in the olfactory bulbs in rabbits exposed to 0.65 mg/m^3 formaldehyde following a previous exposure (2-4 weeks) to $3.7-6.5 \text{ mg/m}^3$ of the gas. In another experiment with rabbits subjected to 24-hour exposures to 0.1 mg/m^3 formaldehyde for 1 1/2 months, statistically significant changes were observed in amplitude and time curves or in the character of the primary and secondary responses of induced potentials in the visual center of the brain.

5. Behavioral Effects

No data reported in the literature surveyed to date.

6. Cardiovascular System

No data reported in the literature surveyed to date.

7. Biochemical and Histochemical Changes

No data reported in the literature surveyed to date.

8. Effects on Tissues and Organs

Eye

Rabbits and guinea pigs subjected to 40-70 ppm airborne formaldehyde for 10 days developed lachrymation and a slight discharge from the eyes, but showed no evidence of corneal injury (9).

Fifty workers in a large wood product plant who were exposed to formaldehyde at an average level of 0.4 ppm in the workroom air for 20 working days (4 weeks), showed no eye disorders or toxic symptoms on the basis of tests for visual acuity, depth perception, peripheral vision, accommodation, fixation, and color vision (21).

9. Cytologic and Cytogenetic Effects

No data reported in the literature surveyed to date.

10. Molecular Effects

No data reported in the literature surveyed to date.

11. Reproductive and Teratogenic Effects

Gofmekler (51) studied the effect of formaldehyde in air on pregnancy and embryonic development in female rats continuously exposed to concentrations of 1 mg/m^3 (0.8 ppm) or 0.012 mg/m^3 (0.01 ppm) for 10-15 days before mating and pregnancy. Both treatment groups showed adverse effects on embryonic development involving gestation time, embryo organ weights, and litter size. Mean duration of pregnancy in both groups was increased by 14-15% over that of the control group. Total body weights and weights of adrenal glands of progeny from both groups were greater than those of the controls. Kidney and thymus weights of offspring from females exposed to 1.0 mg/m^3 were also greater than those of offspring of control dams. In contrast, lung and liver weights of the progeny of both treatment groups were less than those of offspring of the control group.

12. Metabolism

No data reported in the literature surveyed to date.

13. Carcinogenesis

Early structural changes were observed in the respiratory tract, but no tumors were found in 35 C3H mice exposed a total of 11 times to 0.2 mg/liter (162 ppm) formaldehyde in air over a 4-week period. Four animals showed basal-cell hyperplasia; squamous-cell metaplasia was noted in 16 mice; 5 showed atypical metaplasia; and stratification was noted in 8 mice. Seven mice died during the experiment (52).

CHRONIC TOXICITY (Long-term tests, up to 2 years or lifetime)

1. Hematologic Effects

Hematologic values were normal in rats, guinea pigs, rabbits, dogs, and monkeys exposed to formaldehyde in air at a concentration of 4.6 mg/m³ over a period of 90 days (53).

2. Bone Marrow Changes

No data reported in literature surveyed to date.

3. Immunologic Response

Allergic dermatitis affecting the face, neck, arms, and hands occurred in 6 of 13 nurses in a hemodialysis unit, who were exposed to formaldehyde gas daily during sterilization of open tanks with a 2% formalin solution. The solution was allowed to stand in the tanks for several hours during which time considerable formaldehyde gas was released into the room. The first case occurred 6 months after opening the unit. Sensitivity tests with formalin were positive in 4 of the nurses. All skin lesions attributable to formaldehyde improved when another sterilizing agent was substituted for formalin. The author stated that exposure to gaseous formaldehyde was the most likely cause of sensitization (54).

4. Central Nervous System (CNS) Effects

Rats exposed continuously to formaldehyde in air at concentrations of 0.012-3.0 mg/m³ (0.0098-2.45 ppm) over a period of 3 months exhibited local focal proliferation of the glial elements in the parietal area of the cerebral cortex, together with many satellites of oligodendrocytes and astrocytes at doses of 1.0 and 3.0 mg/m³ (7).

5. Behavioral Changes

No data found in literature surveyed to date.

6. Cardiovascular Effects

Coon et al. (53) found focal inflammatory changes in the hearts of rats and guinea pigs exposed to 4.6 mg/m^3 airborne formaldehyde over a period of 90 days. The authors were uncertain, however, whether the microscopic changes observed resulted from inhalation of formaldehyde or from some other cause.

7. Biochemical and Histochemical Changes

Fel'dman and Bonashevskaya (7) detected a moderate decrease in the glycogen content of liver cells in male rats exposed to formaldehyde gas in air at levels of 1 and 3 mg/m^3 over a period of 3 months.

8. Effects on Tissues and Organs

Skin

Prolonged industrial use of dilute (less than 0.5%) formaldehyde solutions and pastes caused fingernails of some workers to become brown and soft with eventual decay. In other cases, the nails became scaly and friable; inflammation of the skin with suppuration also occurred. In some cases, there was an occasional sensitivity accompanied by a "tightening pain" extending up the arm (1).

Lungs

There have been numerous reports on the varied adverse effects of formaldehyde on the lungs of experimental animals and man. Coon et al. (53) observed varying degrees of interstitial inflammation in rats, guinea pigs, rabbits, dogs, and monkeys exposed to formaldehyde in air at

a level of 4.6 mg/m^3 over a period of 90 days. Fel'dman and Bonashevskaya (7) detected proliferation of lymphohistiocytic elements in the interalveolar walls and in the peribronchial and perivascular spaces, as well as moderate hyperemia in rats during a 3-month study in which the animals were exposed continuously to 1 or 3 mg/m^3 formaldehyde.

Stomach

Human beings as well as animals can tolerate relatively large amounts of formaldehyde by the oral route (5). Yonkman et al. (55) observed no significant toxic effects in humans from ingestion of 22 to 200 mg each day over a period of 13 weeks. Mild pharyngeal and gastric discomfort were experienced when the formaldehyde reached a concentration of 1:2500 to 1:3500. These effects were not experienced, however, if the dosage was diluted sufficiently (approx. 1:7000).

Liver

Fel'dman and Bonashevskaya (7) observed the following changes in the livers of rats exposed to $1-3 \text{ mg/m}^3$ formaldehyde in air over a period of 3 months: polymorphism of nuclei, a profusion of binuclear cells surrounding the triads, and focal hyperplasia.

Kidney

Focal chronic inflammatory changes were observed in the kidneys of guinea pigs and rats exposed to 4.6 mg/m^3 formaldehyde for 90 days, but the investigators were uncertain whether the effects were actually due to the formaldehyde treatment (53). In another study, the kidneys of rats subjected to concentrations of 1 and 3 mg/m^3 over a 3-month period exhibited dilated vessels in the juxtamedullary zone of the cortex. Lower concentrations of the gas caused no adverse effects (7).

Reticuloendothelial System

Fel'dman and Bonashevskaya (7) reported an activation of elements of the reticuloendothelial system in the livers of male rats exposed to formaldehyde in air at levels of 1 and 3 mg/m³ over a 3-month period.

9. Cytologic and Cytogenetic Effects

No data reported in the literature surveyed to date.

10. Molecular Effects

Fel'dman and Bonashevskaya (7) reported that liver cells in rats continuously exposed to formaldehyde (1 and 3 mg/m³) for 3 months exhibited enlargement and rarefaction of RNA granules, in addition to the other changes reported in the study described above.

11. Reproductive and Teratogenic Effects

No data reported in literature surveyed to date.

12. Metabolism

No data reported in the literature surveyed to date.

13. Carcinogenesis

Horton et al. (52) conducted a carcinogenicity study with formaldehyde in C3H mice. One group of 60 animals was exposed to formaldehyde in air at a level of 0.5 mg/liter, one hour per day, 3 times per week, for 35 weeks. Twenty-three animals were then sacrificed for microscopic examination. The remaining 37 mice were exposed to the gas at a concentration of 0.15 mg/liter, one hour per day, 3 times per week for 29 weeks and sacrificed after 64 weeks. A second group of 60 mice was exposed to 0.1 mg/liter (81 ppm) formaldehyde, 1 hr/day 3 times/week

for 35 weeks. A group of control animals was sacrificed and examined after 82 weeks. No tumors were found in any of the treated mice or controls. Basal-cell hyperplasia, squamous-cell metaplasia, and stratification were observed in 45 of 120 animals exposed to formaldehyde.

In an earlier carcinogenicity study, Watanabe et al. (56) injected rats sc with 1 ml of 0.4% aqueous formaldehyde solution once each week for 15 months. Injection-site sarcomas were found in four animals 2-5 months after termination of the injections. Three rats had spindle cell sarcomas; one rat had a "fibrous" sarcoma. One of the spindle cell sarcomas had undergone 24 transplants at the time of the authors' report.

The significance of increases in organ weights for rat fetuses observed by Gofmekler (51) should be studied further from the standpoint of carcinogenicity (1).

IV. SIGNIFICANT PHYSICAL AND CHEMICAL PROPERTIES

Formaldehyde (HCHO) is a colorless gas at ordinary temperatures. It has a pungent suffocating odor. In the vapor phase, it exists as a monomer (FM). Important chemical and physical properties are given in Table 1. The pure, dry gas is stable over the temperature range of 80-100° C. At room temperatures, however, polymerization occurs slowly with the formation of polyoxymethylene in the form of a white film on walls of rooms being fumigated or in containers. Stability of the monomer is dependent on freedom from contamination. Traces of polar compounds (water, acids, or bases) can increase the rate of polymerization. Water is the usual contaminant.

The chemistry of formaldehyde in aqueous solutions is complex as indicated in Figure 2 and described in Table 2. In freshly prepared aqueous solutions, formaldehyde exists as a monohydrate form, methylene glycol (MG) (Figure 2). A series of paraformaldehyde and polyoxymethylene glycols (PF) of low molecular weight ($(HO(CH_2O)_xH)$) may be formed depending on age and concentration of the solution. Methylene glycol is the principal form at lower concentrations of formaldehyde; higher concentrations and aging favor the formation of polymeric forms of formaldehyde (PO, PT, PW), which precipitate from solution.

Methanol is usually added to a final concentration of 10-15% to formalin (37% by wt. formaldehyde gas in water), the main commercial solution of the compound, to prevent or slow down polymerization. Formic acid is usually present in commercial formaldehyde preparations as a result of slow oxidation of the aldehyde by air.

Formaldehyde is used as a fumigant, disinfectant, germicide, and fungicide, as well as in a wide variety of industrial processes. It is used in the tanning and preserving of hides, mordanting and waterproofing fabrics, coagulating rubber latex, for hardening gelatin plates, etc. in photography. Formaldehyde is also used in the manufacture of phenolic resins, urea-formaldehyde resins, polyacetal resins, artificial silk, and a variety of other compounds (Table 3).

Table 1

Properties and Characteristics of Formaldehyde (FM) (1)

Formula	HCHO
Molecular weight	30.03
Physical state	Gas
Melting point	-92 C
Boiling point	-21 C
Specific gravity	0.815
Relative vapor density	1.075 (air = 1)
Solubility	Soluble in water, alcohol, and ether
Color	Colorless
Odor	Pungent and irritating
Explosive limits:	
Gas	7.0-73% by volume in air
Aqueous solution	Vapor may be flammable
Flashpoint (closed cup) of aqueous solution	50 C (122 F)
Autoignition temperature	430 C (806 F)
1 mg/cu m = 0.81 ppm	
1 ppm = 1.23 mg/m ³	

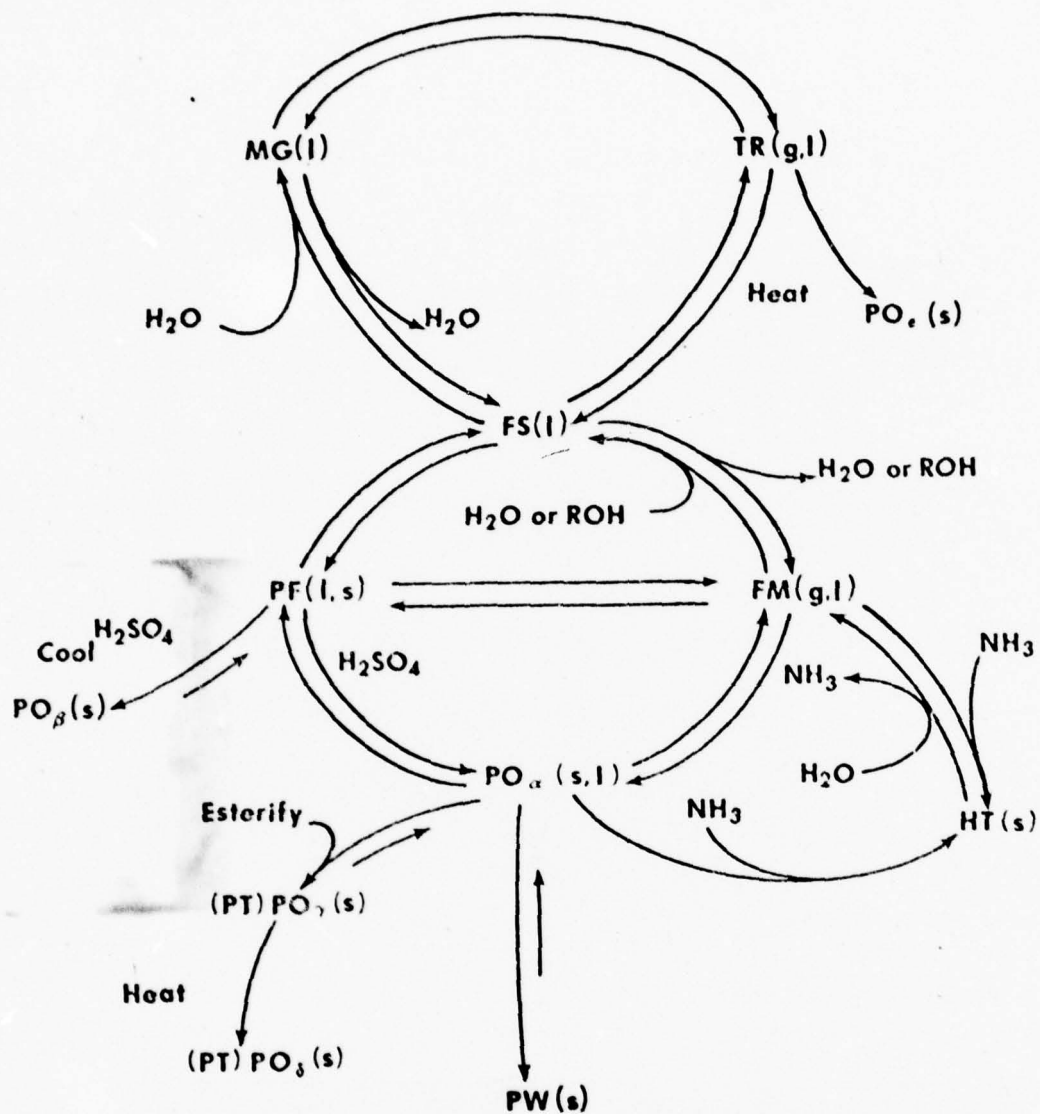


Figure 2

Formaldehyde Reactions and Products (1)

- | | |
|-------------------------------|---------------------------------------|
| FM - Formaldehyde monomer | HT - Hexamethylene tetramine |
| FS - Formaldehyde in solution | PO - Polyoxymethylenes |
| MG - Methylene glycol | PT - Polyoxymethylenes modified |
| PF - Paraformaldehyde | PW - Polyoxymethylenes, high polymers |
| TR - Trioxane | |

Table 2

Forms of Formaldehyde Polymers (1)

Substance	Type Formula	Range of Polymerization, n	CH ₂ O Content, Wt %	Melting Range, C
1. Linear polymers (On vaporization these depolymerize to monomeric formaldehyde gas).				
Lower polyoxymethylene glycols	HO(CH ₂ O) _n H	2-8	77-93	80-120
Paraformaldehyde	HO(CH ₂ O) _n H	8-100	91-99	120-170
alpha-Polyoxymethylene	HO(CH ₂ O) _n H	100-300	99.0-99.9	170-180
beta-Polyoxymethylene	HO(CH ₂ O) _n H +H ₂ SO ₄ (trace)	100-300	98-99	165-170
Polyoxymethylene glycol derivatives*** polyoxymethylene diacetates	CH ₃ COO(CH ₂ O) _n COCH ₃	2-200	37-93	up to ca 1
Lower polyoxymethylene dimethyl ethers	CH ₃ O(CH ₂ O) _n CH ₃	2-200	72-93	up to ca 1
gamma-Polyoxymethylene (higher polyoxymethylene dimethyl ethers)	CH ₃ O(CH ₂ O) _n CH ₃	200-500	93-99	160 to ca
delta-Polyoxymethylene	CH ₃ O(CH ₂ O) _n CH ₂ CH(OH)OCH ₃	g100	96-97	150-170
epsilon-Polyoxymethylene high-molecular weight polyoxymethylenes****	(CH ₂ O) _n HO(CH ₂ O) _n H	probably g100 500-5000	99.7-99.9 99.9-100	195-200 170-185
2. Cyclic polymers (On vaporization these do not depolymerize).				
Trioxane (alpha-trioxymethylene)	(CH ₂ O) ₃	3	100	61-62
Tetraoxymethylene	(CH ₂ O) ₄	4	100	112

Table 2

ms of Formaldehyde Polymers (1)

CH ₂ O Content, Wt %	Melting Range, C	Ace- tone	Water	Dilute Alkali	Dilute Acid
77-93	80-120	s-i	vs	vs	vs
91-99	120-170	s-i	ds	s	s
99.0-99.9	170-180	i	vds	s	s
98-99	165-170	i	vds	ds	ds
37-93	up to ca 165	i for n >10	i	ds	ds
72-93	up to ca 175		i for n >15	i for n >15	ds
93-99	160 to ca 180	i	i	i	ds
96-97	150-170	i	i	i	ds
99.7-99.9	195-200	i	i		
99.9-100	170-185	i	i	vds	vds
100	61-62	s	s	s	s
100	112	s	s	s	s

Table 3

Uses of Formaldehyde (1)

Phenolic resins	25%
Urea-formaldehyde resins	25
Polyacetal resins	8
Melamine	8
Pentaerythritol	7
Hexamethylenetetramine	6
Fertilizers	5
Acetylenics	2
Exports and miscellaneous	<u>14</u>
Total	100%

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Formaldehyde Toxicity - Matrix

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
1. Hematologic	<ul style="list-style-type: none"> o Loss of oxygen combining power (RBC)(4) o Loss of selective permeability (RBC)(4) 	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o No adverse effects (53)
2. Bone Marrow	<ul style="list-style-type: none"> o No effects reported 	<ul style="list-style-type: none"> o No effects reported 	<ul style="list-style-type: none"> o No effects reported
3. Immunologic Effect	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o Allergic sensitization and dermatitis (46,49, 50) (See No. 7) 	<ul style="list-style-type: none"> o Allergic sensitization and dermatitis (54)
4. Central Nervous System (CNS)	<ul style="list-style-type: none"> o Optical chronaxie changes (6) o Changes in cerebral electrical activity (7) o Central nervous system depression (8) o Decreased nasopalatine nerve response (10) o Depression of high frequency - induced activity in olfactory structures (11) o Persistent stress rhythm in cortex and limbic brain structures (11) o Adverse effect on cortical medial nucleus (11) o Inhibition of respiration (14) 	<ul style="list-style-type: none"> o Changes in responses of evoked potential of optic cortex (11). o Decrease in overall EEG of various brain structures (11) o Epileptogenic foci in olfactory analyzer (12) o Paroxysmal responses in olfactory bulbs (12) o Changes in responses of induced potentials in visual center of the brain (12) 	<ul style="list-style-type: none"> o Local focal proliferation of glial elements in parietal area of cerebral cortex; oligodendrocyte and astrocyte satellites (7)

Formaldehyde Toxicity - Matrix

Chronic Treatment Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None Reported	o No adverse effects (53)	None	None
No effects reported	o No effects reported	None	None
Allergenic sensitization and dermatitis (46,49, 50) (See No. 7)	o Allergenic sensitization and dermatitis (54)	Allergenic sensitization (correlates with combination with methyl groups in proteins (see No. 7 below))	a. Immunological tests b. Allergy tests (patch tests)
Changes in responses of evoked potential of optic cortex (11). Decrease in overall EEG of various brain structures (11) Epileptogenic foci in olfactory bulb analyzer (12) Seizure responses in olfactory bulbs (12) Changes in responses of induced potentials in visual center of the brain (12)	o Local focal proliferation of glial elements in parietal area of cerebral cortex; oligodendrocyte and astrocyte satellites (7)	Induced potentials in visual center of brain - changes (subchronic)	a. Induce potential test

2

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
5. Behavioral Effects	o None reported	o None reported	o None reported
6. Cardiovascular Effects	o Hypotension (14) o Circulatory collapse (8)	o None reported	o Focal inflammatory changes in heart (53)
7. Biochemical and Histochemical Effects	o Increased liver alkaline phosphatase activity (16,17) o Decreased nicotinamide-adenine coenzyme content of spermatozoa <u>in vitro</u> (19) o Inhibition of liver glutamic dehydrogenase and heart lactic dehydrogenase <u>in vitro</u> (18) o Inhibition of spermatozoan motility <u>in vitro</u> (19) o Combination with methyl groups in proteins (8) (See No. 3) o Formation of irreversible heme-linked group with hemoglobin (14) o Hyperglycemia (14) o Acidosis (2)	o None reported	o Decreased liver glycogen (7)

1

Formaldehyde Toxicity - Matrix (Cont.)

Chronic Treatment Short-term tests, to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None reported	o None reported	None	None
None reported	o Focal inflammatory changes in heart (53)	None	None
None reported	o Decreased liver glycogen (7)	Combination with methyl groups in proteins	Protein synthesis inhibition

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
8. Effect on Organs and Tissues	o <u>Skin.</u> Irritation and anesthesia (8)	o <u>Skin.</u> None reported	o <u>Skin.</u> Inflammation; suppuration (1); Damage to fingernails (1)
	o <u>Eyes.</u> Irritation and lachrymation (16,20) Intraocular hypertension and protein leakage across blood-aqueous barrier (22) Coagulation of corneal surface (5)	o <u>Eyes.</u> Lachrymation and discharge (9)	o <u>Eyes.</u> None reported
	o <u>Nose.</u> Irritation and severe burning (5,16)	o <u>Nose.</u> None reported	o <u>Nose.</u> None reported
	o <u>Larynx.</u> Irritation, burning, edema, spasm (8)	o <u>Larynx.</u> None reported	o <u>Larynx.</u> None reported
	o <u>Trachea.</u> Irritation and burning (5) Ciliastasis (23)	o <u>Trachea.</u> None reported	o <u>Trachea.</u> None reported
	o <u>Bronchi.</u> Bronchitis (8,15)	o <u>Bronchi.</u> None reported	o <u>Bronchi.</u> None reported

Formaldehyde Toxicity - Matrix (Cont.)

Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
o <u>Skin.</u> None reported	o <u>Skin.</u> Inflammation; suppuration (1); Damage to fingernails (1)	a. Skin irritation	a. Draize test
o <u>Eyes.</u> Lachrymation and discharge (9)	o <u>Eyes.</u> None reported	b. Eye irritation	b. Draize test
		c. Lung changes	c. Lung function test
			d. Ciliary motion test
o <u>Nose.</u> None reported	o <u>Nose.</u> None reported		
o <u>Larynx.</u> None reported	o <u>Larynx.</u> None reported		
o <u>Trachea.</u> None reported	o <u>Trachea.</u> None reported		
o <u>Bronchi.</u> None reported	o <u>Bronchi.</u> None reported		

2

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
8. Effect on Organs and Tissues (Cont.)	<ul style="list-style-type: none"> o <u>Lung.</u> <u>Increased</u> resistance to airflow (26,27) <u>Increased</u> tidal volume (26,27) <u>Decreased</u> respiratory rate (26,27) <u>Decrease</u> in minute volume (27) Irritation (25) Hyperemia (15,25) Hemorrhage (15,24,25) Edema (15,24,25) Alveolar damage (24) Other lesions (25) o <u>Stomach.</u> Inflammation and ulceration (8); Pain (2,8). Hematemesis (2,8); Corrosive damage and strictures (8) o <u>Intestines.</u> Initial inhibition of movement followed by increased tone and amplitude of contraction (14) 	<ul style="list-style-type: none"> <u>Lung.</u> None reported o <u>Stomach.</u> None reported o <u>Intestine.</u> None reported 	<ul style="list-style-type: none"> o <u>Lung.</u> Hyperemia (7); Interstitial inflammation (53) Proliferation of lymphohistiocytic elements in alveolar walls (7) o <u>Stomach.</u> Irritation (55) o <u>Intestines.</u> None reported

Formaldehyde Toxicity - Matrix (Cont.)

Chronic Treatment Short-term tests, to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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g. None reported	o <u>Lung.</u> Hyperemia (7); Interstitial inflammation (53) Proliferation of lymphohistiocytic elements in alveolar walls (7)		
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<u>Stomach.</u> None reported	o <u>Stomach.</u> Irritation (55)		
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<u>Intestine.</u> None reported	o <u>Intestines.</u> None reported		
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Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoint
8. Effect on Organs and Tissues (Cont.)	o <u>Liver.</u> Hyperemia, perivascular edema, necrosis (15)	o <u>Liver.</u> None reported	o <u>Liver.</u> Focal hyperplasia (7) Profusion of binuclear cells surrounding the triads; nuclear polymorphism (7) Activation of reticuloendothelial system elements (7)	
	o <u>Kidneys.</u> Hyperemia (15,25) Perivascular edema (15)	o <u>Kidneys.</u> None reported	o <u>Kidneys.</u> Focal inflammatory changes (53); Dilated vessels in juxtamedullary zone of cortex (7)	

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predict Endpoi
8. Effect on Organs and Tissues (Cont.)	o <u>Uterus.</u> Hemorrhage, edema, necrosis of endometrium; injury to myometrium; polynuclear infiltration; obliteration of cavity (30)	o <u>Uterus.</u> None reported	o <u>Uterus.</u> None reported	
	o <u>Bladder.</u> Inflammation, edema, necrosis; Adhesions to viscera Transmural inflammation, mucosal ulceration, hemorrhage (31) Coagulation necrosis (32) Leucocytic infiltration, hemorrhagic cystitis, destruction of transitional epithelium (32)	o <u>Bladder.</u> None reported	o <u>Bladder.</u> None reported	
	o <u>Gonads.</u> See Reproduction and Teratogenic Effects	o <u>Gonads</u> - None reported	o <u>Gonads</u> - None reported.	

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predicted Endpoints
9. Cytologic and Cytogenetic Effects	<ul style="list-style-type: none"> o Coagulation of cellular proteins (8) o Caryorhexis and pyknotic nuclei in epithelial cells of bladder and uterus (30,32) o Cytocidal effect Ehrlich-Landschutz (ELD) diploid ascites tumor cells <u>in vitro</u> (34) o <u>SMr</u> and Trp+ mutations in <u>Escherichia coli</u> (35) o Mutation in <u>Drosophila melanogaster</u> (36,37) o Adverse effects on mouse neuroblastoma cells <u>in vitro</u> (13) 	o None reported	o None reported	None
10. Molecular Effects	o Interstrand cross-links in DNA of <u>E. coli</u> <u>in vitro</u> (38)	o None reported	o Enlargement and rarefaction of RNA granules in liver cells (7)	None

Formaldehyde Toxicity - Matrix (Cont.)

Chronic Treatment Short-term tests, 0 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None reported

o None reported

None

None

None reported

o Enlargement and
rarefaction of RNA
granules in liver
cells (7)

None

None

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
11. Reproductive and Teratogenic Effects	<ul style="list-style-type: none"> o <u>Testis.</u> Inhibition of spermatogenesis; Calcification, degeneration, and atrophy of tubules; Degenerative changes in epididymis; Mild degeneration of Leydig cells; Damage to blood vessels and capillaries; Enlargement and generalized atrophy of organ (17) 	<ul style="list-style-type: none"> o <u>Increased</u> duration of pregnancy (51). <u>Increased</u> total body weight and weights of adrenals, kidneys, and thymus of progeny (51) <u>Decreased</u> lung and liver weights of offspring (51) 	<ul style="list-style-type: none"> o None reported
12. Metabolism	<ul style="list-style-type: none"> o <u>Absorption.</u> Respiratory tract absorption 95-100%! (40) o <u>Biotransformation.</u> <u>In vivo:</u> Oxidation to formic acid (major pathway); Reduction to methanol; Formation of acetic acid; Metabolism of formic acid to CO₂ or labile methyl groups; Esterification of some HCOOH to methyl formate (8,41,42,43) <u>In vitro:</u> Dismutation to formic acid and methanol (44,45) 	<ul style="list-style-type: none"> o None reported 	<ul style="list-style-type: none"> o None reported

Formaldehyde Toxicity - Matrix (Cont.)

Chronic Treatment Short-term tests, (0 to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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<p><u>Increased</u> duration of pregnancy (51). <u>Increased</u> total body weight and weights of adrenals, kidneys, and thymus of progeny (51)</p>	<p>o None reported</p>	<p>None</p>	<p>None</p>
<p><u>Increased</u> lung and liver weights of offspring (51)</p>	<p>o None reported</p>	<p>None</p>	<p>None</p>

<p>None reported</p>	<p>o None reported</p>	<p>None</p>	<p>None</p>
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2

Formaldehyde Toxicity - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)
12. Metabolism (Cont.)	o <u>Excretion.</u> Some formic acid excreted in urine (8)		
13. Carcinogenesis	o None reported	o No tumors in C3H mice exposed 11 times to 200 mg/cu m (162 ppm) HCHO in air over 4-week period; Sacrificed at end of 4th week (52)	o Injection site sarcomas (spindle cell) in rats given 1 ml 0.4% aq. HCHO soln. sc once/week for 15 months, appeared 2-5 months after termination of injections (56) o No tumors in C3H mice exposed to HCHO in air 0.05 mg/liter 1 hr/day for 35 weeks (sacrificed at end of 35th week), followed by 0.15 mg liter 1 hr/day for 3 times/week for 29 weeks (sacrificed after 64 weeks) (52).

Formaldehyde Toxicity - Matrix (Cont.)

Acute Treatment (Short-term tests, 90 days)	Chronic Treatment (Long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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No tumors in C3H
 mice exposed 11
 weeks to
 10 mg/cu m (162 ppm)
 HCHO in air over
 11 week period;
 sacrificed at end of
 11th week (52)

- o Injection site sarcomas (spindle cell) in rats given 1 ml 0.4% aq. HCHO soln. sc once/week for 15 months, appeared 2-5 months after termination of injections (56)
- o No tumors in C3H mice exposed to HCHO in air 0.05 mg/liter 1 hr/day for 35 weeks (sacrificed at end of 35th week), followed by 0.15 mg liter 1 hr/day for 3 times/week for 29 weeks (sacrificed after 64 weeks) (52).

None

None

2

Toxicity of Nitrogen Oxides - Matrix

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Pre End
1. Hematologic Effects	<ul style="list-style-type: none"> o <u>Leucocytes</u> significant decrease in number (2) o <u>Hemoglobin</u> No change (1) o <u>Platelets</u> No change (1) o <u>Methemoglobin</u> increased (6-8) 	<ul style="list-style-type: none"> o <u>Leucocytes</u> No change (33) o <u>Hemoglobin</u> No change (rat) (33) o <u>Platelets</u> None reported o <u>Methemoglobin</u> (46) o <u>Polycythemia</u> (45) 	<ul style="list-style-type: none"> o <u>Leucocytosis</u> which receded with cessation of exposure (71) o <u>Hemoglobin</u> decreased (dog) (71) No change (dog) (72) o <u>Platelets</u> None Reported. 	1.
2. Bone Marrow Changes	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o None Reported. 	
3. Immunologic Effect	<ul style="list-style-type: none"> o Positive reaction to aerosolized antigen (Severe dyspnea) (9) o Increased susceptibility to infection (10-21) 	<ul style="list-style-type: none"> o Positive reaction to aerosolized antigen (severe anaphylaxis) (47) o Increasing susceptibility to infection (high mortality) (48) 	<ul style="list-style-type: none"> o Positive reaction to aerosolized antigen (severe anaphylaxis) (47,71) o Antibody production inhibited (13,14,16, 17,19,74,75) o Increased susceptibility to infection (high mortality) (76) 	1. 2.

Toxicity of Nitrogen Oxides - Matrix

Chronic Treatment Short-term tests, to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<u>Leucocytes</u> No change (33)	o <u>Leucocytosis</u> which receded with cessation of exposure (71)	1. Leucocytosis	1. Complete CBC, differential, hematocrit
<u>Hemoglobin</u> No change (rat) (33)	o <u>Hemoglobin</u> decreased (dog) (71) No change (dog) (72)		
<u>Platelets</u> None reported	o <u>Platelets</u> None Reported.		
<u>Methemoglobin</u> (46) <u>Polycythemia</u> (45)			
None Reported	o None Reported.	None	None
<u>Positive reaction to aerosolized antigen (severe anaphylaxis)</u> (47)	o <u>Positive reaction to aerosolized antigen (severe anaphylaxis)</u> (47,71) o <u>Antibody production inhibited</u> (13,14,16, 17,19,74,75)	1. Hypersensi- tivity 2. Increased suscepti- bility to infection	1. Immunological tests 2. Mouse infectivity test
<u>Increasing susceptibility to infection (high mortality)</u> (48)	o <u>Increased susceptibility to infection (high mortality)</u> (76)		

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)
4. Central Nervous System (CNS)	o None Reported	o None Reported	CNS disturbances (human) (32)
5. Behavioral Effects	o None Reported	o None Reported	o None Reported.
6. Cardiovascular Effects	o Hypotension accompanied by decreased cardiac output and stroke volume; systemic acidosis out of proportion to the rise in arterial CO ₂ pressure. (23)	o None Reported	o None Reported.
7. Biochemical and Histochemical Effects	<ul style="list-style-type: none"> o <u>Lung.</u> o <u>Elevated Peroxidation</u> (24), Lactic dehydrogenase (28) o <u>Depressed Catalase</u> (25), glucose metabolism (26) o <u>No Change,</u> Cathepsin (27), benzpyrene hydroxylase (29) o Chromatographic shift of lactic dehydrogenase to anaerobic band 5 (14,18) 	<ul style="list-style-type: none"> o <u>Lung.</u> o <u>Elevated Lactic dehydrogenase and aldolase</u> (28,49,50); Peroxidation. (34) 	<ul style="list-style-type: none"> o <u>Lung.</u> o <u>Depressed Total saturated phospholipid fatty acids</u> (77)

toxicity of Nitrogen Oxides - Matrix (Cont.)

Chronic Treatment (short-term tests, 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None Reported	CNS disturbances (human) (32)	None	None
None Reported	o None Reported.	None	None
None Reported	o None Reported.	None	None

o Depressed Lactic
dehydrogenase and
aspartate aminotransferase (28,49,50);
oxidation. (34)

o Lung.

1. Lung damage

1. Lipid peroxidase
test

o Depressed Total
saturated phospho-
lipid fatty acids
(77)

2

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (Cont.)	<ul style="list-style-type: none"> o <u>Blood</u> <u>Elevated</u> Serum lactic dehydrogenase (28), aldolase (28). o Chromatographic shift of lactic dehydrogenase to anaerobic band 5 (14,18) o <u>Liver</u> <u>Elevated</u> Lactic dehydrogenase (28) aldolase activity (28), QO_2 Insignificant (28) o <u>Kidney</u> <u>Elevated</u> Lactic dehydrogenase, (28) aldolase (28), QO_2 (28) o <u>Spleen</u> <u>Elevated</u> Lactic dehydrogenase, (28), aldolase (28), QO_2 (28) 	<ul style="list-style-type: none"> o <u>Blood</u> <u>Elevated</u> D-2,3-diphosphoglycerate (51) o <u>Depressed</u> catalase activity (52) o <u>Liver.</u> <u>Elevated</u> Lactic dehydrogenase and aldolase activity (28,49,50) o <u>Kidney</u> <u>Elevated</u> Lactic dehydrogenase activity and QO_2 (28,49,50) o <u>Depressed</u> Aldolase activity 28,49,50 o <u>Spleen</u> <u>Elevated</u> Lactic dehydrogenase, aldolase and QO_2 (28) 	<ul style="list-style-type: none"> o <u>Blood</u> <u>Elevated</u> Serum immunoglobulins (74); lipoproteins, cholesterol and albumin (human) (32) o <u>Depressed</u> serum albumin (71) No Change alkaline phosphatase (72) o <u>Kidney</u> None Reported. o <u>Spleen</u> None Reported.

Toxicity of Nitrogen Oxides - Matrix (Cont.)

Chronic Treatment Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
<u>Blood</u> Elevated D-2,3- phosphoglycerate (51)	o <u>Blood</u> Elevated Serum immunoglobulins (74); lipoproteins, cholesterol and albumin (human) (32)	2. Elevated IG (possibly correlates with hematological effects)	2. Immunological tests
Depressed catalase activity (52)	o <u>Depressed serum</u> albumin (71)		

No Change alkaline
phosphatase (72)

Liver.
Elevated Lactic
hydrogenase and
aldolase activity
(28,49,50)

Kidney
Elevated Lactic
hydrogenase
activity and QO_2
(28,49,50)
Depressed Aldolase
activity 28,49,50

o Kidney None Reported.

Spleen
Elevated Lactic
hydrogenase,
aldolase and
 QO_2 (28)

o Spleen None Reported.

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including in <u>vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (Cont.)	o <u>Heart</u> Chromatographic shift of lactic dehydrogenase to anaerobic band 5 (14,18)	o <u>Heart</u> None Reported	o <u>Heart</u> None Reported
8. Body Weight, Organs	o <u>Body weight</u> , Weight loss dose related (1,3)	o <u>Body weight</u> No difference between experimental and control animals (3,52)	o <u>Body weight</u> Significant decrease (several species incl. rats). (71,78,70)
	o <u>Pulmonary Effect</u> <u>Function.</u> <u>Increased</u> respiratory rate; <u>decreased</u> tidal volume and pulmonary diffusing capacity (reversible) (18,30-32)	o <u>Pulmonary Effect</u> <u>Function</u> None reported.	o <u>No change</u> (rats) (13,72)
	o <u>Pathologic.</u> Edema, epithelial degeneration, inflammation, abnormal mast cells, interstitial fibrosis, hemorrhage, mitochondrial loss in alveolar cells, inhibition and loss of cilia in alveolar epithelium (2,3,23,18,33-38)	o <u>Pathologic.</u> Perivascular chronic inflammation, hyperplastic and hypertrophic changes, cellular abnormalities, hemorrhage, abnormal ciliogenesis, abnormal collagen fibrils (3,33,38,49, 52-62)	o <u>Pathologic.</u> Bronchiolar inflammation (59), hyperplastic and hypertrophic changes (81,82), cellular abnormalities (45,81-83), abnormal collagen fibrils (65), emphysematous changes (53,84,86), abnormal ciliogenesis (83)

city of Nitrogen Oxides - Matrix (Cont.)

Acute Treatment (short-term tests, 30 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None Reported o Heart None Reported

Body weight No
difference between
experimental and
control
animals (3,52)

o Body weight
Significant decrease
(several species
incl. rats).
(71,78,70)

o No change (rats)
(13,72)

Pulmonary Effect
Function None
reported.

o Pulmonary Effect
Function. Increased
respiratory rate
(80): decreased
tidal volume (80).
No change in
respiratory flow
resistance (73),
 $\dot{V}O_2$ (72)

1. Lung damage	1. Pulmonary adema test (labelled albumin)
----------------	--

Pathologic.
vascular
inflammation,
hyperplastic and
hypertrophic
changes, cellular
abnormalities,
hemorrhage, abnormal
angiogenesis,
normal collagen
fibrils (3,33,38,49,
83)

o Pathologic.
Bronchiolar
inflammation (59),
hyperplastic and
hypertrophic changes
(81,82), cellular
abnormalities
(45,81-83), abnormal
collagen fibrils
(65), emphysematous
changes (53,84,86),
abnormal ciliogenesis
(83)

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Pro End
8. Body Weight, Organs (Cont.)	<u>Vascular Permeability and Edema formation</u> (32,39)	<u>Vascular Permeability and Edema formation</u> (38,65,66)	<u>Vascular Permeability and Edema formation</u> (55,59,72,79,81,87) Humans decrease in ventilatory performance in one study (88) but not in others (32)	
	o <u>Liver.</u> <u>In vitro</u> growth inhibition (liver cells) (40), cellular abnormalities (18)	o <u>Liver</u> No adverse effects seen (52)	o <u>Liver</u> No adverse effects seen (59)	
	o <u>Kidney.</u> Edema and lymphocytic infiltration, glomerulae abnormalities (18); <u>No effect</u> on body weight to kidney weight ratio (1)	o <u>Kidney</u> None Reported	o <u>Kidney</u> No adverse effects seen (59)	
	o <u>Spleen.</u> None Reported	o <u>Spleen</u> None Reported	o <u>Spleen</u> No adverse effects seen (59)	
	o <u>Heart.</u> Interstitial fibrosis and edema (18)	o <u>Heart</u> None Reported	o <u>Heart</u> No adverse effects seen (50)	

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)
8. Body Weight, Organs (Cont.)	<ul style="list-style-type: none"> o <u>Skin</u> None Reported o <u>Other organs</u> Eye and nose irritation (3), change in dark adaption (human) (3,28,41) 	<ul style="list-style-type: none"> o <u>Skin</u> None Reported 	<ul style="list-style-type: none"> o <u>Skin</u> disorder 32 o <u>Other organs.</u> Trachea, lymph, adrenal, thyroid, pancreas, duodenum, bladder, brain, not affected (72)
9. Cytologic and Cytogenetic Effects	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o No adverse effect on mitosis of alveolar epithelial cells (67) 	<ul style="list-style-type: none"> o None Reported
10. Molecular Effects	<ul style="list-style-type: none"> o <u>Protein</u> molecular structures of collagen and elastic altered (42) 	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o None Reported
11. Reproductive and Teratogenic Effects	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o Adverse effects on genital function (68,69) delayed littering (52) 	<ul style="list-style-type: none"> o None Reported

5 Nitrogen Oxides - Matrix (Cont.)

Treatment Tests,)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Reported	o <u>Skin</u> disorder 32		
	o <u>Other organs.</u> Trachea, lymph, adrenal, thyroid, pancreas, duodenum, bladder, brain, not affected (72)		

Effect on alveolar cells	o None Reported	None	None
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Reported	o None Reported	None	None
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Effects on function	o None Reported	None	None
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irritating

Toxicity of Nitrogen Oxides - Matrix (Cont.)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (Short-term tests, up to 90 days)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Pred. Endp.
12. Metabolism	<ul style="list-style-type: none"> o In human 81-87% of exhaled nitrogen was absorbed during normal ventilation and more than 90% during periods of maximal ventilation (43,44), glycolysis was stimulated but stimulation was not persistent (25) 	<ul style="list-style-type: none"> o Increased excretion of albumin, alpha-, beta- and gamma globulins in the urine (70) 	<ul style="list-style-type: none"> o None Reported 	
13. Carcinogenesis	<ul style="list-style-type: none"> o None Reported 	<ul style="list-style-type: none"> o Adenomatous proliferation of bronchial and bronchiolar tissue (mice) (48) 	<ul style="list-style-type: none"> o Type of tumors produced in rats, Spindle-cell and ganglioneuroma of adrenal (29,72,80, 89,90) 	

of Nitrogen Oxides - Matrix (Cont.)

Treatment m tests, ays)	Chronic Treatment (long-term tests, up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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ed excretion min, alpha-, and gamma ins in the (70)	o None Reported	None	None
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matous feration of hial and hiolar tissue) (48)	o Type of tumors produced in rats, Spindle-cell and ganglioneuroma of adrenal (29,72,80, 89,90)	None	None
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Tracor Jitco

EXPERIMENTAL OXIDES OF NITROGEN INTOXICATION

Backup Report to Oxides of Nitrogen Matrix

Toxicity of Oxides of Nitrogen

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12. Metabolism
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I. INTRODUCTION

There is ample evidence to show that nitrogen oxides produce adverse effects on experimental animals and humans. Most of this evidence is based on the relationship between exposure to nitrogen oxides and the *resultaat pulmonary dysfunction (emphysema or bronchitis)*, or on the potentiating effect of such exposure on an already present chronic respiratory disease.

Although there are several oxides of nitrogen present in the atmosphere (nitrous oxide, dinitrogen trioxide, dinitrogen pentoxide and nitrate ions) besides nitric oxide (NO) and nitrogen dioxide (NO₂), they are present in very small quantities, and except for nitrous oxide, (N₂O) are not of toxicological interest. Information concerning effects of nitrogen oxide on experimental animals and humans is scarce, except for N₂O.

Nitrous oxide possesses strong anesthetic and analgesic properties. At concentrations of 80% (1.44×10^6 mg/m³), nitrous oxide is effective as a general anesthetic. Inhalation of 10-20% of this oxide ($1.8 \times 10^5 - 3.6 \times 10^5$ mg/m³) provides effective analgesia.

Nitric oxide has been shown to produce adverse effects in animals and humans. Although no cases of human poisoning due to nitric oxide have been reported in the literature, it is undoubtedly included in occupational exposure to NO_x. When present in high concentrations, nitric oxide is readily converted to nitrogen dioxide, a property which makes it difficult to control its concentrations in laboratory experiments. Exposure to very high concentrations of nitric oxide results in central nervous system effects, decrease in pulmonary function, increase of methemoglobin and enzymatic inhibition.

Nitrogen dioxide (NO_2) is by far the most significant biologically. It is about 4 times more toxic than nitric oxide and its primary effect is on the lung where it can alter the configuration of the lung tissue structural proteins, collagen and elastin. Its systemic effects include extensive tissue changes, weight loss, hematologic abnormalities and inhibition of enzymes. Exposure to NO_2 results in increased susceptibility to infection.

Although the biological effects in humans of repeated exposure to NO_2 (and other oxides of nitrogen) are of interest, the review by the committee on Threshold Limit Value (TLV) (1962) led to the conclusion that data derived from human industrial settings were not sufficiently precise to be conclusive in setting a threshold value for nitrogen dioxide. At best, the human data pertain to exposures of 8 hours/day, 5 days/week.

The LC_{50} values for rats and rabbits exposed to NO_2 for 15 minutes ranged from $191-212 \pm 4.1$ ppm and $290-342 \pm 5.9$ ppm, respectively (1). Hine et al. (2) studied the mortality threshold concentrations of $75-94 \text{ mg/m}^3$ ($40-50$ ppm) after 1 hour exposures of rats, mice, guinea pigs, rabbits and dogs, and observed that the rats, mice and guinea pigs are more susceptible to nitrogen dioxide than the rabbit and dog. Other investigators observed the nonhuman primate to be slightly more susceptible to nitrogen dioxide (122 mg/m^3 (65 ppm) for 8 hours) than similarly exposed rodents (3).

Length of exposure and concentration are critical factors in the determination of the LC_{50} of NO_2 in various animal species. For example short exposures of rats to high concentrations were more toxic than equivalent exposures to low concentrations for longer periods, as illustrated below.

Comparison of Lethal Levels of Acute Exposure of Male Rats to NO₂ (4)

Number of Experiments	Exposure in Minutes	LC ₅₀ a,b		LC ₅₀ b,c ppm x min
		PPM	mg/m ³	
3	2	1,445 ^d	2,715	2,890
2	5	833 ^d	1,566	4,165
6	15	420 (362-487)	790 (680-916)	6,300 (5,430-7,305)
10	30	174 (154-197)	325 (290-370)	5,220 (4,620-5,910)
10	60	168 (153-185)	315 (290-350)	10,080 (9,180-11,100)
7	240	88 (79-99)	165 (140-185)	21,120 (18,960-23,760)

^aLC₅₀ represents the concentration lethal to 50% of the animals

^b95% confidence limits in parenthesis

^cLC₅₀ represents the exposure (concentration x time) lethal to 50% of the animals

^dNo confidence limits

The American Conference of Governmental Industrial Hygienists has been publishing lists of TVL's from time to time over many years. These TLV's have been adopted as the official maximum exposure levels by NIOSH. The current established TLV for NO₂ is 5 ppm (about 9 mg/m³) (5).

The information contained in this document pertains to the most biologically active of the three nitrogen compounds mentioned above - NITROGEN DIOXIDE (NO₂).

For the purpose of this review, acute toxicity is defined as that resulting from a single exposure, injection, administration, or application (skin). Under acute effects will also be included in vitro observations. Subchronic toxicity is defined as that resulting from repeated (continuous or intermittent) treatment during a 90-day experimental period or less (short-term tests). Chronic toxicity is defined as that resulting from continuous treatment over a period of 2 years or more (long-term tests or lifetime tests).

Typical examples will be selected to illustrate toxic endpoints.

Information Sources - The information contained in the body of this document is derived from the following sources:

- o Nitrogen oxides - Committee on Medical and Biologic Effects of Environmental Pollutants. National Academy of Sciences, Washington, D.C. 1977.
- o Low-Level Air Pollution Effects on Experimental Animals. A Review S.D. Talisayon Cornell University, Ithaca, N.Y. 1972. Distributed by NTIS.

- o Conference on Health Effects of Atmospheric Salts and Gases of Sulfur and Nitrogen in Association with Photochemical Oxidant, Held at Newport Beach, California, January 7-8, 1974. Vol. II., T. Timothy Crocker, Distributed by NTIS.
- o Six Years of Research in Air Pollution. 1955-1961. DHEW, Washington, D.C.
- o Citations identified through a Tracor Literature Search.
- o Occupational Diseases, a Guide to Their Recognition Revised Edition, 1977. DHEW Ed. M. M. Key, A.F. Henschel, J. Butler, R.N. Ligo, I.R. Tabershaw.
- o Report of the Ad Hoc Committee Meeting on Nitrogen Dioxide Air Quality Standard April 18, 1962. State Department of Public Health, California.
- o Health Effects of Motor Vehicle Exhaust. J.R. Goldsmith. Presented to Motor Vehicle Pollution Control board, Los Angeles, August 11, 1961.

Matrix. The pertinent effects of NO₂ contained in this document will be summarized (see appended matrix). Where experimental data were not available, human data were included both in the text and in the matrix.

II. ACUTE TOXICITY (including in vitro tests)

1. Hematologic Effects

Leucocytes. Guinea pigs exposed to $123 \text{ mg/m}^3 \text{ NO}_2$ showed a 50% decrease in leucocyte counts ($6000/\text{mm}^3$ to $3000/\text{mm}^3$) in animals that survived to 2 weeks (13 of 15 animals) (2).

Blood Platelets

In one study, a group of dogs exposed to 73.3 and 99.6 mg/m^3 (39 and 53 ppm) for 60 minutes, 97.8 and 160 mg/m^3 (52 and 85 ppm) for 15 minutes and 235 and 308 mg/m^3 (125 and 164 ppm) for 5 minutes resulted in no changes in the hematocrits and blood platelet counts determined 4, 24, 48, 72 hours after exposure (1).

Methemoglobin (MeHb). Methemoglobin was detected in the blood of cats exposed to 188 mg/m^3 (100 ppm) after approximately 1 hour of exposure. However at 282 mg/m^3 (150 ppm) NO_2 and above, the blood of the animals tested contained MeHb after 1 hour of exposure, but the MeHb was no longer measurable 1 to 2 hours after animals were returned to clean air. In these experiments, the NO_2 values reflected the total oxides of nitrogen present in the exposure chamber; the concentration of NO_2 was not evaluated (6).

Dogs exposed to NO_2 (2%) by inhalation or iv showed marked increase in MeHb levels (7, 8). Lower increases were found at lower doses (0.1 - 0.5%). There was no evidence for the formation of other compounds such as nitric oxide hemoglobin, nitric oxide methemoglobin and nitrate methemoglobin (7).

2. Bone Marrow Changes

There were no reports on NO_2 effects on bone marrow in experimental animals or humans.

3. Immunologic Effects

Female guinea pigs were given intraperitoneal (ip) injection of 0.25 mg of egg albumin and 2.5 mg of bovine serum every other day. The injection was repeated 5 times. Three days after the last injection, the animals were exposed to 40 and 80 ppm of nitrogen dioxide for 30 minutes. Thirty minutes later they were exposed to the aerosolized antigen. Immediately after the initiation of inhalation of antigen, almost all the animals pre-exposed to more than 40 ppm of nitrogen dioxide had severe dyspnea attacks and many of them died, whereas most of the controls showed only a slight, labored breathing (9).

Role of Specific Immune Mechanisms. The role of the alveolar macrophage as a central mechanism in the lung for defense against inhaled bacteria provides a logical mechanism for the expression of specific cellular and humoral immunity. The presence of immune globulin in alveolar macrophages has been demonstrated. Studies on pyrogenic pulmonary infections in diseases of immunoglobulin deficiency, as in congenital or acquired hypo- or dysgammaglobulinemia and in lymphatic leukemia, suggest that macrophage effectiveness is solely dependent on specific humoral immunity (10).

Valand et al. (11) showed that alveolar macrophages harvested from rabbits that had received intratracheal injections of para-influenza-3 virus were resistant to in vitro challenge with rabbit pox virus. However, if the rabbits were exposed to NO_2 (47 mg/m^3 ; 25 ppm) for 3 hours soon after challenge with the virus, during the challenge or up to 24 hours before the challenge, this resistance did not develop. This refractory stage lasted for 96 hours. In vitro the macrophages isolated from exposed rabbits were unable to produce interferon when inoculated with the influenza virus. Exposure to NO_2 also increased the adsorption rate of the virus in the lungs of rabbits but did not enhance infectivity by the virus.

Ehrlich and his coworkers demonstrated in a series of studies that acute exposure to low concentrations of nitrogen oxide reduces the resistance in mice (12, 13, 14, 15) and in squirrel monkeys (16, 17, 18, 19) to bacterial pneumonia. This increased susceptibility is evidenced by increased mortality rate, reduced lifespan, and reduced ability to clear the viable bacteria from the lungs. In their experiments, Ehrlich et al. exposed animals to atmospheres containing measured quantities of NO_2 before and after respiratory challenge with aerosols of virulent Klebsiella pneumoniae, (K. pneumoniae).

In studies of acute exposure, mice were exposed to 2 hours of NO_2 at concentrations of 2.8-47.0 mg/m^3 (1.5-25 ppm), and then challenged with an aerosol of K. pneumoniae within 1.6 or 27 hours after NO_2 exposure. The minimal NO_2 concentration required to produce a statistically significant (p less than 0.05) rise in mortality (2 hrs exposure) was 6.6 mg/m^3 (3.5 ppm), when the infectious challenge occurred within 1 hour after that exposure. When the infectious challenge was delayed, a statistically significant effect was noted after 6 hours, but not after 27 hours following exposure to NO_2 at concentrations of 9.4 mg/m^3 (5 ppm) and above (12). When mice were infected with K. pneumoniae following prior exposure to 47.0 mg/m^3 (25 ppm) for 2 hours, the mortality was increased significantly. This effect was not observed at 4.7 mg/m^3 (2.5 ppm) (15).

A similar increase in mortality was observed in hamsters exposed to 65.8 mg/m^3 (35 ppm) or more of NO_2 for 2 hours and then challenged within less than 1 hour with K. pneumoniae (14).

Henry et al. (18) exposed squirrel monkeys to NO_2 at 18.8-95.0 mg/m^3 (10-50 ppm) for 2 hours then challenged with K. pneumoniae introduced intratracheally. Neither the infectious challenge nor the 2 hour NO_2 exposure (94.0 mg/m^3 , 50 ppm) alone was fatal. When the two were combined, all three monkeys died after treatment.

It was also demonstrated that susceptibility of a species to respiratory infection was partially responsible for the increased mortality rate after NO_2 exposure (20). It was found that the rate of clearance of bacteria from the lungs of mice and hamsters decreased with NO_2 exposure (18). Autopsies of mice that died after bacterial challenge revealed high incidence of purulent exudate in the pleural cavities (14). The lungs of squirrel monkeys exposed to NO_2 and challenged with K. pneumoniae developed massive infection which extended to the kidney, heart, liver, adrenal, spleen and lung. Exposures to increasing NO_2 concentrations produced alveolar abnormalities.

4. Central Nervous System (CNS) Effects

No reports were found on the CNS effects of NO_2 in experimental animals or humans.

5. Behavioral Effects

Six hour exposures of 6.9-39.3 mg/m^3 (3.7-20.9 ppm) NO_2 depressed the voluntary running activity of male mice, when the concentration was 14.5 mg/m^3 (7.7 ppm) or greater. Although the threshold was not identified, it could be narrowed to between 6.9 and 14.5 mg/m^3 . In all cases, activity returned to normal on the first post-exposure day (22).

6. Cardiovascular Effects

Exposure of beagle dogs to 7-16 ppm NO_2 for 1 hour resulted in hypotension, accompanied by a decrease in cardiac output and stroke volume. The heart rate tended to become slower. Systemic acidosis was out of proportion to the rise in arterial CO_2 pressure (PaCO_2) and paralleled the decrease in cardiac output and arterial O_2 pressure (PaO_2) (23).

7. Biochemical and Histochemical Effects

Lung. Thomas et al. (24) exposed rats to NO_2 (1 ppm) for a single 4-hour period. Controls were exposed to ambient air. The animals were killed 2 and 3 days after exposure, and the lungs removed and the mitochondrial fraction assayed for lipid peroxidation. The peroxidative changes did not occur immediately but appeared to reach a maximum between 24 and 48 hours after exposure.

Vasallo et al. (25) measured the in vitro effect of NO_2 on the catalase activity of alveolar macrophage during killing of Staphylococcus epidermidis and Pseudomonas aeruginosa in rabbits. Exposure to 10-15 millimoles of NO_2 for 15 minutes caused a decrease in catalase activity.

While testing the antibacterial activity of extracts of alveolar macrophages (exposed to 50 ppm NO_2 for 3 hours), following phagocytosis by Mycobacterium smegmatis (BCG strain) in rabbits, Myrvik and Evans (26) observed impairment of phagocytic activity of the alveolar macrophage. The results indicated that the impairment could be the result of an inhibition of either glucose metabolism per se or inhibition of energy transfer.

Milanesi et al. (27) studied the activity of cathepsin D in vitro in the lung tissue of rats. The lungs of untreated rats were removed, homogenized and prepared with sucrose-edetate and adjusted to pH 7.2 with KOH. Aliquots of the homogenate were exposed to NO_2 (1 liter/min or 3 liters/min flow rate) for 5 to 10 minutes. No significant change in cathepsin activity was noted as a result of NO_2 exposure.

Guinea pigs were exposed to 40 ppm NO_2 for a total of 4 1/2 hours at one half hour intervals. At the time of death, the lungs were removed and analyzed for LDH and aldolase activities. Levels of both enzymes were significantly elevated (28).

Palmer et al. (29) studied the effect of NO_2 on benzpyrene hydroxylase activity in the tracheobronchial mucosa of rabbits. Rabbits (18 animals) were exposed for 3 hours to 9.4 - 94.1 mg/m^3 (5-50 ppm) of NO_2 . Within 30 minutes of removal from exposure chambers the animals were killed and their tracheae and bronchi (main stem) removed and assayed for enzymatic activity. Exposures to NO_2 even at levels as high as 94.1 mg/m^3 (50 ppm) had no significant effect on benzpyrene hydroxylase activity. The lack of any significant drop in enzyme activity over the week following exposure to NO_2 , supported the anatomical observations that immediate or delayed cellular destruction after exposure was minimal or nonexistent.

Following a 2-hour exposure to 65.8 and 94.0 mg/m^3 (35 and 50 ppm) NO_2 , LDH isoenzyme from lung tissue of squirrel monkeys shifted to predominantly anaerobic band 5 when chromatographed. This change in isoenzyme pattern was present at 2 days, but returned to normal 8 days after termination of the NO_2 exposure (18, 14).

Serum. Guinea pigs were exposed to 40 ppm of NO_2 for a total of 4 1/2 hours at one half hour intervals. The animals were killed (time not specified), the blood removed by cardiac puncture, and the serum analyzed for LDH and aldolase activities. Both enzymes were significantly elevated. The increase in levels of LDH probably indicated tissue damage with subsequent release of tissue enzyme into the blood stream (28). Oxygen consumption was also found to be elevated.

Squirrel monkeys were exposed to 18.8 mg/m^3 (10 ppm) NO_2 for 2 hours. The LDH of lung tissue was measured. It was found that the LDH shifted to a predominantly anaerobic band 5 when chromatographed. This change in enzyme pattern persisted for 2 days but returned to normal 8 days after termination of NO_2 exposure (18, 14).

Liver. Buckley and Balchum (28) exposed guinea pigs to 40 ppm of NO_2 for 4 1/2 hours. The animals were killed (time not specified) and the livers removed and assayed for LDH and aldolase activities. Both enzymes were elevated. Oxygen consumption was measured, and the difference between the treated and control livers was not significant (slight elevation).

Kidney. Guinea pigs exposed to 40 ppm of NO_2 for 4 1/2 hours showed marked increase in O_2 consumption and significantly elevated LDH and aldolase activities (28).

Spleen. Guinea pigs exposed to 40 ppm of NO_2 for a total of 4 1/2 hours showed significantly elevated levels of LDH and aldolase activities (28). Oxygen consumption was found to be accelerated.

Heart. Hamsters were exposed to 9.4-65.8 mg/m^3 (5 and 35 ppm) NO_2 for 2 hours and LDH measured. The enzyme in the heart shifted to predominantly anaerobic band 5 when chromatographed. This change lasted for 2 days but returned to normal 8 days after termination of the NO_2 exposure (18, 14).

8. Effects on Body Weight, Tissues and Organs

Body Weight. Rats, guinea pigs, rabbits, monkeys and dogs exposed acutely to 123 mg/m^3 of NO_2 for 8 hours showed weight loss in the survivors up to 20 days following exposure. High mortality rate was observed among the treated animals. Death occurred as a result of anoxia (3). Mortality and weight loss produced by NO_2 was shown to be dose and time related in rats and rabbits (1). No toxic effects were seen in rats exposed for 5 and 15 minutes to 74 and 33 ppm of NO_2 , respectively (1).

Pulmonary Effects

Pulmonary Function. The effect of short term exposure to NO_2 on pulmonary function has been investigated in the guinea pig (30), monkey (18) and rabbit (31). The guinea pigs showed an increase in respiratory rate and a decrease in tidal volume after 4 hours of exposure to 5.2 ppm or 2 hours of exposure to 6.2 ppm of NO_2 (30). Similar observations were made on monkeys exposed for 2 hours to 28 mg/m^3 (15 ppm) of NO_2 (18). These abnormalities were reversible and exposures to lesser concentrations did not disrupt functional activities. Pulmonary diffusing capacity was reduced in rabbits exposed to 56 mg/m^3 (30 ppm) for 15 minutes (31).

In humans, NO_2 exposure at $1.32\text{-}3.76 \text{ mg/m}^3$ (0.7-2.0 ppm) resulted in increased respiratory and expiratory flow resistance and alveolar - arterial pO_2 gradient and a decrease in compliance and single-breath diffusing capacity (32).

Pathologic changes. Kleinerman and Wright (33) exposed rats, rabbits, and guinea pigs to a single high dose (rabbits, 100 ppm; rats and guinea pigs to 80 ppm) or a low dose (rabbits 25 ppm; rats and guinea pigs 15 to 20 ppm), all for 2-hour periods. Following exposure the animals were sacrificed at 1, 2, 4, 7, 14 and 21 days and the lungs examined histologically. A moderate degree of pulmonary edema, epithelial degeneration and inflammatory exudate in the region of the respiratory bronchioles were observed at 24 hours. By the 4th day macrophage infiltration and intense epithelial degeneration was observed. Repair was almost complete by the 2nd week, as evidenced by disappearance of amorphous cellular debris and fibrin and healing of injured cells, despite continued exposure. This suggested a development of tolerance.

With acute exposure to low concentrations (0.5 ppm for 4 hours or 1.0 ppm for 1 hour) of NO_2 , rats sustained reversible lung tissue changes (31). In tissues of animals sacrificed immediately after exposure the

mast cells were ruptured and disoriented and showed loss of cytoplasmic granules. This occurred primarily in the pleura, bronchi and surrounding tissues but most markedly in the media-stinum. This response seemed reversible in animals sacrificed 24 and 27 hours after exposure, since there were smaller numbers of ruptured mast cells in animals sacrificed at these times. The authors considered that the release of granular material from the lung mast cells in response to NO_2 inhalation signified the potential onset of an acute inflammatory reaction.

Guinea pigs and rats exposed to 123 mg/m^3 of NO_2 for 8 hours showed hemorrhagic and edematous lungs. Histologically, the lungs were not much different from the controls (3).

Eleven beagle dogs were exposed to NO_2 at concentrations of 3-16 ppm for 1 hour. Two control animals were air ventilated for 1 hour. Exposure to 7-16 ppm NO_2 was frequently associated with acute pulmonary edema within 60 minutes, and the mean dry weight/wet weight ratio was 12.9 in the animals (control ratio was 18.2). Ultrastructural examination revealed damaged cell membranes, swelling of mitochondria in alveolar cells with loss of pinocytic vesicles and swelling of endothelial cells (3-12 ppm NO_2 for 1 hour). The inner membranes and cristae of mitochondria disappeared, and blebs appeared in endothelial cells reducing capillary lumina. Intra-alveolar edema examined by light microscopy was associated with impaired surfactant activity and lung compliance. Exposure of 5 ppm or more resulted in decreased recovery of saturated lecithin from lung lavage (23).

Pulmonary changes in five species (mice, guinea pigs, rabbits and dogs), exposed to 5-250 ppm of NO_2 for 5-1440 minutes was studied by Hines et al. (2). Fifty parts per million was a critical concentration; below this value mortality rarely occurred with exposures up to 8 hours. The pathologic changes in the lung developed sequentially with grading of edema, congestion, interstitial irritation bronchiolitis and interstitial fibrosis. These changes persisted in some animals for periods up to 6 months. Toxic responses were separated into four clinical and pathologic

entities: acute asphyxia, secondary to laryngeal edema and spasm; acute pulmonary lung edema, bronchiolitis and pneumonia; and permanent, non-incapacitating residue in the lung. These findings were essentially in agreement with and confirm those of Carson et al. (1) and Kleinerman and Wright (33).

Twelve dogs were anesthetized and exposed to 0.1 NO₂ in O₂ or 0.5% undiluted NO₂. Three dogs recovered; and one dog was killed at 24 hours and 2 dogs at 48 hours. The lungs were examined for histopathologic changes. The changes were focal, including edema, hyperinflation, hemorrhage, desquamation of mucosa and bronchopneumonia. The severity of the lesions was proportional to the duration and concentration of NO₂ (35).

Primary lesions appeared in lung alveoli of squirrel monkeys exposed for 2 hours to 18.8-94.0 mg/m³ (10-50 ppm) NO₂. Progressive alveolar expansion occurred with increasing concentrations of NO₂. At 18.8 mg/m³ (10 ppm) NO₂, many septal breaks appeared and the alveoli expanded markedly. In some areas, large air vesicles with extremely thin septal walls were seen. Other tissues appeared to be normal. At 28.2 mg/m³ (15 ppm) NO₂, alveolar tissue was expanded with minimal wall thinning and patchy interstitial infiltration with lymphocytes. The bronchioles were normal. At 65.8 mg/m³ (35 ppm), NO₂ areas of the lung were collapsed and alveolar septa became very basophilic. The bronchi were moderately inflamed and some showed epithelial proliferation (18). Exposure to 94.0 mg/m³ (50 ppm) NO₂ resulted in extreme vesicular dilatation or total collapse of alveoli, along with extensive edema and lymphocyte infiltration. The bronchi showed epithelial surface erosion and absence of cilia.

In other studies NO₂ exposure at 188 and 280 mg/m³ (100-150 ppm) caused cessation of ciliary movement in rabbit alveolar epithelium (36), inhibition of the rate of clearance of particles by the mucociliary apparatus (790 mg/m³ or 420 ppm for 18 seconds) (37), and marked loss of cilia from the terminal bronchiolar surface (38).

Vascular Permeability and Edema Formation

Sherwin and Richters (39) used the tritium-labeled method for detecting small changes in pulmonary capillary permeability. They used tritium-labeled serum to assess intrabronchial protein leakage, and found that mice exposed to 9.4 mg/m^3 (4.0 ppm) of NO_2 for 14-72 hours showed transient increases in intrabronchial radioisotope. Due to the preliminary nature of this investigation, the data cannot be used for conclusions about edema formation.

Studies with human volunteers on airway resistance indicated acute effects after 15-45 minute exposures to NO_2 concentrations of 2.8-3.8 mg/m^3 (1.5-2.0 ppm). No airway resistance was observed at concentrations less than 2.8 mg/m^3 (1.5 ppm). Changes in airway resistance were reversible. The fatal level for humans is 282 mg/m^3 (150 ppm) and above. These high concentrations caused pulmonary edema or bronchiolitis fibrosa obliterans that resulted in death. Lower concentrations (47 and 140 mg/m^3 ; 25 and 75 ppm) produced pneumonia and bronchitis, which were reversible. Permanent sequelae, resulting from acute exposure, were not found in the follow-up reports (32).

Liver cells. Pace et al. (40) exposed strain L, mouse liver cells and HeLa cells to various concentrations of NO_2 and found that proliferation was retarded at 8600 ppm and 4100 ppm NO_2 . At 2400 ppm, proliferation was retarded in a majority of strain L cultures, but in one strain there appeared to be actual stimulation. At lower concentrations (1500 and 800 ppm) no adverse effects were observed; in fact at times there appeared to be increased proliferation. At 10 ppm of NO_2 for a single 8 hour exposure, or two to three 8 hour exposures on separate days, the final cell population in the experimental groups exceeded those of the control groups. At the same time, the total number of dead cells in the experimental groups was 35-40% greater. It appears therefore that NO_2 had a slight stimulatory effect

concomitant with a cytotoxic effect. This reduced the number of viable cells in the cultures exposed to 10 ppm NO₂ to below that found in control cultures. The net result was cell growth inhibition, which increased with the duration of exposure (40).

Liver tissue of squirrel monkeys given 2-hour exposures to NO₂ were damaged in proportion to the dose. At 28.2 mg/m³ (15 ppm), cells ballooned and developed a clear cytoplasm with displaced nuclei and congested interstitial spaces. At 94 mg/m³ (50 ppm), the lymphocytes infiltrated liver tissue and there was centrilobular necrosis (18).

Kidney. Exposure of rats to low concentrations of NO₂ (90, 72 ppm) for 15 and 60 minutes did not affect the kidney to body weight ratio (1). In the squirrel monkey, 2-hour exposure to 65.8 mg/m³ (15 ppm) NO₂ produced swollen renal glomerular tufts and at 94 mg/m³ (50 ppm), there was edema and lymphocyte infiltration (18).

Heart. In the squirrel monkey 2 hours of exposure to NO₂ produced cardiac lesions comprised of interstitial fibrosis (65.5 mg/m³ (35 ppm)) and edema (94 mg/m³ (50 ppm) (18)).

In a series of experiments with healthy male volunteers (20-35 years of age), individuals were exposed to 0.225-56.8 mg/m³ (0.12-30.2 ppm) of NO₂ and the olfactory threshold measured under various conditions. At 0.225 mg/m³ (0.12 ppm) of NO₂, only a few subjects responded to the odor.

Eye and Nose. Fifteen rats, 15 guinea pigs, 3 rabbits, 3 monkeys and 2 dogs were exposed for 8 hours to 123 mg/m³ of NO₂. Signs of eye and nose irritation were noted during the hours of exposure. At the end of the 8-hour exposure period, the rabbits' eyes showed signs of corneal opacity (at 8 hours post-exposure) although still reactive to light. The opacity was persistent during the 20-day post-exposure observation period (3). Exposure at lower doses (90, 72 ppm) for shorter periods (15 and 60 min) resulted in eye irritation and respiratory distress in rats, lasting over a period of 2 days, but there were no deaths (1).

Studies on human volunteers show that NO_2 odor is perceivable at concentrations of 0.23 mg/m^3 (0.12 ppm), while changes in dark adaptation occur at $0.14\text{-}0.5 \text{ mg/m}^3$ (0.075-0.26 ppm). These responses were not accompanied by pathological effects and were in all cases immediately reversible (28, 41).

9. Cytologic and Cytogenetic Effects

No reports were found on the effects of NO_2 on mitosis and chromosomes.

10. Molecular Effects

Structural Proteins. Nitrogen dioxide can alter the configuration of lung proteins, collagen and elastin (42). Rabbits were exposed to 1.9 mg/m^3 (1 ppm) (1 rabbit) or 9.4 mg/m^3 (5 ppm) (2 rabbits) for 1 hour. Four animals served as controls. All animals were killed immediately after exposure, except one receiving 9.4 mg/m^3 (5 ppm) which was sacrificed at 24 hours after exposure. The lungs were excised, and converted to a lipid-free powder from which collagen and elastin were extracted. Differential ultraviolet spectrophotometry indicated that the molecular structures of both collagen and elastin were altered in both exposure groups. These alterations were reversible within 24 hours. The authors suggested that the metabolic activity of the lung tissue was reduced in the highly acidic environment produced by exposure to NO_2 and speculated that repeated exposure to NO_2 , with concurrent repeated denaturation of elastin and collagen, may be a factor in the etiology of pulmonary emphysema.

11. Reproductive and Teratogenic Effects

No reports were found on the effects of NO_2 on reproduction in the adult experimental animal, nor was any information found on its effect on the fetus.

12. Metabolism

The few studies on inhaled NO_2 indicate that it is probably distributed throughout the lung and that a high percentage of it is absorbed (43, 44). Von Nieding (43) and his co-workers exposed volunteers to NO_2 and analyzed the gas concentration in exhaled air to determine the extent of absorption. The results indicated that 81-87% of exhaled nitrogen is absorbed during normal ventilation and that more than 90% is absorbed during periods of maximum ventilation.

Ichioka (43) designed a model airway to simulate the dynamic behavior of NO_2 within the respiratory system. When NO_2 at 9.4 mg/m^3 (5.0 ppm) flowed through the model, most of the pollutants penetrated the more distal regions. More exacting studies of intrapulmonary distribution and absorption, such as those available for SO_2 , have not been performed with NO_2 .

The effects of NO_2 on $^{14}\text{CO}_2$ production from ^{14}C -labeled substrates was studied by Vassallo et al. (25). The results indicated that NO_2 in high concentrations (10 mM or higher) increases glucose and pyruvate oxidation in resting alveolar macrophages. These effects are present also in phagocytosing alveolar macrophages except for comparable $^{14}\text{CO}_2$ production rates from ^{14}C -6-glucose in alveolar macrophages during phagocytosis in the presence or absence of NO_2 . Since ingestion rates were impaired by these concentrations of NO_2 , even the similar $^{14}\text{CO}_2$ production from ^{14}C -6-glucose could still represent stimulation of glucose oxidation by NO_2 . These effects on glucose were not persistent, as evidenced from the lack of stimulation of ^{14}CO production from either ^{14}C -1-glucose or ^{14}C -6-glucose when the labeled precursors were added 90 minutes after NO_2 (10 mM). The mechanisms of these actions are not known. It was hypothesized that the oxidation of the pyridine nucleotides regulates the pentose shunt and glycolytic pathway.

13. Carcinogenesis

No reports were found on the acute carcinogenic effects of NO₂ in experimental animals and humans.

II. SUBCHRONIC TOXICITY (Short-term tests)

1. Hematologic Effects

Leucocytes counts. Steadman et al. (3) exposed rats, guinea pigs, rabbits, dogs and monkeys to 67 mg/m^3 of NO_2 for 8 hours/day, 5 days/week for 30 exposures. There was no difference in the leucocyte counts between treated and control animals at the end of the experiment. A similar observation was made on the same 5 species exposed continuously (24 hours/day) for 90 days to lower NO_2 concentrations (0.9, 1.0, 9.2, 21.3 and 21.6 mg/m^3) (3).

Hemoglobin. Steadman et al. (3) also determined hemoglobin and hematocrit levels in the blood under the circumstances described above and found no difference between control and treated animals.

Polycythemia. Freeman et al. (45) found polycythemia in rats allowed to breathe 3.8 mg/m^3 (2 ppm) NO_2 or more continuously. The concentration of the erythrocytes rose within 2-3 weeks and achieved levels which were 40-100% above baseline values. After some delay, both hematocrit and hemoglobin level rose, but to a lesser degree so that the fully developed polycythemia was characterized by a reduction in mean and corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The cellular diameters were normal. The blood of monkeys (*M. speciosa*) exposed to 3.8 and 16.9 mg/m^3 (2 and 9 ppm) NO_2 followed a similar course (44, 45).

Methemoglobin (MeHb). MeHb appeared in the blood of rabbit and rats exposed to fumes produced in the electric arc welding process (46). Rats exposed for 6 hours/day 5 days/week for 43 days to fumes containing 45.1 mg/m^3 (24 ppm) NO_2 developed 4.5 to 21.7% MeHb (mean of 13.6%). Detectable levels were still present on the 11th day of the post-exposure period. Rabbits similarly exposed for 45 days formed an average of 2.8% MeHb (range 0.9-4.5%). On the 6th day of post-exposure period, detectable amounts remained in male rabbits only.

Male rats exposed to 132 mg/m^3 (70 ppm) NO_2 for 6 hours/day formed 2.6% MeHb after the first day of exposure and 3% by the 3rd day. Three days after exposure, the levels were within the control range. In these experiments, it was estimated that between 12-17% of the nitrous fumes existed as NO (46).

2. Bone Marrow Changes

No reports were found which showed the effects of NO_2 exposure on bone marrow of experimental animals or humans.

3. Immunologic Effects

Female guinea pigs (12-18 animals) were exposed to 40 and 70 ppm of nitrogen dioxide. Thirty minutes later they were exposed to aerosolized antigen for 45 minutes. This treatment was repeated 5-7 times at intervals of a few days, or every day to avoid systemic anaphylaxis. Blood was drawn 2 weeks after the last inhalation of the antigen. The hemagglutination test, a passive cutaneous anaphylaxis test and a radioimmunodiffusion test were used to examine the serologic effects. Immediately after the 5th inhalation of the aerosolized antigen, a severe anaphylactic reaction occurred in guinea pigs exposed to 70 ppm of nitrogen dioxide but not with 40 ppm. It was concluded that repeated 30 minute pre-exposure to 70 ppm of nitrogen dioxide enhanced sensitization via the airways in the guinea pigs (47).

It has been demonstrated that exposure to low concentrations of NO_2 reduces the resistance of several laboratory animals to bacterial infection (see acute studies). This reduced resistance is reflected in the increased mortality rate, reduced life span, and reduced ability to clean viable bacteria from the lungs. Mice challenged with mouse-adapted type A influenza virus strain PR 8_x 2 hours after intermittent exposure to 10 ppm NO_2 for 2 hour/day for 3 or 5 days or after continuous exposure to 0.5-1.0 ppm (NO_2) for 39 days, showed increased mortality, and interstitial pneumonia (48).

4. Central Nervous System (CNS) Effects

No reports were found on subchronic effects of NO_2 in experimental animals or humans.

5. Behavioral Effects

No reports were found on subchronic effects of NO_2 in experimental animals or humans.

6. Cardiovascular Effects

No reports were found on the subchronic effects of NO_2 in experimental animals or man.

7. Biochemical and Histochemical Changes

Lung. Studies of alteration in enzyme activity levels in lung tissue resulting from inhalation of NO_2 have shown that these changes tend to appear early and may precede clinical signs. Buckley and Balchum (28, 49, 50); exposed male guinea pigs (70 animals) continuously for up to 6 weeks to 10 and 15 ppm of NO_2 . The animals were killed at various time during the experiment (26-42 days) and their lungs assayed for lactic dehydrogenase (LDH), and aldolase activities. There was a consistent elevation of LDH activity in the lung tissue throughout the experiment, whereas aldolase activity was significantly elevated at 12 days and significantly reduced after 26 days of exposure.

Rats were exposed to 1.0 ppm NO_2 for 4 hours daily during a 6-day period. Controls were forced to inhale ambient air. The animals were sacrificed immediately after exposure and at various times thereafter. The livers were removed and the mitochondrial fraction quantitated for lipid peroxidative changes. The changes reached peak values between 24-48 hours after exposure (34).

Blood. In a preliminary test, 4 guinea pigs were exposed to 0.36 ppm NO_2 continuously for 1 week to determine the effect on D-2, 3-diphosphoglycerate (2, 3-DPG) content of red blood cells. Results showed a significant increase (p less than 0.05) for exposed animals as compared with nonexposed controls (4 animals) (51).

Ripperton and Johnston (52) exposed weanling rats continuously and dynamically to concentrations of NO_2 of 0.5 ppm and less for 2, 4, 5 and 6 weeks (8, 7, 11 and 20 rats, respectively). At the time of sacrifice, blood catalase concentrations were measured. The catalase activity in the blood was decreased at all times with the decrease being statistically significant for the 5th and 6th week. Assay of glutamic acid and aspartic acid did not show significant differences between mean control and experimental values for glutamic acid, but for aspartic acid the difference between the mean values of the two groups was statistically significant.

Liver. Buckley and Balchum (28, 49, 50) studied the effect of NO_2 on the LDH and aldolase activities of guinea pig liver. The animals were exposed to 10 or 15 ppm of NO_2 for varying lengths of time (26 days-10 weeks), after which they were killed and the livers removed for enzyme determination. The response of the liver was variable with LDH and aldolase activity levels showing increase with exposure, while QO_2 data were variable.

Kidney. Kidneys from guinea pigs exposed to 10 and 15 ppm of NO_2 for varying periods of time (26 days-10 weeks) showed elevated QO_2 and LDH activity while aldolase activity was decreased significantly following 26 and 32 days of exposure (28, 49, 50).

Spleen. Guinea pigs exposed to 15 ppm of NO_2 for 10 weeks showed significant acceleration of O_2 consumption, LDH and aldolase activities in the spleen (28).

8. Body Weight, Tissues and Organs

Body Weight. Rats, guinea pigs, dogs, rabbits and monkeys were exposed to NO_2 at 67 mg/m^3 for 8 hours/day, 5 days/week for a total of 30 exposures. All animals exhibited varying degrees of lethargy and dyspnea. The monkeys were most susceptible and the mortality rate among all species was high. The body weight gains in surviving animals in all species did not differ significantly from those of controls. A similar observation was made on the same five species exposed continuously (24 hours/day for 90 days) to NO_2 concentrations of 0.9, 1.0, 9.2, 21.3 and 21.6 mg/m^3 (3) or to 0.5 ppm and less (rat) for 2, 4, 5 and 6 weeks (24 hour/day) (52).

Pulmonary Effects

Pathologic Changes. Kleinerman and Wright (33) exposed rats, rabbits and guinea pigs to 15-25 ppm of NO_2 for 2-hour periods for 5 successive days. The animals were sacrificed at 1-21 day intervals after the last exposure and the lungs examined histologically. Edema and inflammation were less severe in the early stage, but peribronchial and perivascular chronic inflammation were very marked in the rat and guinea pig. Epithelial regeneration was not prominent and repair was almost complete by 7 days post-exposure, but traces of inflammation persisted.

In another study hamsters were exposed to NO_2 continuously for 10 weeks at NO_2 concentration of $85-103 \text{ mg/m}^3$ (45-55 ppm) for 21-23 hours daily (53). At the end of the exposure period, animals were sacrificed and the lungs removed and examined histologically. Control animals were exposed to air without addition of NO_2 and sacrificed according to the same schedule as the experimental animals. The tissues of the NO_2 exposed animal did not show emphysematous changes; however, hyperplastic, hypertrophic changes and desquamation of epithelial cells were observed.

An ultrastructural quantitative study of the lungs of 12 guinea pigs exposed continuously to 10 ppm of NO_2 for 6 weeks and 8 control lungs revealed increased ratio of type 2 pneumocytes to other cells in the exposed group. The findings, derived from 304 electron microphotographs indicated thickening of blood gas barrier through replacement of ultrathin type 1 cells by cuboidal or columnar type 2 pneumocytes. The lungs of the exposed animals also exhibited more frequent intracellular and extracellular lipid bodies (54).

Rats, rabbits, guinea pigs, dogs and monkeys exposed to 67 mg/m^3 of NO_2 for 8 hours/day 5 days/week for a total of 30 exposures showed vascular congestion and focal hemorrhage in the lungs. This was also seen in controls but to a lesser degree and may have been in part due to terminal anoxia (3). No adverse effects were seen with lower doses of NO_2 (0.9, 1.0, 9.2, 21.3 and 21.6 mg/m^3) on any of the above five species following continuous exposure (24 hours/day for 90 days) (3).

Hamster and guinea pigs exposed to NO_2 for 2 hours/day, 5 days/week for 3 consecutive days at 41 mg/m^3 (22 ppm) concentration developed multifocal type of emphysema 11 months or more from initiation of exposure (guinea pigs) (55). An ultrastructural study of the lung of 12 guinea pigs exposed continuously to 18.8 mg/m^3 (10 ppm) for 6 weeks showed great increase in type II pneumocytes, presumably resulting from hyperplasia (54).

Giordano and Marrow (56) studied the effect of NO_2 on the mucociliary clearing rate in anesthetized rats after a 6-week exposure to NO_2 at "first edge time" and a "20% transport time". Exposure to 11.3 mg/m^3 (6 ppm) produced a significant inhibition of the mucociliary clearance mechanism. This decrease in mucociliary activity was not accompanied by any observable abnormality of the airways. The authors suggested that the point of interaction between NO_2 and the mucociliary apparatus might be the energy source of the cilia. This possibility was

borne out by experiments which showed that NO_2 can affect lung homogenate enzymes linked to ATP formation (49). Other possible reactions affecting the mucus are those which modify the cross linking of polymer chains that affect the viscosity of the mucus (57).

Mice are more susceptible to the toxic effects of NO_2 than rats. Continuous exposure to NO_2 at 0.94 mg/m^3 (0.5 ppm) for 3 months caused loss of cilia, alveolar cell disruption and respiratory bronchiolar obstruction (58). Longer exposures result in more severe bronchiolar inflammation, pneumonitis and increased alveolar surface, secondary to alveolar expansion rather than septal breakage (59).

Continuous exposure for 3 days to 3.8 mg/m^3 (2 ppm) NO_2 resulted in changes in the terminal bronchiolar epithelium of rats. The epithelium changed from an active, inhomogeneous lining layer to a uniform layer of enlarged cells. This change was further documented by the demonstration of abnormal ciliogenesis. Although ciliary basal bodies developed normally, they failed to orient appropriately at the apical surfaces of the cells. They either formed no cilia or directed them intracytoplasmically into vacuoles. Intracytoplasmic, crystalloid inclusion bodies also developed in time (60, 61, 62).

Distal airways from lungs of healthy (control) rats and from rats exposed to 15 ppm of NO_2 for 2 and 7 days were dissected out and examined with a scanning electron microscope. Three types of bronchiolar epithelial cells were identified (type I, type II and alveolar brush cells). Morphological surface changes in response to NO_2 exposure included loss of cilia and increased uniformity of surface in the terminal bronchioli. The type I cell was substantially replaced by microvilli-covered cells, and there was an increase in the amount of debris and macrophages in response to NO_2 exposure (38).

Although pathologic changes in the lung and alveolar epithelium were seen in a majority of rat studies, in a few rats the lung tissues were not affected following NO_2 exposure (0.5 ppm, 24 hours/day for 2, 4, 5 and 6 weeks) (52).

Cell proliferation in the peripheral bronchus occurred in mice exposed to $0.94-1.5 \text{ mg/m}^3$ ($0.5-0.8 \text{ ppm}$) for 30-45 days (63). Electron microscopic examination of the lungs revealed slight ciliary abnormalities, mitochondrial degeneration of clara and alveolar cells and edematous changes within the cytoplasm. Germ free mice exposed to 75 mg/m^3 (40 ppm) for 6-8 weeks developed epithelial abnormalities in the bronchial tree. These were most prominent at the terminal bronchioles and in the alveoli immediately surrounding the terminal bronchioles (64). Inflammation and edema, and exudation of white blood cells were also observed. Striking changes in both collagen fibrils and basement membranes were revealed with the electron microscope in the lungs of rats exposed to 17 ppm of NO_2 for 90 days (continuous exposure). The exposed animals showed mild respiratory distress when killed after 3 months exposure. In control animals collagen fibrils underlying the terminal bronchiole increased in size with age and appeared stellate in cross-section. In marked contrast, 3-month-old rats exposed to 17 ppm of NO_2 developed very large fibrils, up to 15 times the normal diameter (65).

The collagenic changes in the lung were detectable as early as 6 days following daily exposure of rabbits to 0.25 ppm for 21 hours/day. Electron microscopy of isolated collagen showed that some denaturation remained 7 days after the final exposure (42).

Vascular Permeability and Edema Formation

In one ultrastructural study, early edematous changes occurred within alveolar epithelial cells and alveolar interstitium in mice that were exposed to continuous NO_2 at $0.94-1.5 \text{ mg/m}^3$ ($0.5-0.8 \text{ ppm}$) for 30 to 45 days.

Sherwin and Carlson (66) used the disc-gel electrophoresis method to measure intrabronchial protein in guinea pigs that were exposed for a week to nitrogen dioxide at 0.75 mg/m^3 (0.4 ppm). Their preliminary results showed that protein transudation resulted from this exposure.

Parkinson and Stephen (38) exposed rats to 15 ppm NO_2 for 1, 2 and 7 days, and Stephen et al. (65) who exposed rats to 17 ppm for 90 days; no pulmonary edema was observed.

Liver. Ripperton and Johnston (52) exposed weanling rats to 0.5 ppm or less of NO_2 continuously (24 hours/day) for 2, 4, 5 and 7 weeks and found no histopathologic effect in the livers of exposed animals.

9. Cytologic and Cytogenetic Effects

The effects of acute exposure to NO_2 on in vivo alveolar epithelial cells was studied by Evans et al. (67). Rats were exposed to 15 ppm NO_2 for 48 hours. They were then removed from the chambers and injected with tritiated thymidine and killed at hourly intervals thereafter up to 12 hours. Light autoradiographic study of labeled cells indicated that type 2 cells are stimulated to divide. The mitosis is normal with both sister cells moving apart over the basement membrane at anaphase. Following division, the evidence suggests that one or both type 2 sister cells may transform into type 1 cells.

10. Molecular Effects

No reports were found on the effects of NO_2 on DNA, RNA, and protein synthesis in experimental animals or humans.

11. Reproductive and Teratogenic Effects

Shalamberidze and Tsereteli (68) reported on the effect of NO_2 on the reproductive functions in albino rats exposed to NO_2 concentrations of 23.6 mg/m^3 daily for 1 hour/day for 3 months. The exposure caused disturbances of the estrual cycle and had an adverse effect on genital functions. Exposure to lower concentrations under similar experimental conditions did not result in any adverse effects. No teratogenic observations were reported.

In another study male and female (5 animals) rats were kept together during the period of continuous exposure to 0.5 ppm of NO_2 . Four other females along with males were exposed to ambient air (controls). Of the latter group, 1 female had no litter. In terms of littering, a delay was observed in the experimental group as compared to controls (no other information given) (52). This was interesting in light of the earlier studies of Kotin et al. who observed a definite decrease in the ability of mice to conceive after chronic exposure to air pollutants (69).

12. Metabolism

Information on the metabolism of NO_2 in experimental animals is scarce. Sherwin and Layfield (70) analyzed urinary proteins in the guinea pig following exposure of 7 animals to continuous 0.5 ppm NO_2 : 3 animals for 7 days and 4 for 14 days. All treated animals had consistently higher levels of urinary protein than control animals (p less than 0.01). The increase involved all 3 of the major protein groups demonstrated by the disc-gel procedure, putatively albumins, combined alpha-, beta-globulins and gamma-globulins. The same results were obtained with shorter exposure time (4 hours/day). Histological examination of the kidney failed to show any overt abnormality.

13. Carcinogenesis

Ito et al. (48) have reported on the occurrence of adenomatous proliferations of bronchial and bronchiolar epithelium in mice challenged with influenza virus after continuous exposure to low levels of NO_2 (0.5-1.0 ppm for 39 days).

III. CHRONIC TOXICITY (Long-term Tests)

1. Hematologic Effects

Leucocytosis. Leucocytosis occurred in the peripheral blood of rabbits exposed to both 2.5 and 5.6 mg/m³ (1.3 and 3.0 ppm) NO₂ for 15 to 17 weeks, but it receded with cessation of exposure. Phagocytic activity was depressed and both leucocytic and phagocytic alterations were greater at the higher NO₂ exposure (71).

Hemoglobin. Reductions in hemoglobin levels were observed at 9-10 weeks exposure to 2.5 and 5.6 mg/m³ (1.3 and 3.0 ppm) of NO₂. This condition was maintained until the end of the experiment (15-17 weeks) (71). In another study, dogs exposed to 1, 5, and 25 ppm of NO₂ daily for 18 months showed no effects on the hemoglobin or hematocrit (72).

2. Bone Marrow Changes

No reports were found on the effect of NO₂ on bone marrow in experimental animals or humans.

3. Immunologic Effects

A circulating substance with properties similar to a lung antibody appeared in the serum of guinea pigs exposed to 9.4 mg/m³ (5 ppm) NO₂ either 4 hours/day, 5 days/week or 7.5 hours/day, 5 days for up to 5.5 months. In a second group, exposed to 28.2 mg/m³ (15 ppm) NO₂ continuously for 1 year, the antibody reacted in vitro with protein extracted from the lung tissue of control animals. The titers of reactive substances increased with the intensity and duration of exposure, but no absolute values were ascribed to the data because the latex agglutination method employed was not quantitative (73).

Matsumura (1970) reported results of studies on guinea pigs that were sensitized to egg albumin. A 30 minute exposure to NO_2 at 132 mg/m^3 (70 ppm) increased the susceptibility of those guinea pigs to systemic anaphylaxis after inhalation of egg albumin aerosols. Exposure to NO_2 (75 mg/m^3 ; 40 ppm) caused an increase in dyspnea (47).

The effects of chronic exposure to low concentrations of NO_2 on the immunologic response were studied by vaccinating mice with 279 CCA units of chick embryo $\text{A}_2/\text{Taiwan}/1/64$ influenza virus vaccine (74). One group of mice were exposed continuously to NO_2 at 3.8 mg/m^3 (2 ppm); another group was exposed to 0.94 mg/m^3 (0.5 ppm) with daily 1-hour long peaks of 3.8 mg/m^3 (2 ppm) for 5 days a week. Three months after the exposures, the mice were vaccinated with the influenza virus and held in the exposure conditions described for up to 7 months. At various times during the vaccination, the hemagglutination inhibition and SN antibody titers and the concentration of the immunoglobulins were measured. Two weeks after vaccination the SN antibody decreased and seroconversion rates were markedly lower, especially among mice exposed to 0.5 ppm with the daily 1-hour peaks of 3.8 mg/m^3 (2 ppm). After 4 weeks of exposure the SN titers and seroconversion rates did not differ significantly from those in control mice which received filtered air.

Squirrel monkeys (males) continuously exposed to 1.0 ppm of NO_2 for 493 days were challenged 5 times with monkey adapted influenza A/PR/8/34 virus. All monkeys exposed to NO_2 produced serum neutralization antibody within 21 days after virus infection (4 animals), whereas only 1 animal of 3 exposed to filtered air showed a similar response. The difference observed in hemagglutination inhibition antibody titers between NO_2 -exposed and control monkeys was not significant. Examination of lung tissues indicated slight emphysema and thickened bronchial and bronchiolar epithelium only in monkeys exposed to NO_2 and challenged with the influenza virus. No ultrastructural abnormalities were observed in exposed animals (17).

Another study in which squirrel monkeys were challenged with 4 periodic intratracheal injections of mouse-adapted influenza A/PR/8 virus, while exposed to filtered air or NO₂ for 169 days (24 hours/day, 7 days/week) showed no change in the action of the hemagglutination - inhibition antibody. The production of serum neutralizing antibody, however, was delayed. After 133 days, no significant difference was observed in the production of the hemagglutination-inhibition, or serum neutralizing antibodies, body temperature, respiratory function, body weight and hematologic values between control and experimental monkeys. Histopathological examination revealed focal alveolar edema in all but one monkey exposed to NO₂, but no additive effect of NO₂ exposure and influenza virus challenge was apparent (16).

In several studies, mice (14, 13) and squirrel monkeys (19, 75), were exposed to NO₂ for extended periods, and at various intervals during exposure challenged with K. pneumoniae aerosols. Exposure was intermittent (6 hours/day, 5 days/week) or continuous (24 hours/day, 7 days/week). Intermittent exposures appeared to cause greater effects than continuous exposures.

Aranyi and Porta (76) observed a significant decrease in the phagocytic function of alveolar macrophages (against heat killed Escherichia coli) obtained from mice exposed to 0.94 mg/m³ (0.5 ppm) of NO₂ with daily 1-hour peaks of 3.8 mg/m³ (2 ppm) 5 days/week for 3.5-7 months. This did not occur in mice exposed to 3.8 mg/m³ (2 ppm) for the same period.

4. Central Nervous System (CNS) Effects

No reports were found on the effect of NO₂ on the CNS of laboratory animals, but in humans health clinic records of residents living in close proximity (1 kilometer) to a chemical plant showed that nervous system effects (effects not defined) were seen more frequently in this group

then in another group living further away (3 kilometers). In this area NO_2 levels in the atmosphere exceeded maximum permissible concentration (MPC) by a factor of 5.8-12/MCP, 0.10 mg/m^3 ; 0.053 ppm for 24-hour average (32).

5. Behavioral Effects

No reports were found on the effect of NO_2 on the behavior of experimental animals or humans.

6. Cardiovascular Effects

No reports were found on the effect of NO_2 on the cardiovascular system in laboratory animals or humans.

7. Biochemical and Histochemical Changes

Lung. Male rats were exposed chronically to an average of 2.9 ppm of NO_2 , 24 hours/day, 5 days/week for 9 months. The major effects of NO_2 were a significant decrease in lung lipid content and a marked decrease in the percentage of total saturated phospholipid fatty acids. This reduction in saturation was the result of a decrease in the percentage of palmitic acid. The authors suggested that lung lipid metabolism may be the underlying mechanism which leads to some of the pulmonary effects following long-term exposure to small amounts of NO_2 (77).

Blood. The serum immunoglobulin concentration in non-vaccinated mice was altered during the 3 months exposure period to NO_2 . In general, IgA decreased while IgM, IgG, and IgG₂ increased. During the 28 weeks after vaccination, exposure to NO_2 did not further influence IgA concentration. Serum IgM, IgG, and IgG₂ were higher in mice exposed to NO_2 than in those maintained in filtered air. More specifically, mice consistently exposed to 0.94 mg/m^3 (0.5 ppm) with daily 1-hour peaks of 3.8 mg/m^3 (2 ppm) showed consistently higher immunoglobulin

concentrations before and after vaccination. Furthermore, mice exposed to filtered air before vaccination and to NO_2 at 3.8 mg/m^3 (2 ppm) after vaccination and those exposed to NO_2 at 3.8 mg/m^3 (2 ppm) before vaccination and to filtered air after vaccination showed similar increases in serum immunoglobulins (74).

These results suggest that fluctuation in atmospheric NO_2 has more influence on the immune response than constant (although high) concentrations of NO_2 . Continuous exposure of mice to 3.8 mg/m^2 (2 ppm) for 10 months did not influence the antibody formation or the immunoglobulin concentrations. Conversely, exposure to 0.94 mg/m^3 (0.5 ppm) with daily 1 hour peaks of 3.8 mg/m^3 (2 ppm) decreased the ability to form SN antibody and significantly altered the concentrations of serum IgM and IgG₂ (74).

Increased serum levels of beta lipoproteins cholesterol and albumin have also been reported in humans exposed to NO_2 levels of 0.10 mg/m^3 (0.053 ppm) or less for 3-hours/day for 150 days (32).

Wagner et al. (72) exposed dogs to 1 and 5 ppm of NO_2 for 18 months and found no significant alterations of basic alkaline phosphatase and magnesium activated phosphatase activities in sera of animals exposed to these concentrations of NO_2 .

In another study in which rabbits were exposed to 2.5 and 5.6 mg/m^3 (1.3 and 3.0 ppm) NO_2 for 2 hours daily for 15 to 17 weeks, a decrease in serum albumin and increase in globulin fractions was observed (71).

8. Effects on Body Weight, Tissues and Organs

Body Weight. Studies on the effects of chronic exposure of NO_2 have produced conflicting results with respect to weight loss.

No significant reduction in the rate of weight gain was observed in rabbits, guinea pigs, rats or hamsters exposed to 1.9, 9.4 or 47 mg/m^3 (1, 5 and 25 ppm) NO_2 and dogs to 1.9 and 9.4 mg/m^3 (1 and 5 ppm)

NO₂ for 6 hours a day for 18 months (72). Furthermore, no significant difference in weight gains appeared in mice exposed to 940 ug/m³ (0.5 ppm) NO₂ for 6, 18 or 24 hours/day, 5 days/week for up to 12 months (13).

In contrast, rats exposed continuously to 22.6 mg/m³ (12 ppm) NO₂ for 9 months continued to grow, but their body weights remained 20% less than those of control animals (78, 79).

One observer reported (71) a 10% weight loss in rabbits exposed to 5.6 mg/m³ (3 ppm) NO₂ for 15 weeks, while controls gained 11% in their weight. The effect was apparently dose-dependent; rabbits exposed to 2.5 mg/m³ (1.3 ppm) NO₂ for 17 weeks increased their weight by 2% as compared to an 8% increase by controls.

Pulmonary System

Pulmonary function. Continuous (lifetime) exposures of rats to NO₂ caused respiratory rates to increase and tidal volumes (volume of air inhaled in an average single breath) to decrease during exposures to concentrations of 1.5 mg/m³ (0.8 ppm) (80).

Other pulmonary functions have been examined, but showed no evidence of NO₂ effects. Guinea pigs exposed to 9.4 mg/m³ (5 ppm) NO₂ for either 4 or 7.5 hours/day, 5 days/week for up to 5.5 months had no changes in respiratory flow resistance (73). Four rabbits exposed to 47.0 mg/m³ (25 ppm) NO₂ continuously for 18 months showed a transient increase in the rate of oxygen consumption, which reverted to normal within 48-72 hours after exposure. In 16 rabbits exposed to 1.9 and 9.4 mg/m³ (1 and 5 ppm) NO₂ for the same length of time, no change in oxygen consumption occurred (72).

Pathologic Changes. Histopathology of mouse lung tissue following chronic exposure to 0.5 ppm of NO₂ (0.94 mg/m³) for 6, 18 and 24 hours daily for 3-12 months revealed expanded alveoli which increased

with exposure time. The general impression was of early bronchiolar inflammation with reduction of distal airway size and a concomitant expansion of alveoli. The overall lesions appeared to be consistent with the development of early focal emphysema (59).

In rats exposed to NO_2 at 18.8, 23.5 or 47 mg/m^3 (10, 12.5 or 25 ppm) for 3 or more months, the thoracic cavities became larger, dorsal kyphosis developed, and the animal acquired a bloated appearance. There was distention of the alveolar ducts, dilation of alveoli and hyperplasia of bronchiolar epithelium. Alveolar septa were missing occasionally, but the parenchyma was unusual (81, 82). These pathologic conditions were similar but not identical to those of human emphysema, with the major difference being the absence of alveolar necrosis.

The main feature of emphysema is the development of destructive bulbous lesions. These were never found even in rats exposed for a lifetime to NO_2 concentrations of 1.5 and 3.8 mg/m^3 (0.8 and 2.0 ppm). The lungs from these animals were grossly normal, but microscopic examination revealed minor ciliary loss, epithelial hypertrophy and "cytoplasmic blebbing" (45, 82). These animals had a normal life span and died of diseases which were unrelated to NO_2 exposures (81).

Electron microscopic examination of the lungs of rats exposed to NO_2 at 3.8 mg/m^3 (2.0 ppm) revealed hypertrophy and focal hyperplasia in the epithelium of the terminal bronchiole and a loss of cilia. These abnormalities, which appeared on the 3rd day of exposure, disappeared by day 21 despite continued NO_2 exposure (83). After a lifetime exposure to this same concentration, the only abnormality observed was thickening of the basement membrane under the epithelium of the terminal bronchiole due to enlargement of the collagen fibers (65).

Exposure to higher doses (32 mg/m^3 ; 17 ppm) was found to cause more severe ciliary loss, injury to the epithelial lining of the alveoli adjacent to the terminal bronchiole, sloughing of type I alveolar cells, thickening of air-blood barrier and deposit of fibrin along the basement

membrane (83). The most important abnormality in a 2 year-old rat exposed to 32 mg/m^3 (17 ppm) for 610 days was the massive increase in the size of the collagen fibers (65).

Haydon et al. (84) exposed rabbits to continuous atmosphere containing NO_2 at $15\text{-}23 \text{ mg/m}^3$ (8-12 ppm) for 3-4 months and reported destructive changes in alveolar walls and abnormal distention of distal air spaces. These observations are similar to human emphysematous lesions.

Investigations in rabbits ($28\text{-}47 \text{ mg/m}^3$; 15-25 ppm) exposed for 2 years (2 hours/day) to NO_2 failed to find emphysematous changes in the lung (53).

Dogs were found to be resistant to emphysematous changes following exposure to 9.4 mg/m^3 (5.0 ppm) of NO_2 for 15-18 months (72). This observation was confirmed by other investigators (3, 85). Bulbous emphysema has been produced in dogs but only after prolonged (6 months) exposures to high concentrations ($48.9 \text{ mg/m}^3 = 26 \text{ ppm}$) (86).

Stephen et al. (65) exposed rats to 2 ppm NO_2 continuously for two or more years and observed that the rats developed very large collagen fibrils (up to 15 times the normal diameter) underlying the terminal bronchioles. The basement membrane under the epithelium of the terminal bronchiole was also thickened.

Vascular Permeability and Edema Formation. The most important pathophysiologic consequence of exposure to NO_2 is damage of the vascular membranes which results in increased capillary permeability, transudation of protein into alveoli and edema formation. The most commonly used method for estimating edema fluid is the wet: dry weight ratio. This ratio remained unaltered in rats that were exposed to NO_2 (81). Pulmonary weights, both dry and wet, increased proportionally in

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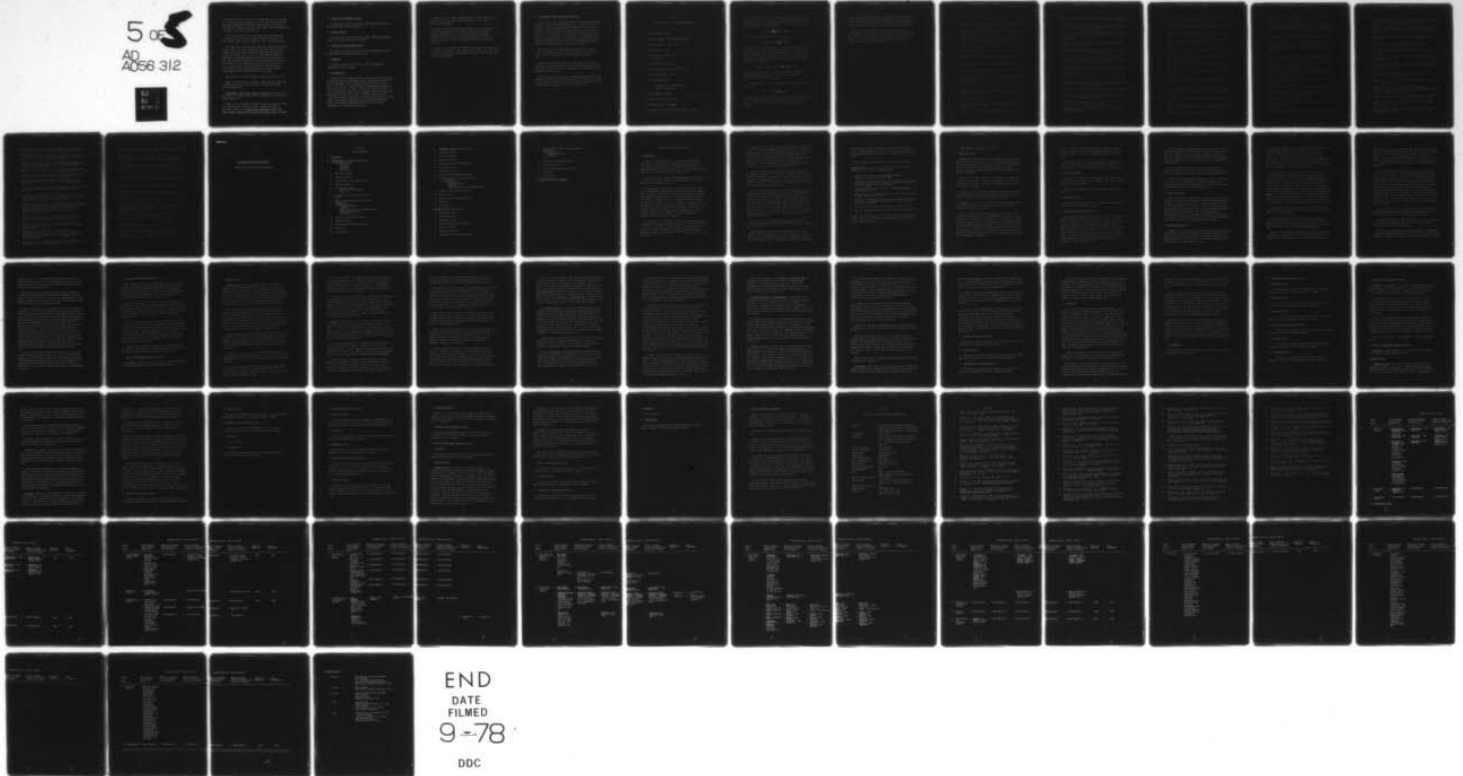
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these animals indicating that mass (cell and fibrous tissues) increased as a consequence of NO₂ exposure (79, 81, 87). Histologic and electron microscopic examination of pulmonary tissue provide a more accurate determination of edema; nevertheless, small quantities of edema fluid can go undetected even by these methods (55).

In squirrel monkeys focal alveolar edema was observed following continuous exposure to NO₂ at 9.4 mg/m³ (5.0 ppm) for 169 days. In other studies, edema was either absent (81, 72) or not reported (59).

The significance to human health of presently encountered atmospheric levels of NO₂ is not known. Recent investigations involving healthy populations as well as patients with chronic pulmonary disease have yielded conflicting results. Shy et al. (88) compared neighboring communities in Chattanooga exposed to high and low concentrations of NO₂. They reported a decrease in ventilatory performance and an excess of respiratory illness among families exposed to increased levels of NO₂. Since the source of NO₂ pollution was a factory producing trinitrotoluene, other automobile-associated pollutants (lead, CO, hydrocarbons) were not considered factors in this study.

Other studies on volunteers exposed to NO₂ showed no effect (32).

Skin. Skin disorders were reported in humans exposed to NO₂ levels exceeding MPC by a factor of 5.8-12 (MPC, 0.10 mg/m³; 0.053 ppm; 24-hour average) (32).

Other Organs. Heart, liver, kidney and spleen of mice exposed to 0.5 ppm of NO₂, 6, 18 and 24 hours daily for 3-12 months did not reveal any unique pathology (59).

Wagner et al. (72) exposed six species (rats, mice, hamsters, guinea pigs, rabbits, dogs) to 5 ppm of NO₂ for 15 months and found no histologic changes in the trachea, hilar lymph nodes, heart, liver, spleen, kidney, adrenal, thyroid, pancreas, duodenum, bladder and brain.

9. Cytologic and Cytogenetic Effects

No reports were found on the cytologic and cytogenetic effects of NO_2 in experimental animals or humans.

10. Molecular Effects

No reports were found on the effects of NO_2 on DNA, RNA and protein synthesis in experimental animals or humans.

11. Reproductive and Teratogenic Effects

No reports were found on the effects of NO_2 on pregnancy and fetal abnormalities in experimental animals or man.

12. Metabolism

No reports were found on the effect of NO_2 on metabolism in experimental animals or humans.

13. Carcinogenicity

A possible relation between exposure to NO_2 and pulmonary neoplasia has been the subject of preliminary studies. Exposure of rats to 0.8 ppm NO_2 for approximately their lifetimes was found to result in large tumors in 4 of 21 rats. Three of these were subcutaneous, spindle-cell tumors containing foci of calcium. Two also had areas of necrosis. The cells were arranged in orderly nests and whorls. The fourth tumor was a ganglioneuroma of the adrenal (80). In another study by the same investigators in which rats (15 rats) were exposed continuously to 12 ppm of NO_2 , one developed a ganglioneuroma, one had focal nodular metaplasia of the bronchial epithelium, and two focal had bronchial adenomatosis in the periphery of the lung.

Wagner et al. (72) exposed the CAF/Jax mice to 5 ppm of NO for 12-16 months and found an increased rate of tumorigenesis in the group sacrificed at 12-months.

Gardiner (89) obtained evidence that NO₂ exposures may contribute to lung tumor development in mice. Because benzeno-pyrene hydroxylase presumably inactivates the carcinogenic potential of polyaromatic hydrocarbons, the effect of an acute exposure to NO₂ on this enzyme system was evaluated in rabbit tracheobronchial mucosa. No effect was found (29).

Heuschler and Ross (90) exposed NMRI mice to 40 ppm, 2 x 48 hours/week or 48 hours every 10 days, or every 30 days for 1.5 years and found a high incidence of lung adenomas, skin adenomas and leukosis in the animals that survived (100% survival).

IV. SIGNIFICANT PHYSICAL AND CHEMICAL PROPERTIES

Nitrogen dioxide and its polymer nitrogen tetroxide are always found together at normal environmental temperatures. Nitrogen dioxide is a by-product of many operations and results whenever nitric acid acts upon metals, as in bright dipping, pickling, and etching, or upon organic material, as in the nitration of cotton or other cellulose. It is also a by-product of the manufacture of many chemicals including explosives, dyes, lacquers, and celluloid. It also results, in significant amounts, from the slow burning of explosives or the detonation of explosives having a high oxygen balance, or from electric arcs (91).

Nitrogen dioxide is a reddish brown gas above 21.3° C, a nearly colorless liquid between 21.3° C and -9.3° C, and a white solid below -9.3° C. At 40° C, approximately 30% is NO_2 and 70% is N_2O_4 (92).

Nitrogen dioxide absorbs light over a wide range of the visible wavelengths causing the characteristic light yellowish-orange to reddish brown colors of gaseous nitrogen dioxide seen at relatively low and high concentrations, respectively.

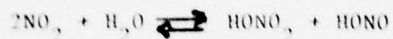
Nitrogen dioxide does not burn but supports the combustion of carbon, phosphorus, and sulfur. It is very corrosive to steel when wet, but may be stored in steel cylinders when moisture content is 0.1% or less. Its significant physical properties are given in Table I (93).

Table 1

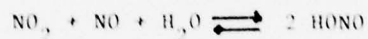
Physical Properties of Nitrogen Dioxide (93)

- o Chemical formula = NO_2
 - o Molecular weights = 46.01 (100% NO_2 assumed)
 - o Specific gravity = 1.448 at 20°C
 - o Boiling point = 213°C
 - o Relative vapor density = 1.59
(air = 1)
 - o Critical pressure = 100 atm.
 - o Heat by vaporization = 99.0 Cal/g at 21°C
 - o Critical temperature = 158.2°C
 - o At 25°C and 760 mm Hg:
 - 1 ppm of vapor = 0,00188 mg/liter
 - 1 mg/liter = 532 ppm
 - o Odor = pungent, sweetish
 - o Threshold of detection = 5 ppm
 - o Visual threshold = 75-150 ppm
 - o Is soluble in concentrated sulfuric and nitric acids
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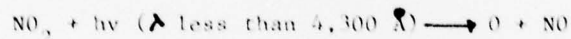
Nitrogen dioxide has a high oxidation rate. Above 150° C it starts to become dissociated into nitrogen oxide and oxygen, with decomposition complete at 620° C. Nitrogen dioxide may react with water to generate nitric acid (HONO₂) and nitrous acid:



and with nitric oxide and water to give nitrous acid:

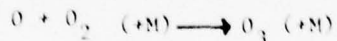


The homogeneous gas-phase reactions of nitrogen dioxide and nitric oxide with water, forming nitric and nitrous acids, appear to be very slow at ambient temperature. When a quantum of sunlight of wavelength less than 4,300 Å is absorbed by a nitrogen dioxide molecule, the excited molecule formed is of sufficient energy to dissociate into ground state oxygen atoms and nitric oxide.



Nitrogen dioxide photolysis in sunlight is believed to be largely responsible for the generation of ozone (O₃) in the sunlight-irradiated, polluted atmosphere because:

o Oxygen atoms in air react predominantly with molecular oxygen to form ozone.



The M represents a third molecular species such as nitrogen, oxygen, or water. Nitrogen dioxide reacts with alkalies to form nitrate and nitrite. The effects of NO₂ on alveolar tissue is interpreted as follows:

The relative dry NO_2 -air mixture reacts little with the slightly moist surfaces of the respiratory passages, whereas after reaching the alveoli the humid air, moist surfaces, and extended time promote almost complete hydrolysis in intimate contact with the alveolar tissue (91).

The odor of nitrogen dioxide is characteristic and distinct in concentrations as low as 5 ppm. In concentrations of 10 to 20 ppm, the gas is mildly irritant to the eye, nose, and upper respiratory mucosa. There is very little difference in intensity of odor and irritation between concentrations of 20 and 100 ppm (91).

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EXPERIMENTAL PHOSGENE INTOXICATION

Backup Report to Phosgene Toxicity Matrix

Phosgene

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Experimental Phosgene Intoxication

I. INTRODUCTION

Phosgene or carbonyl chloride is a colorless gas under normal temperatures and pressure which is obtained by passing chlorine and excess CO over activated carbon or by decomposition of chlorinated hydrocarbons by ultraviolet radiation or heat. Its physical and chemical properties are discussed in Section V of this report.

It was first used as a chemical warfare agent during World War I. It is currently used in the synthesis of isocyanates, which are the basic materials for polyurethane resins (1).

The primary action of phosgene is on the respiratory tract where it causes an inflammatory edema, which is followed by a cellular exudate, both in the upper respiratory passages and in the pulmonary parenchyma. While the highest mortality occurs in the acute (incipient) stage, a second peak in the mortality curve is encountered with the pneumonia process (critical stage). The latter inflammatory response is largely determined by the extent of the primary destruction. Generally, if death does not ensue in the incipient or critical stages, regressive and reparative changes set in. Despite the proliferative activity of the pulmonary tissue, scattered emphysema and focal atelectasis are detectable long after exposure (6 months) (2).

The threshold limit value (TLV) of phosgene adopted by the American Conference of Governmental Industrial Hygienists (3) for 1971 was 0.1 ppm or 0.4 mg/m³. This figure is probably based on the finding that rats exposed to 0.5 ppm for 2 hours and to 2 ppm for 80 minutes showed pathologic changes in the lung 96 hours and 3 months, respectively, after gassing (4).

Although there are no data to correlate the toxicity of phosgene in man to that in laboratory animals at low concentrations, there have been estimates made by observers in World War II that the LC_{50} for man is 3200 mg min/m^3 . In dogs the measured LCt_{50} was 8400 mg min/m^3 for a 1-minute exposure and 3450 mg min/m^3 for a 1-minute exposure in the mouse. From these values, it would appear that man is twice as susceptible as the dog and about equally as susceptible as the mouse. The monkey LCt_{50} was approximately 1000 mg min/m^3 (5).

The above LCt_{50} values were obtained at unspecified temperatures (presumably room temperature). There are indications, however, that increased temperature, at high doses at least, increases the toxicity of phosgene in the mouse but not in the rat; studies in dogs demonstrated no consistent changes (5). No information is available on the effects of humidity or altitude on phosgene toxicity.

There are no quantitative data indicating what dosage might cause permanent lung damage in humans, since most of the reports dealing with acute accidental exposure or long-term exposure (in industrial situations) do not specify the dosage. Since the lowest experimental level available for long-term exposure suggests that 0.8 mg/m^3 (0.2 ppm) for 5 hours for 5 days may cause slight pulmonary changes (6) it would seem that a value of 0.1 ppm (0.4 mg/m^3) would not be sufficiently safe, if a tenfold safety margin is not to be exceeded for working personnel for an 8-hour day, 5 days per week at sea level (i.e., 0.08 mg/m^3 or 0.02 ppm).

The toxic effects of phosgene on various organ systems will be described in detail in this report under the headings, acute, subchronic, and chronic toxicity.

Acute toxicity is defined as that which results from a single exposure, injection, administration or application (skin). In vitro observations are included under acute effects. Subchronic toxicity is defined as that which results from repeated (continuous or intermittent)

treatment during a 90-day experimental period or less (short-term tests). Chronic toxicity is defined as that resulting from continuous treatment over a period of 2 years or more (long-term tests or lifetime tests).

Typical examples will be selected to illustrate toxic endpoints.

Information Sources. The information contained in the body of this document is derived from the following sources:

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Matrix. The pertinent effects of phosgene described in this document will be summarized in the matrix. Where animal data were not available, human data were included.

II. ACUTE TOXICITY (including in vitro tests)

1. Hematologic Effects

Erythrocytes. Meek and Eyster (20) (7) exposed dogs to 80-100 ppm of phosgene for 30 minutes and made red cell counts over a 10-hour period after gassing. There was a decrease over the first 3 hours and a sharp rise over the next few hours until the animal died. A similar observation was made by Cameron and Courtice (8) in dogs exposed to 440 mg/m³ for 30 minutes.

Daly et al. (9) studied the effect of phosgene (610-11,500 mg/m³) on perfused lungs of dogs. Continuous measurements of red cell counts were made every half hour. No change in cell number was found at any time during the experiment.

Leucocytes. While studying the erythrocytes, Daly et al. (9) also counted the leucocytes. The results showed that dogs exposed 610-11,500 mg/m³ of phosgene did not show abnormal white cell counts.

Hemoglobin. Daly et al. (9) also measured blood hemoglobin levels in dogs exposed to 610-11,500 mg/m³ of phosgene and found no change between pre- and post-gassing levels.

Exposure of dogs to 80-1000 ppm of phosgene for 30 minutes (7) resulted in below normal levels of hemoglobin during the first 4 hours after gassing. Thereafter, the hemoglobin levels rose sharply, reaching 125% above normal at 6 hours. The interpretation of hemoglobin readings in terms of blood volume would mean that during the first 4 hours there is an increase of blood volume followed by a marked decrease thereafter. These observations confirmed earlier findings of Underhill (10) and were also later confirmed by Cameron and Courtice (8) who exposed dogs to 440 mg/m³ of phosgene for 30 minutes.

A rise (100%) in plasma hemoglobin was also observed in humans exposed accidentally to phosgene (concentration and exposure time not specified) by Hegler (11). These changes were noted 6-36 hours post exposure.

Other effects. Daly et al. (9) studied the effect of phosgene (610-11,500 mg/m³) on perfused dog lungs and found no change in sedimentation rate, viscosity, and conductivity of blood and plasma.

2. Bone Marrow Changes

Comen et al. (2) exposed dogs, cats, rabbits, guinea pigs, and rats to 0.9-3.25 mg/liter of phosgene for 30 minutes. The exposure produced congestion in the bone marrow.

Humans exposed accidentally to phosgene showed hyperemia in bone marrow (12).

3. Immunologic Effects

No reports were found on the effect of phosgene on the immunological system of experimental animals or humans.

4. Central Nervous System (CNS) Effects

New Zealand white rabbits (both sexes) were exposed to phosgene at 50 ppm for 14 minutes and 200 ppm for 25 minutes. Changes in the total electrical activity of the cervical sympathetic nerve before and after gassing were recorded. The data showed a significant decrease in sympathetic nervous system activity in exposed animals. In every case, gross observation of the lungs at the end of the experiment revealed congestive changes of the patchy hyperemia type, usually limited in extent. The authors concluded that phosgene toxicity was an example of acute pulmonary edema resulting from a hypoactive-sympathetic or neuroparalytic state in the host (13).

The observations of Ivanhoe and Meyer (13) were confirmed (in part) by the work of Froselono (14) who studied rat lungs following exposure to 1000- 4320 ppm/min. Electron microscopic examination indicated the presence of interstitial edema; the authors believed that the autonomic nervous system might indeed play a significant role in the appearance of this abnormality.

Dogs, cats, rabbits, guinea pigs, and rats exposed to 0.4-3.25 mg/liter of phosgene for 30 minutes showed hyperemia and edema in the brain tissues between the 7th hour and 3-day post-exposure period (2).

In humans, accidental exposure to phosgene resulted in subarachnoid hemorrhage, cellular degeneration in gray matter and generalized hyperemia extending up to the white matter (12). Other observers have reported "ring hemorrhages" in the brain (15,16,17).

5. Behavioral Effects

Galdston et al. (18) reported studies of 6 cases of acute exposure to phosgene. Each patient received physical and psychiatric examinations. The men were exposed for brief periods to phosgene (exposure time and dose were not specified). The authors felt that psychological factors were contributory (to some degree) to the lingering symptomatology when patients were examined 19 months later. Some nervousness and hypochondriacal behavior were also observed in patients who survived acute exposure to phosgene (dose and duration of exposure not specified) (11). These reactions were present 4 months after exposure.

6. Cardiovascular Effects

Pulmonary irritation and pathology incident to phosgene poisoning may produce marked circulatory disturbances not unlike those seen in shock. Many of these effects have been described in papers by Underhill (19), Delepine (20), Groll (15), Patt et al. (21), Cameron and Courtice (8), Meek and Eyster (7), and Gibbon et al. (22).

In dogs, the immediate effect of phosgene poisoning (310-350 mg/m³ (75-87 ppm, MLC)) was the lowering of pulse rate (from 90 to 75 beats/min), which returned to normal by the 4th or 5th hour after gassing. The heart rate also increased (150 beats by 12 hours post-gassing). There was evidence in some cases that the high heart beat rate resulted in a circulatory failure. In such cases the pulse rate dropped very rapidly to a point considerably below normal, which caused a drop in body temperature and resulted in death (19).

In another study (7), dogs were subjected for 30 minutes to air containing 80-100 ppm of phosgene. This was sufficient, with rare exceptions, to produce death within 24 hours. Arterial pressure was recorded every half hour. As a rule the blood pressure gradually rose during the first half of the experiment (5 hours after gassing) increasing about 10% above the normal. Thereafter, it fell gradually reaching normal at the 8th hour after gassing. After the 8th hour, the decline became extremely rapid until the animal died. The venous blood pressure was found to be rather variable, conforming on the whole, however, to what one might expect from the arterial. During the long period of increased arterial pressure, venous pressure was either normal or slightly below. In terminal stages, however, there was a rapid rise.

The pulse rate fell shortly after gassing in all animals (95 to 70 beats/min), but rose gradually thereafter (2 hours after gassing) until death, 10 hours after gassing.

A relative enlargement of the right auricle and ventricle was observed immediately after gassing. This was followed by gradual reduction in size (4-6 hours post-gassing) during the onset of pulmonary edema. Just before death, the heart was again enlarged (7).

Patt et al. (21) studied the hemodynamics in dogs following exposure to phosgene in concentrations of 0.4-0.7 mg/liter for 30 minutes. The indices studied included pulse, arterial and venous blood pressures,

right ventricular pressure, circulation time, and arterial-venous oxygen difference. The measurements were made every 2-4 hours until the animal died or recovered. Similar measurements were made in untreated controls. In some dogs (treated and untreated), several vessels were directly visualized to explore the possibility of vascular spasm or red cells' agglomeration after phosgene poisoning. This was also done on gassed rats.

Heart rate decreased precipitously with gassing, then slowly rose to or above the initial value. The early bradycardia which occurred was probably a reflex response to irritation of the respiratory passages. It was not observed following bilateral vagotomy or atropinization. No clear difference in pre- or post-gassing pulse was seen between the various groups of treated animals, based on survival time or treatment. Comparable tachycardias were seen in surviving and dying animals. Although the tachycardia observed in the later stages after gassing was probably due partly to the severe anoxia, no clear relationship was found between the degree of anoxia and the extent of tachycardia. Heart rate decreased and was irregular as death approached, but respiration failed before circulation, the heart continuing to beat for some minutes after respiration ceased.

The arterial pressure fell distinctly and progressively with time after gassing, while the venous pressure was not significantly affected. No rise was found in the right ventricular pressure (26 dogs).

Pulmonary circulation time was increased on the average by two-thirds at 8-12 hours after gassing. Spasms were observed in the systemic vessels and scleral vessels of gassed dogs.

The nature of phosgene death was basically asphyxial. Gassed animals died when the venous oxygen approached zero, with arterial oxygen falling in parallel. Death was occasionally preceded by enlargement of the right

and left ventricle. It is believed that the change in the heart size (particularly the right side) is due to clogging of the capillaries and veins with red blood cells, which greatly increases pulmonary resistance and the work of the right heart (7).

Cameron and Courtice (8) exposed dogs to 440 mg/m^3 of phosgene for 30 minutes and noted that during the breathing of phosgene, the blood pressure fell, probably due to the vagal reflex slowing the heart. After exposure to phosgene, the blood pressure generally rose somewhat and then fell as hemoconcentration and anoxia progressed.

Pulmonary arterial pressure in phosgene poisoned cats was measured by Gibbon et al. (22) using the marsupialized heart preparation technique. The prepared animals, each paired with a control, were exposed to varying doses of phosgene. The concentration-time (CT) products of exposure, in milligrams/minutes per liter, were selected to produce varying degrees of severity of poisoning and edema. The mean concentration was $0.29 \text{ mg/liter} \pm 7\%$, the duration of exposure (up to 30 hours) being varied from 8.5-13 minutes. At intervals after gassing, blood pressures (pulmonary and femoral, right and left auricles), pulse rates, and respiratory rates were taken. The pulmonary arterial pressure rarely rose above the pre-gassing values except terminally; the tendency was toward a fall, synchronous with the systemic pressure. There was typical bradycardia immediately after exposure, but thereafter the pulse respiratory rates were variable. Measurements of pressure in the right and left auricles indicated no essential changes from gassing.

In one report of two men who died 19 and 22 hours after accidental exposure to phosgene, cardiac dilatation was observed at autopsy (20). Autopsy reports of World War I warfare gas victims showed the occurrence of subendocardial and subpericardial hemorrhages, and thrombosis of the pulmonary vessels with thrombi attached to the cardiac valves (15). Additionally, in one human study decreased blood pressure and tachycardia were seen (11).

7. Biochemical and Histochemical Effects

Blood. Adult male rabbits (62 animals) were exposed to .570 mg/m³ (67 ppm) of phosgene for 30 minutes. The animals that survived were anesthetized and blood sampled for sodium, chloride, total lipids, neutral fats, total fatty acids, total cholesterol, ester cholesterol, free cholesterol and phospholipids. The levels of chloride and all lipids except neutral fat were above control levels (23).

In rabbits, dogs, and goats exposed to .440 mg/m³ of phosgene for 30 minutes, a fall was observed in the albumin and globulin fractions of the blood plasma between 5-24 hours post-exposure. This was associated with a decrease in plasma volume during formation of pulmonary edema (see Section 8 - *pulmonary effects - vascular permeability and edema formation*) (8).

Daly et al. (9) studied the effect of phosgene (610-11,500 mg/m³) on perfused dog lungs. Continuous measurements were made on hemoglobin iron levels, urea, total nitrogen, non-protein nitrogen, chloride, and protein of plasma. The results showed that doses of phosgene sufficient to cause desquamation of the bronchial mucosa, severe bronchioconstriction, and increase in circulatory blood volume of the lungs, exert no significant effects (as compared to control lungs) on the physiochemical state of the blood.

In humans, acute exposure to phosgene (time and concentration not specified) resulted in degenerative changes and presence of hematin in the serum in patients examined 2-5 hours after exposure (24).

8. Effects on Body Weight, Organs and Tissues

Body Weight. There were no reports on the effect of acute phosgene exposure on body weight of experimental animals or humans.

Pulmonary Effects

Pulmonary Function. The earliest physiological consequence of pulmonary edema is, undoubtedly, an increase in the resistance to gas diffusion across the lung membrane. Long and Hatch (25) reported that reduction in the rate of CO by the lung tissue was an early and sensitive test of impaired pulmonary function following exposure to irritants such as phosgene. In this test rats were exposed to phosgene levels of 0.5-5 ppm for 30 minutes. There was a decreased uptake of CO, which was progressive for 6-8 hours, followed by gradual recovery thereafter.

The assessment of microscopic changes seen in the lungs showed a rough correlation with the magnitude of the loss in the capacity to absorb CO, but the agreement was best for the higher exposure levels which produced severe lung damage. The lungs of animals exposed to lower concentrations (1.3 ppm) were histologically not very different from controls, but their capacity to absorb CO was significantly lower than controls. This suggests that the change in CO uptake provides a more sensitive index of the beginning of pulmonary impairment (25).

Dogs (31 animals) were exposed to phosgene concentrations averaging 0.29 mg/liters (72.0 ppm) for 30 minutes. Immediately after exposure to phosgene, there was a transient fall in the oxygen saturation of the arterial blood, presumably due to bronchiolar constriction. This was followed by progressive pulmonary edema, more profound anoxemia, and death (26).

An increase in respiratory rate was observed by Meek and Eyster (7) in dogs exposed to 80-100 ppm of phosgene for 30 minutes. This increase continued throughout the 10-hour post-gassing period until the death of the animal.

The action of phosgene on isolated perfused lungs of dogs exposed to 610-11,500 mg/m³ of phosgene was studied by Daly et al. (9). These studies indicated a marked reduction in tidal air and an increase in the circulating blood volume of the lung.

Galdston and Luetscher (27) compared the pO_2 in arterial blood and alveolar air in humans exposed to phosgene (concentration not given) with pO_2 in unexposed subjects. The average pO_2 values for non-exposed subjects (15 subjects) were 97 mm Hg for alveolar air and 96 mm Hg for arterial blood, while in the exposed group (8 subjects) the alveolar air and arterial blood pO_2 averaged 94 mm Hg.

In one instance, accidental exposure of a 40-year-old man to phosgene (concentration not specified) resulted in a severe decrease in vital capacity (pulmonary) with a gradual return to normal (1300 ml by the 2nd hospital day; 6174 ml by the 5th hospital day). There was an associated decrease in plasma volume, as indicated by the high hematocrit value (57%) and the fluid intake-output ratio. This fluid was almost positively sequestered in the lungs. Studies of arterial blood disclosed severe hypoxemia and glucosuria (28).

Pathology. Male Wistar rats were exposed to 0.5-4.0 ppm of phosgene for varying lengths of time ranging from 5 minutes to 8 hours (117 animals). There were 18 unexposed controls. All animals were killed in groups of 3 at 5 minutes to 8 hours after exposure. One group of rats, which was exposed to 2.2 ppm phosgene for 80 minutes, was killed 3 months after exposure and another, exposed to 1.7 ppm of phosgene, was killed at 4, 8, 24 and 48 hours after exposure (4).

The earliest detectable lesion involved the respiratory bronchiole which had thickenings and increased cellularity of its walls that increased with time. Alveolar edema was infrequently encountered at high doses, but where found, it was commonly associated with acute pneumonia. Inhalation of small doses resulted in chronic reversible pneumonitis.

Boyd and Perry (23) exposed male rabbits to 270 mg/m^3 (67 ppm) of phosgene for 30 minutes. The survivors were examined histologically at 6-7 and 16-20 hours after exposure. A mild edema of the trachea, bronchioles, and alveolar tissues, associated in the last case with some congestion, emphysema and contraction of arteries, was observed at 6-7

hours after exposure. At 16-20 hours, edema had markedly increased, there was more emphysema and hemorrhage, the pulmonary arteries were less contracted and there was less edema in the bronchioles and trachea.

Meek and Eyster (7) exposed dogs for 30 minutes to phosgene at 80-100 ppm concentration. Histological examination of the lungs of gassed animals revealed severe injury to the lower respiratory passages. There was constriction or spasm of the small bronchioles with the accompanying atelectasis and emphysema. There was edema of connective tissue; the alveoli were irregular, their membranes were injured, and in many cases they contained exudate. There was clogging of capillaries with red cells, resulting in increased pulmonary resistance.

Adult mongrel dogs were exposed to 24-40 ppm of phosgene in air for 30 minutes and sacrificed 1-2 days later. The lungs were removed and examined histologically. Except for the presence of bronchiolitis and peribronchiolitis involving terminal and respiratory bronchioles, the lungs were otherwise normal (29).

Koontz (30) gassed dogs with phosgene at the minimal lethal dose (not specified) and studied 95 survivors for gross pathological changes. The animals were killed at intervals from 2-60 weeks. About 72% of the animals showed no gross abnormalities, while the remaining showed emphysema, pneumonia, congestion, hemorrhage, edema, transient bronchiolar plugging, and atelectasis.

Durlacher and Bunting (26) exposed 31 dogs (mongrels) to 0.29 mg/liter of phosgene for 30 minutes. Gross findings included edema, emphysema, atelectasis, and consolidation of one or more lobes of the lung 4-9 days after exposure. Additionally, "obliterative bronchiolitis" was found with the involved lobes showing diffuse mononuclear exudate within the alveoli, plus foci of thickening and organization of the alveolar walls composed of large mononuclear cells and young fibroblasts.

Comen et al. (2) exposed dogs, cats, rabbits, guinea pigs and rats to varying concentrations of phosgene (0.4-3.25 mg/liter) for 30 minutes and killed them at different time intervals after exposure (0.38-8.54 hours). The lungs were removed in toto and examined histologically. The course of phosgene poisoning was divided arbitrarily into three phases: the incipient stage extending from gassing up to 2-6 hours; the critical stage, during which the majority of deaths occurred, extending to day 3 after gassing; and the regressive or reparative stage, extending from the 4th day onward. The time of completion of repair was uncertain, and the residue of phosgene was regarded as the terminal stage of repair.

The incipient stage was characterized by emphysema, necrosis, and sloughing of bronchiolar mucosa, perivascular and peribronchial edema, congestion and alveolar edema, thickening of bronchiolar membrane and bronchial-bronchiolar constriction. Neither bronchiolar plugs nor emphysema could be related spatially with alveolar edema, and all lesions in this phase tended to be without preferential localization. There was *no way of deciding whether the fluid in a given alveolus arose from surrounding or more distant capillaries*. No lesions were found in the extrapulmonary tissue in this incipient stage, and there was no evidence of damage to the capillary endothelial cells.

A similar observation was made by Daly et al. (9) on infused dog lung preparations exposed to phosgene (610-11,500 mg/m³). Under the conditions of these experiments, the pathological changes produced by gassing with phosgene were confined to the bronchial tubes.

In the critical stage of phosgene poisoning, the animals that died during the first 48 hours after exposure had froth-filled trachea and fluid-filled bronchi. This finding was related to the abrupt release of large amounts (500 ml) of fluid via the trachea immediately before death. The mucosal surfaces of the trachea and larger bronchi were usually smooth, and the pleural cavity was usually free of fluid (2).

Grossly, the lungs were large and mottled. The microscopic pulmonary lesions included presence of eosinophilic granular material and fibrin in the fluid filling the alveoli, hemorrhagic areas on the alveolar surface, necrosis, and sloughing of bronchiolar epithelium and intense inflammatory lesions in the bronchioles (pneumonitic response) which were generally focal but occasionally diffused with scattered cellular and fibrinous exudate. These lesions were identical regardless of species and the varying conditions of exposure and appeared to represent merely the full development of the earlier changes (2).

In animals that survived until the third day regression of the edema and congestion appeared to have begun, but the cellular exudate frequently increased in severity and extent. These animals (121 dogs) were examined between 4 and 138 days after exposure. Typically, the regressive changes consisted of gradual diminution of the amount of fluid in the alveoli and reduction of congestion so that by 2 weeks post-gassing the lungs failed to drip fluid on sections. There were also decreases in leucocyte, monocyte, and fibroblast infiltration (bronchopneumonia) into the alveolar exudate, a return to normal of the lung/body weight ratio, and an increased focal fibroblastic proliferation by 2 weeks post-exposure. The fibroblastic proliferation appeared to be the means of repair of the alveolar structure, since animals that survived for one month after gassing showed emphysema, atelectasis, and fibrous scars. By day 60 (post-exposure), only scarring, together with scattered emphysema and foci of atelectasis, was seen consistently in the sections. These lesions were also observed in the dogs killed 6 months after gassing (2).

In humans (2 workers) who died 19 and 22 hours after exposure to phosgene (amount not stated), the following abnormalities were observed at autopsy: subpleural emphysema, fibrotic adhesions at apices of both lungs, hemorrhage in the parenchyma, interstitial emphysema, desquamation of bronchi, limited soft atheroma, and general congestion (20). Pleural effusions were also reported in the autopsy records of 105 World War I soldiers who were gassed with phosgene (15). The above changes occurred during the critical stage of phosgene poisoning.

Autopsy data on humans in the regressive or reparative stage are fragmentary and described mainly in terms of "bronchopneumonia" (15,32,33). In one man who died 11.5 days after exposure, the combination of fibroblastic proliferation, macrophages in the alveoli, chronic bronchitis and bronchiectasis, and residual edema was almost identical with that seen in dogs (11,12).

Vascular Permeability and Edema Formation. Boyd and Perry (23) exposed adult male rabbits to 270 mg/m^3 (67 ppm) of phosgene vapors for 30 minutes. This dose killed more than 80% of the animals. The survivors were anesthetized and respiratory tract fluid measured at hourly intervals and expressed as ml/kg/24 hours. Samples collected near the time of the animals' deaths were analyzed for sodium, chloride, and lipid contents.

Measurements on respiratory tract fluid showed that during the latent period (6 hours after exposure), sodium and chloride levels were elevated but the volume remained normal. During the early symptomatic period, levels of sodium and chloride were definitely elevated but the volume was still normal. Just prior to death (late symptomatic period), respiratory tract fluid poured out of the lungs at a rate that averaged 60 times the normal. Animals in the last stage had intense pulmonary edema, hemoconcentration, and little or no resistance to the lethal effect of urethane (23).

Cameron and Courtice (8) investigated phosgene poisoning in rabbits, dogs, and goats to obtain further information regarding the process of edema formation. The animals were exposed to 440 mg/m^3 of phosgene for 10-30 minutes. In the rabbit the fluid loss in the lung was rapid, but the withdrawal of fluid from the undamaged tissues into the blood was so rapid that the plasma volume usually fell very little. For example, the mean level of plasma proteins, albumin, and globulin were, respectively, 5.57, 3.22, and 2.35 g /100 ml of plasma before exposure and 4.37, 2.63 and 1.74 g/100 ml of plasma after exposure (6 hours).

In the dog and goat, however, the plasma loss is more rapid than the withdrawal of fluid from the tissue so that the plasma volume falls considerably at 12 hours after exposure and remains at this level for another 12 hours, after which it slowly rises. Associated with this fall in plasma volume is a rise in hemoglobin and red cell count. Six hours after exposure, hemoconcentration begins and rapidly develops. The fall in plasma protein is due to the fall in the albumin and globulin fractions, as in the case of the rabbits (8).

The lymph drainage of the lung after exposure to phosgene was determined for the dog in the above studies (8). The first 2-5 hours after exposure the rate of flow remained approximately the same, varying slightly in different animals, but thereafter the rate of lymph flow rose fairly steeply to reach a considerable level, which usually lasted until death. The severity of the edema was reflected by an increased heart/lung weight ratio seen at the time of death.

Cameron (33) noted that the mitochondria of the endothelium lining the capillaries disintegrate and disappear before the capillaries begin to leak.

Nose (odor perception). Wells et al. (34) exposed 56 military personnel (without respiratory problems) to increasing concentrations of phosgene, until all subjects could detect phosgene by odor. Of the "technically trained" (no further clarification) personnel, 50% detected phosgene at 6.1 mg/m^3 (1.5 ppm), 30% at 4.7 mg/m^3 (1.2 ppm), and none at 1.5 mg/m^3 (0.4 ppm). Effects other than odor detection were not described.

Another study involving 4 subjects reported the "detection threshold" for phosgene as 1.0 ppm (35).

Other Organs. Dogs, cats, rabbits, guinea pigs, and rats exposed to varying concentrations of phosgene (0.4-3.25 mg/liter) for 30 minutes and killed thereafter at various time intervals (0.38-8.54 hours) after

exposure showed congestion of the liver, spleen, kidney, and intestines (2). The adrenal glands occasionally showed small hemorrhages in the medulla or at the corticomedullary junction. These changes were observed between the 7th hour and 3-day post-treatment period.

In humans, acute exposure (concentrations and time not given) did not produce adverse effects on the liver, kidney, spleen, stomach or small intestine in one study (20), but in another, hyperemia was observed in the kidney, spleen, thyroid and thymus, although not in the liver and adrenals (12).

Filatova et al. (36) investigated the general health of people who worked in an isocyanate factory. Among the pollutants in the factory atmosphere were phosgene (10.1 ppm; 0.4 mg/m^3), hexamethylen diisocyanate (0-0.1 ppm), hexamethylen diamine (0.05-0.81 ppm), chlorobenzene (0.6-8.0 ppm), and dilyn (0-4.6 ppm). There was a strong indication of abnormalities in the hearts and livers of the workers. It was not possible to say how much of this was attributable to phosgene, especially since no abnormalities of the liver have been noted in animals exposed to benzene.

9. Cytologic and Cytogenetic Effects

No reports were found on the effects of phosgene on mitosis nor on chromosomes in experimental animals or humans.

10. Molecular Effects

No reports were found on the acute effects of phosgene on the DNA, RNA, and protein synthesis in experimental animals or humans.

11. Reproductive and Teratogenic Effects

No reports were found on the acute effects of phosgene on the reproductive and embryonic tissues of experimental animals.

A case has been reported of a 38-year-old woman, in her 7th month of pregnancy, who was exposed accidentally to phosgene for 3 hours in the afternoon. That evening hemoptysis occurred with symptoms worsening the next morning. On admission to the hospital, a chest film was taken and pulmonary edema was seen. She was released from the hospital before her condition returned to normal. After 2 months, she gave birth to a healthy child. This is the only case found in the literature that reported on phosgene exposure during pregnancy (37).

12. Metabolism

Very little is known regarding the metabolism of phosgene. Its inhalation toxicity is probably enhanced by its low solubility, which allows it to penetrate further into the lungs. Phosgene is poorly absorbed in the nasal passages of the rabbit or monkey (8). The gas damages the blood-air barrier in some way, allowing leakage of plasma into the alveoli. It was believed that phosgene damage was the result of its direct action with sensitive groups in the tissues or due to acid release. However, Nash and Prattle (38) have shown phosgene is not absorbed in acid solution, but is absorbed in alkaline solutions and solutions containing non-ionized amines. It was concluded from the data on the solubility and rate of hydrolysis that phosgene in molecular form can penetrate all three layers of the blood air barrier and that its effects are caused by molecular phosgene and not by hydrochloric acid (39). The upper respiratory tract is protected against phosgene by hydrolysis in the mucous layer.

A number of experiments carried out in World War II showed that phosgene affects tissues because the carbonyl group combines with the free amines of cell enzymes or other critical substances (40).

The primary effect of phosgene appears to be an alteration in lung capillary permeability so that there is a loss of fluid with high protein content from the circulation and into the pulmonary tissues. This fluid is free of cells and resembles plasma in composition (8). The accumulation of this fluid in the lung results in lowered plasma volumes,

but there is little or no hemoconcentration. This is readily seen in the dog and goat, but may not be seen in the rabbit unless the latter is kept on a diet drier than normal for a few days before exposure to phosgene (42).

Cameron and Courtice (8) have shown that as the pulmonary edema increases, the lymph flow from the lung is increased. This drainage route appears to be the only one available for the resolution of protein transudates from the air passages. Thus Courtice and Phipps (42) showed that, whereas saline can be rapidly absorbed directly into the lung capillaries from the air spaces, serum was only slowly absorbed and then via the lymphatics. This latter process is slow and is unable to keep pace with the plasma outflow in phosgene poisoning. Thus edema progresses, oxygen transfer is disturbed, and anoxia results.

Phosgene produces profound shifts in body water by acting on local exposed capillary vessels. The main toxic action of this compound is confined to the lung, since it is rapidly hydrolyzed in the pulmonary tissues so that none passes into the general circulation. The underlying mechanism of the effect on the lung capillaries is not known. It may be enzymic, especially since the mitochondria of the endothelium have been shown to disintegrate and disappear before the capillaries begin to leak (33).

13. Carcinogenesis

No reports were found on the possible carcinogenic effect of phosgene in experimental animals or humans.

III. SUBCHRONIC TOXICITY (short-term tests)

1. Hematologic Effects

No reports were found on the subchronic effects of phosgene on the hematologic system in experimental animals or humans.

2. Bone Marrow Changes

No reports found on the subchronic effects of phosgene on the bone marrow elements in experimental animals or humans.

3. Immunologic Effects

No reports were found on the immunologic effects of subchronic exposure of experimental animals or humans to phosgene.

4. Central Nervous System (CNS) Effects

No reports were found on the CNS effects of subchronic exposure of experimental animals or humans to phosgene.

5. Behavioral Effects

No reports were found on the behavioral effects of subchronic exposure of experimental animals or humans to phosgene.

6. Cardiovascular Effects

No reports were found on the cardiovascular effects of subchronic exposure of experimental animals or humans to phosgene.

7. Biochemical and Histochemical Effects

Tolerance. The development of tolerance to phosgene was studied in the guinea pig by Cordier and Cordier (43). The animals exposed to 10 mg/m^3 (1.5 ppm) for 10 minutes for 7 days became relatively resistant to toxic levels (140 mg/m^3 (35 ppm) for 10 minutes) of phosgene.

Similar repeated exposures of cats to $10\text{-}15 \text{ mg/m}^3$ (1.5-3.8 ppm) or to $20\text{-}25 \text{ mg/m}^3$ (5-6 ppm) for 10 minutes every day caused no greater lung damage after 40 days than after 2 days (44). They were unable to tolerate a total ct (concentration-time) of 9000 mg/min/m^3 (total time, 400 minutes), even though the LC_{50} for cats is about 2000 mg/min/m^3 for one minute.

There is some evidence that 1 ppm of phosgene produces tolerance to other irritants (43). Rats exposed for 6 hours to this concentration survived ct's of ozone and nitrogen dioxide that killed almost all rats not previously exposed to phosgene (5). The exact mechanism of this tolerance is not known, but it is believed that tolerance to high doses of phosgene represents manifestation of pathologic changes in the lungs.

There is no information on the development of tolerance to phosgene in man.

8. Effects on Body Weight, Organs and Tissues

Body Weight. Repeated exposure (2-41 exposures) of cats to phosgene ($20\text{-}25 \text{ mg/m}^3$) resulted in weight loss (44).

Pulmonary Effects

Pulmonary function. The effect of subchronic exposure of dogs to phosgene was studied by Rossing (45). Fourteen healthy adult mongrel dogs were exposed to $2.91\text{-}4.86 \text{ mg/l}$ of phosgene in air for 30 minutes at the rate of 1-3 exposures a week for up to 12 weeks. Transpulmonary

pressure, airway resistance, tidal volume, and dynamic elastance were measured prior to exposure (control values) and weekly thereafter during exposure. Animals were sacrificed at intervals during exposure and at the termination of the experiment. The lungs were removed and examined histologically.

The dynamic elastance rose very quickly, reaching a maximum mean value of four times the control in the 1st week of exposure and remained elevated up to the 3rd week. Fluctuations set in thereafter, which resulted in rise and fall in levels between the 4th-9th week.

Mean lower airway resistance also rose by the 1st week, and this rise continued throughout the exposure period until at the 12th week the values were 20 times that of controls.

Accompanying the change in lower airway resistance, the uniformity of distribution of inspired air was progressively disturbed as evident from nitrogen washout curves. The decrease in overall efficiency is best demonstrated by the continued increase in the mean number of breaths necessary to reduce the end expiratory nitrogen concentration below 1% (45).

Box and Collumbine (46) exposed rats to 80 mg/m^3 (20 ppm) for 10 minutes. Five days later, the pre-exposed animals and an equal number of controls were exposed to lethal concentrations ($230\text{-}440 \text{ mg/m}^3$; 55-110 ppm) of phosgene, and the respiration rates were counted for 15-second periods every 2 minutes during a 10-minute exposure. Results showed a marked increase in respiratory rate in the pregassed animals, but no significant difference between the CO_2 outputs of pregassed and control rats during the 5-day exposure.

Pathology. Exposure of dogs (20 animals) to 24-40 ppm of phosgene in air for 30 minutes, 3 times a week for up to 12 weeks resulted in acute bronchiolitis with peribronchiolar edema, hemorrhage, and inflammatory reactions. Extensive areas of pulmonary edema were also observed. In animals surviving for 4 weeks and longer, the inflammatory reaction

was much less severe despite continued exposure. There was chronic bronchiolitis of varying degree, and associated with the obliterated bronchioles were alternating areas of atelectasis and air space over distribution. Grossly recognizable emphysema was not evident (45).

In another study (29), dogs were exposed 2-3 times a week for 30 minutes each time to 24-40 ppm of phosgene. The animals were killed after 4-10, 15-25, and 30-40 exposures, and their lung examined histologically.

Four to ten exposures resulted in chronic bronchiolitis, partial or complete obliteration of bronchioles, dilatation of terminal respiratory bronchioles, flattened alveoli and fragmented or contracted septa. Lungs from dogs exposed 15-25 times to phosgene developed fibrosis in addition to the above changes. Animals exposed for longer periods (30-40 times) showed changes most suggestive of emphysema. The lungs of control animals were normal except for some pulmonary edema and congestion probably caused by the anesthetic vascular permeability and edema formation.

Cats and guinea pigs were exposed to phosgene at 20-25 mg/m³ (5.0-6.25 ppm) and 10-15 mg/m³ (2.5-3.75 ppm) for 10 minutes/day for 2-41 days (44). At the higher concentration (20-25 mg/m³; 5-6.25 ppm), the animals developed pulmonary edema, bronchitis and bronchiopneumonia. A slight increase in lung weight was seen. Since the degree of pulmonary damage did not increase with the number of exposures, the authors concluded that there was no cumulative effect of phosgene at these concentrations. At the lower dose (10-15 mg/m³; 2.5-3.75 ppm), pulmonary edema was produced only in the cats (50%) but not in the guinea pigs. The alveoli were also affected, but the changes were insignificant.

9. Cytologic and Cytogenetic Effects

No reports were found on the cytologic and cytogenetic effects of phosgene on mitosis and on chromosomes in experimental animals or humans.

10. Molecular Effects

No reports were found on the subchronic effects of phosgene on DNA, RNA, and protein synthesis in experimental animals or humans.

11. Reproductive and Teratogenic Effects

No reports were found on the subchronic effects of phosgene on reproduction and teratogenesis in experimental animals or humans.

12. Metabolism

See acute studies.

13. Carcinogenesis

No reports were found on the possible carcinogenic potential of phosgene in experimental animals or humans.

IV. CHRONIC TOXICITY (long-term tests)

1. Hematologic Effects

In 5 cases of human exposure to low amounts of phosgene (amounts not specified) during a 1.5-3.5 year period, the red blood count, level of hemoglobin, total and differential white blood counts were normal (47).

2. Bone Marrow

No reports were found on the chronic effects of phosgene on the bone marrow in experimental animals or humans.

3. Immunologic Effects

No reports were found on the chronic effects of phosgene on the immunologic system in experimental animals or humans.

4. Central Nervous System (CNS) Effects

Five workers exposed repeatedly to low doses (dose not specified) of phosgene over a 16-50-month period experienced dizziness, mental confusion, blurred vision, severe headaches, a sense of constriction in the chest, and muscle twitching at various times during the exposure prior to entering the hospital (47).

5. Behavioral Effects

Galdston et al. (47) studied the psychiatric status of 5 workers exposed to phosgene for 16-50 months. Four of the five patients were stable and did not show undue distress or serious alteration in their personal functioning; however, one patient showed considerable emotional stress.

6. Cardiovascular System

Humans (5 workers) exposed to small amounts of phosgene (dose not specified) over a period of 16-50 months showed tachycardia (1 patient) and lower cardiac output (1 patient). Pulse rate appeared to be normal in all 5 patients (47).

7. Biochemical and Histochemical Effects

No reports were found on the chronic effects of phosgene on the biochemical and histochemical responses of tissues and organs in experimental animals or humans.

8. Effects on Body Weight, Organs and Tissues

Body Weight

No reports were found on the adverse effects of chronic exposure to phosgene on body weights of animals or humans.

Pulmonary Effects

Pulmonary function. During a pilot study, dogs (number not specified) were exposed to 0.097-0.162 mg/liter (24-40 ppm) of phosgene for 20 minutes in a chamber of 600-liter capacity. After the 30-minute exposure to phosgene, the blower was then operated for an additional 20 minutes to assure complete evacuation of phosgene from the chamber before the dogs were removed. The intervals between exposures were 2 and 7 days, but no concerted effort was made to keep this time interval or the dose per exposure constant. The lower airway resistance was measured at least twice before and after exposure. The normal lower airway resistance in the dog was usually less than 1.0 cm/liter/second. As the animals were repeatedly exposed (over 5.5 months) the lower airway resistance tended to rise, and the rise was roughly proportional to the cumulative dose. The resistance however, fell off appreciably with the interval between gassing exposures (48).

Galdston et al. (47) reported on 5 workers who had repeated exposures to small amounts of phosgene (doses not specified) during the course of 16-50 months. There was impaired ventilatory function of the lungs as indicated by a decrease in vital capacity, slow dilution rate, impaired intrapulmonary mixing of gas, high residual air and mid-capacity volumes, and slow pulmonary emptying rate. These findings were consistent with pulmonary emphysema.

Pathology. One worker reportedly exposed to small amounts of phosgene (amount not given) over a period of 16-50 months was found to have voluminous clear lungs and an emphysematous chest. Re-examined six months after the initial examination, the patient still showed the symptoms presented at the first admission. Another worker under similar circumstances showed rales at the base of each lung and these also were present 6 months later (47).

Eye. Conjunctivitis, blurred vision, and a burning sensation have been reported in cases of humans exposed over long periods (16-50 months) to small amounts (amounts not specified) of phosgene (47).

9. Cytologic and Cytogenetic Effects

No reports were found on the chronic effect of phosgene on mitosis and on chromosomes of experimental animals or humans.

10. Molecular Effects

No reports were found on the chronic effects of phosgene on the DNA, RNA, and protein synthesis in experimental animals or humans.

11. Reproductive and Teratogenic Effects

No reports were found on the chronic effects of phosgene on the reproductive and embryonic tissues of experimental animals or humans.

12. Metabolism

See acute studies.

13. Carcinogenesis

No reports were found on the carcinogenic potential of chronic phosgene treatment in experimental animals or humans.

V. PHYSICAL AND CHEMICAL PROPERTIES

Phosgene (carbonyl chloride, carbon oxychloride or chloroformyl chloride) is a colorless gas at ordinary temperatures. In commerce it is liquified under pressure and/or refrigeration and is classified by the U.S. Department of Transportation as a Class A poison. Its commercial grade and strength is 100%.

The properties and characteristics of phosgene are summarized in Table I.

Phosgene (COCl_2) can be prepared by reacting chlorine and CO, CO and nitronyl chloride, or CCl_4 and oleum. Besides being soluble in water, it is soluble in benzene, toluene, glacial acetic acid and most liquified hydrocarbons (50). One mg/liter of phosgene approximates 247 ppm, and 1 ppm approximates 4.05 mg/m^3 at 25°C , 760 mm Hg (51).

The harmful effects of phosgene are primarily due to irritation. This action is not immediately apparent when exposure is mild, and a person may breathe the gas deeply into the lungs before he is aware of the hazard. This results in irritation of the bronchial tubes and the lung. When the exposure is heavy, the effects become almost immediately apparent but because phosgene produces spasms in the air passages, deep penetration in the lungs is still possible. The gas also has a local irritant action on the eyes, nose, and throat (52).

A concentration of 20 ppm causes coughing, respiratory tract irritation, and severe lung injury in just 1-2 minutes of exposure, which may be fatal within 24 hours or less. At low concentrations, development of pulmonary edema may be delayed as long as 16 hours (52).

Table I

Properties and Characteristics of Phosgene (49)

Appearance	Colorless gas (at atmospheric temperature and pressure) and colorless to light yellow liquid (under pressure/and or refrigeration)
Boiling Point	8.2°C (46.7 F)
Corrosivity	Not corrosive unless exposed to moisture. Reacts readily with water to form HCl and CO ₂ . The HCl is corrosive to metal containers
Critical Pressure	823 lbs/sq inch
Critical Temperature	182°C (360°F)
Density (gas)	4.39 g/liter at 20°C
Flammability	Nonflammable
Heat of Decomposition	1544 Btu/lb
Heat of Evaporation	5832 cal/mol at 7.5°C
Hygroscopicity	Reacts with moisture
Melting (freezing) point	- 127.8°C
Molecular Weight	98.9
Odor	Sweet, in low concentrations; Sharp, pungent, in high concentrations
Specific Gravity (liquid)	1.392 at 19°C
Specific Heat	Gas at 20°C- 0.1462 Btu/lb per °F; Liquid at 20°C - 0.242 Btu/lb per °F.
Vapor Density (air = 1)	3.4
Vapor Pressure	11801 mm at 20°C
Viscosity	Gas - 0.011 cps at 20°C Liquid - 0.47 cps at 0°C

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Phosgene Toxicity - Matrix

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
1. Hematologic Effects	<ul style="list-style-type: none"> o <u>Erythrocytes</u> - Decreased counts (7, 8); No change (9). o <u>Leucocytes</u> - No change (9). o <u>Hemoglobin</u> - Decrease (first 4 hours after exposure) followed by increase (125%) by end of experiment (7, 8, 10). No change (9). o In humans a rise (100%) was observed 6-36 hours following accidental exposure (11). o <u>Other effects.</u> No change in sedimentation rate, viscosity and conductivity of blood (9). 	<ul style="list-style-type: none"> o <u>Erythrocytes.</u> None Reported.* o <u>Leucocytes.</u> None Reported. o <u>Hemoglobin.</u> None Reported. 	<ul style="list-style-type: none"> o <u>Erythrocytes.</u> No adverse effects (human) (47). o <u>Leucocytes.</u> No adverse effects (human) (47). o <u>Hemoglobin.</u> No adverse effects (humans) (47).
2. Bone Marrow Changes	<ul style="list-style-type: none"> o <u>Congestion</u> (2). o <u>Hyperemia</u> (humans) (12). 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported.
3. Immunologic Effects	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported.

* - No references found

Phosgene Toxicity - Matrix

Chronic Treatment Short-term tests; to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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<u>Erythrocytes.</u> None Reported.*	o <u>Erythrocytes.</u> No adverse effects (human) (47).	None	None
<u>Leucocytes.</u> None Reported.	o <u>Leucocytes.</u> No adverse effects (human) (47).		
<u>Hemoglobin.</u> None Reported.	o <u>Hemoglobin.</u> No adverse effects (humans) (47).		

None Reported.	o None Reported.	None	None
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None Reported.	o None Reported.	None	None
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Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
4. Central Nervous Systems (CNS) Effects	<ul style="list-style-type: none"> o Decreased sympathetic nervous system activity (13, 14). o Hyperemia and edema in brain tissue (2, 12). o Subarachnoid hemorrhage, cellular degeneration in grey matter (human) (12). o "Ring hemorrhages" in the brain (15-17). 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o Dizziness, mental confusion, severe headaches, twitching (human) (47).
5. Behavioral Effects	<ul style="list-style-type: none"> o Nervousness, hypochondriasis (human) (11, 18). 		<ul style="list-style-type: none"> o Emotional stress (47)
6. Cardiovascular Effects	<ul style="list-style-type: none"> o Lowered pulse rate up to 4-5 hours post gassing (19, 21). o Tachycardia after gassing followed by bradycardia (7, 11, 19, 21). o Increased blood pressure (10% at 5 hr post-gassing) returning to normal (8th hour) and then rapidly declining before death (7, 8). 	<ul style="list-style-type: none"> o None Reported. o None Reported. o None Reported. 	<ul style="list-style-type: none"> o None Reported. o Tachycardia (human) (47). o None Reported.

1

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment (short-term tests; 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None Reported.	o Dizziness, mental confusion, severe headaches, twitching (human) (47).	None	None
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	o Emotional stress (47).	None	None
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None Reported.	o None Reported.	None	None
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None Reported.	o Tachycardia (human) (47).		
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None Reported.	o None Reported.		
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Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
6. Cardiovascular Effects (Cont'd)	<ul style="list-style-type: none"> o Enlarged right and left ventricles (7). o Increased pulmonary circulation (21). o Spasms in systemic vessels (21). o <u>In vitro</u> studies using perfused heart preparation were inconclusive (22). o Cardiac dilatation (humans)(15, 20). o Subendocardial and subpericardial hemorrhage (humans) (15). 	<ul style="list-style-type: none"> o None Reported. o None Reported. o None Reported. o None Reported. o None Reported. o None Reported. 	<ul style="list-style-type: none"> o None Reported. o None Reported. o None Reported. o None Reported. o None Reported. o None Reported.
7. Biochemical and Histochemical Effects	<ul style="list-style-type: none"> o <u>Blood Elevated</u> chloride, total lipids, neutral fats, total fatty acids, total cholesterol, phospholipid (23). o <u>Depressed</u> albumin and globulin fractions of blood plasma (8). 	<ul style="list-style-type: none"> o <u>Blood.</u> None Reported. 	<ul style="list-style-type: none"> o <u>Blood.</u> None Reported.

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment Short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
None Reported.	o None Reported.		
None Reported.	o None Reported.		
None Reported.	o None Reported.		
None Reported.	o None Reported.		
None Reported.	o None Reported.		
None Reported.	o None Reported.		
Blood. None Reported.	o Blood. None Reported.		

1. Depressed
A/G

1. A/G Ratios

2

Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including in vitro tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
7. Biochemical and Histochemical Effects (Cont'd)	<p><u>No change.</u> Urea, total nitrogen, nonprotein nitrogen, chloride, and protein levels (9).</p> <p>Presence of hematin in serum (24).</p>	<p>o <u>Development of Tolerance to phosgene in animals</u> (5, 43, 44). No information on same in humans.</p>	<p>o None Reported.</p>
8. Body Weight, Organs and Tissues	<p>o <u>Body Weight.</u> None Reported.</p> <p>o <u>Pulmonary Effects Function. Increased</u> resistance to gas diffusion across lung membrane (25), respiratory rate (7), blood volume in lung (9).</p> <p><u>Decreased CO uptake</u> (25); oxygen level in arterial blood (26, 27); tidal air (9); vital capacity (humans) (28).</p>	<p>o <u>Body Weight.</u> Decreased (44).</p> <p>o <u>Pulmonary Effects Function. Increased</u> bronchial resistance to gas diffusion across lung membrane (45), respiratory rate (46).</p>	<p>o <u>Body Weight.</u> None Reported.</p> <p>o <u>Pulmonary Effects Function. Increased</u> bronchial resistance to gas diffusion across lung membrane (48).</p> <p><u>Decreased vital capacity</u> (human) (47).</p>

Phosgene Toxicity - Matrix (Cont'd)

Acute Treatment (short-term tests; 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Development of tolerance to phosgene in animals (5, 43, 44). No information on tolerance in humans.	o None Reported.		
<u>Body Weight.</u> Decreased (44).	o <u>Body Weight.</u> None Reported.		
<u>Pulmonary Effects</u> <u>Function.</u> Increased bronchial resistance to gas diffusion across lung membrane (45), respiratory rate (46).	o <u>Pulmonary Effects</u> <u>Function.</u> Increased bronchial resistance to gas diffusion across lung membrane (48).	1. Resistance to gas diffusion	1. Gas diffusion test 2. Pulmonary edema test (labelled albumin)
	<u>Decreased vital capacity</u> (human) (47).		

Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
8. Body Weight, Organs and Tissues (Cont'd)	<p><u>Pathology.</u> Bronchiolar lesions (2, 4, 7, 26, 29, 30); alveolar edema (4, 9, 30); emphysema and hemorrhage (2, 23, 30); focal fibrosis (2).</p> <p><u>In humans,</u> pleural effusions, interstitial emphysema, desquamation of bronchi, fibrotic adhesion in lung (11, 12, 15, 20, 31, 32).</p> <p><u>Vascular permeability and edema</u> (8, 23, 33).</p> <ul style="list-style-type: none"> o <u>Nose</u> (odor perception) (human) (34, 35). o <u>Liver</u> congested (2). o <u>Kidney</u> congested (2). o <u>Spleen</u> congested (2). o <u>Intestines</u> congested (2). o <u>Adrenal</u>, occasional medullary hemorrhage (2). 	<p><u>Pathology</u> same as acute (29, 45).</p> <p><u>Vascular permeability and edema</u> (44).</p> <ul style="list-style-type: none"> o <u>Nose</u> (odor perception) None Reported. o <u>Liver.</u> None Reported. o <u>Kidney.</u> None Reported. o <u>Spleen.</u> None Reported. o <u>Intestine.</u> None Reported. 	<p><u>Pathology</u> voluminous clear lungs, emphysematous chest (human) (47).</p> <ul style="list-style-type: none"> o <u>Nose</u> (odor perception). None Reported. o <u>Liver.</u> None Reported. o <u>Kidney.</u> None Reported. o <u>Spleen.</u> None Reported. o <u>Intestine.</u> None Reported. o <u>Adrenal.</u> None Reported.

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment Short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Pathology same as acute (29, 45).

Pathology voluminous clear lungs, emphysematous chest (human) (47).

Vascular permeability and edema (44).

Nose (odor perception) None Reported.

Liver. None Reported.

Kidney. None Reported.

Spleen. None Reported.

Intestine. None Reported.

o Nose (odor perception). None Reported.

o Liver. None Reported.

o Kidney. None Reported.

o Spleen. None Reported.

o Intestine. None Reported.

o Adrenal. None Reported.

Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Pre-End
8. Body Weight, Organs and Tissues (Cont'd)	<ul style="list-style-type: none"> o In <u>humans</u>, no adverse effects were observed on <u>liver, kidney, spleen, intestines</u> and <u>stomach</u> in one study (19), but in another study, hyperemia was observed in <u>kidney, spleen, thyroid</u> and <u>thymus</u>. In a third study, <u>heart</u> and <u>liver</u> abnormalities were reported (36). 		<ul style="list-style-type: none"> o In <u>humans</u>. None Reported on <u>liver, kidney, spleen, stomach, intestine, thyroid</u> <u>thymus</u>. o <u>Eye</u> conjunctivitis, blurred vision, burning sensation (human) (47). 	
9. Cytologic and Cytogenetic Effects	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	
10. Molecular Effects	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	
11. Reproductive and Teratogenic Effects	<ul style="list-style-type: none"> o <u>Humans</u>. No adverse effects on fetus (37). 	<ul style="list-style-type: none"> o None Reported. 	<ul style="list-style-type: none"> o None Reported. 	

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment Short-term tests; (up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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	<ul style="list-style-type: none"> o In <u>humans</u>. None Reported on <u>liver</u>, <u>kidney</u>, <u>spleen</u>, <u>stomach</u>, <u>intestine</u>, <u>thyroid</u> <u>thymus</u>. 		
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	<ul style="list-style-type: none"> o <u>Eye</u> conjunctivitis, blurred vision, burning sensation (human) (47). 		
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None Reported.			
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	o None Reported.		
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		None	
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None Reported.			
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None Reported.			
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	o None Reported.		
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			None
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Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
12. Metabolism	<ul style="list-style-type: none"> o The inhalation toxicity of phosgene is probably enhanced by low solubility which allows it to penetrate further into the lungs. It is poorly absorbed in the nasal passages in animals (8). The data on the solubility and rate of hydrolysis indicate that phosgene in molecular form can penetrate all three layers of the blood-air barrier and that its effects are caused by molecular phosgene and not by HCl (38, 39). The upper respiratory tract is protected against phosgene by hydrolysis in the mucous membrane (39). 	<ul style="list-style-type: none"> o See acute studies. 	<ul style="list-style-type: none"> o See acute studies.

Genotoxicity - Matrix (Cont'd)

Acute Treatment Short-term tests; (days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
Acute studies.	o See acute studies.	None	None

Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
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12. Metabolism
(Cont'd)

Experiments indicate phosgene affects tissues because the carbonyl group combines with the free amines of cell enzymes or other critical substances (40). Phosgene's primary effect appears to be an alteration in lung capillary permeability, resulting in loss of fluid with high protein content (plasma-like fluid) from the circulation and into the pulmonary tissues (8, 41). The rate of resorption of the plasma-like fluid from surrounding tissue is slow and is unable to keep pace with the plasma outflow. Thus edema progresses, O₂ transfer is disturbed, and anoxia is caused (42).

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment (short-term tests; 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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Phosgene Toxicity - Matrix (Cont'd)

System or Effect	Acute Treatment (including <u>in vitro</u> tests)	Subchronic Treatment (short-term tests; up to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)
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12. Metabolism
(Cont'd)

Phosgene produces profound shifts in body water by acting on local exposed capillaries. Its action is confined to the lung since it is rapidly hydrolyzed in the pulmonary tissues so that none passes into the general circulation. The underlying mechanism of its action on lung capillaries is not known. It may be enzymic, since the mitochondria of the endothelium disintegrate and disappear before the capillaries begin to leak (33, 42).

13. Carcinogenesis o None Reported. o None Reported. o None Reported.

Phosgene Toxicity - Matrix (Cont'd)

Chronic Treatment Short-term tests; to 90 days)	Chronic Treatment (long-term tests; up to 2 years or lifetime)	Predictive Endpoints	Tests Recommended
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None Reported.

o None Reported.

None

None

2

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