III. HEALTH HAZARDS FROM EXPOSURE IN PESTICIDE MANUFACTURE AND FORMULATION

As defined by the Federal Environmental Pesticide Control Act (FEPCA), a pesticide is (1) "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (insect, nematode, fungus, weed, other forms of terrestial or aquatic plant or animal life or viruses, bacteria, or other microorganisms, except microorganisms on or in man or other living animals) which the Administrator (EPA) declares to be a pest, and (2) any substance or mixture of substances intended for use as a plant regulator, defoliant or dessicant" (40 CFR 162). There are approximately 1,500 active ingredients in common use and registered by EPA (Federal Register 41:7218-7375, February 17, 1976). There are also numerous other pesticide active ingredients for which tolerance levels on food have been established, which are registered on an experimental basis only at this time, or which are produced in the United States for export. These substances range in acute toxicity from lethal at low doses (strychnine) to edible in relatively large quantities (sodium chloride). Certain of these compounds have produced carcinogenic, teratogenic, mutagenic, and neurotoxic effects, and alteration of reproductive processes or functions in experimental animals. Consequently, this chapter is not intended

to be a comprehensive review of any individual pesticide or to cover the biologic effects of any substantial fraction of the total number of substances currently registered and used as pesticides.

The earliest known pesticides were organic materials of natural origin. Inorganic compounds, particularly the salts of arsenic, lead, mercury, copper, and zinc, came into wide use as pesticides in the mid-19th century. The era of synthetic organic pesticides began with the discovery of the insecticidal activity of dichlorodiphenyltrichloroethane (DDT) in 1939. The success of DDT during World War II against lice carrying typhus and against mosquitos carrying malaria created great enthusiasm for commercial use when the war ended. Subsequent research developed a wide variety of synthetic organic pesticides, including the organochlorine (OC), organophosphorus (OP), and carbamate pesticides. The 1,500 active pesticide ingredients are now formulated into more than 40,000 registered pesticide products [1].

Since the discovery and use of pesticides, their potential for producing harmful effects in humans has been recognized along with their beneficial effects. This recognition has resulted in a vast expenditure of government funds for scientific research and in a voluminous amount of literature pertaining to the biologic effects of pesticides. Much has been learned of the biologic effects of these substances, but the research effort has been disappointing in some important respects. For instance, probably no substance has been more intensively studied than DDT,

especially in terms of potential for carcinogenic effects. Yet, it is unknown whether the very small amounts of DDT to which we have all been exposed are responsible for any portion of the overall human cancer problem.

While the results of studies on the effects of exposure of humans to small quantities of DDT and other pesticides remain inconclusive, larger exposures to certain pesticides are conclusively harmful. The recent incidents of severe neurologic disorders in employees involved in the manufacture of Kepone and leptophos provide clear evidence of harmful effects. Based on these episodes and other less spectacular incidents discussed later in this chapter, every effort must be made to minimize human exposure to pesticides, especially during their manufacture and formulation when the opportunity for excessive exposure is potentially the greatest.

In the development of pesticides today, an effort should be made to achieve a selective toxic effect by exploiting physiological and biochemical differences between cells of pest organisms and those of nontarget organisms. Such differences are infrequent and are overshadowed by the abundance of similarities existing among most species. Moreover, while a particular substance may be thought of as having one main toxic action, eg, inhibition of chitin synthesis in insects, the normal functions of the mammalian body involve so many enzyme systems and physiologic interactions that the probabilities are high that the substance may also affect mammalian systems.

DDT, at the time of its development as an insecticide, was

one of the two substances (the other being penicillin) thought to have achieved the ultimate in selective action. A very high ratio exists between the acute toxicities of DDT for the mosquito and for man. However, recent research has demonstrated that the compound is capable of producing tumors in some species of experimental animals [2]. Furthermore, there is convincing evidence that DDT and its metabolites accumulate in food chains by a process of biologic concentration in ecosystems [3]. These findings have severely limited the acceptability of DDT use in many countries.

Similarly, when mirex was developed as an effective weapon against the fire ant a few years ago, it was believed to be a safe pesticide with regard to man and the environment. However, recent animal tests indicate this substance is a carcinogen [4,5], further demonstrating that pesticides, although specifically designed to attack undesired insects and other pests, present potential hazards to human beings.

Observations in both humans and experimental animals have clearly shown the ability of certain pesticides to produce delayed irreversible effects. Induction of cancer in humans is a primary concern, and may require as many as 20-40 years to appear. Due to the inability to detect most cancer and to relate it to a specific etiologic agent, as well as a desire to prevent its occurrence, the results of animal studies must be relied upon extensively in assessing the toxic effects of pesticides. The same argument holds for other types of delayed actions including carcinogenesis, mutagenesis, teratogenesis, other effects on

reproductive processes, chronic toxic effects on parenchymatous organs, eg, aplastic anemia, chronic nephrosclerosis and cirrhosis, and interference with neuronal integrity.

Industry Characteristics and Extent of Exposure

(a) Pesticide Production

In 1975, the latest year for which figures are available, 1.61 billion pounds of pesticide active ingredients (excluding creosote) were produced in the United States, an increase of 13.5% over 1974 [1]. Of this total, 788 million pounds were herbicides, 666 million pounds were insecticides, and 156 million pounds were fungicides [1]. The growth in pesticide production is expected to continue according to a 1976 survey conducted by the US Department of Agriculture's (USDA) Economic Research Service [6]. Expansion in the industry should boost domestic capacity in 1976 by 12% and by another 7% by 1978 [1].

Approximately 90% of all present-day pesticides are organic compounds. Insecticides consist primarily of OC, OP, and carbamate compounds. Fumigants include halogenated hydrocarbons and inorganic gases. Herbicides include amides, arsenicals, carbamates and thiocarbamates, OP compounds, and substituted ureas. Fungicides include thiocarbamates, phthalimides, and orgarotin compounds. The production of these compounds involves many chemical processes including chlorination, alkylation, nitration, phosphorylation, sulfonation, and bromination.

Creosote, a mixture of phenols derived from either wood or bituminous coal by destructive distillation, is used as a wood

preservative because of its ability to kill both the fungi and the boring insects and arthropods that are likely to infest wood. In 1972, approximately 1.15 billion pounds of creosote were produced, an amount almost equal to the total amount of synthetic organic pesticides produced that year [7].

Inorganic pesticides account for the remaining 10% of pesticide production and include calcium arsenate, lead arsenate, sodium flouride, arsenic acid, borate, and sulfur. Of all inorganic pesticides, 55% are fungicides, 38% are herbicides, and 7% are insecticides [1]. The estimated 1975 production levels and uses of major pesticides are shown in Table XIV-1.

(b) Description of Industry

Commercial pesticide products are produced in two sequential operations: manufacturing and formulating. The manufacturing operation produces the active pesticide ingredients by chemical synthetic procedures. The active ingredients are then transformed into formulated products by diluting them with solvents, by spraying them onto clay, or by mixing them with other carriers. By their nature, formulating operations are primarily batch mixing and blending operations [8(pp 1,70)].

(1) Pesticide Manufacturers

Pesticide manufacturers usually operate capital-intensive, integrated chemical synthesis plants and, with a few exceptions, produce many other chemical products in addition to pesticides. In 1972, the average pesticide manufacturing plant employed approximately 185 workers, 100 of whom were employed directly in production operations [9]. The

employees normally include chemists, engineers, managers, skilled chemical operators, pipefitters, electricians, and laborers.

Manufacturing includes pretreatment of reactants (change of size, temperature, and state), reaction, purification, and post-treatment of products (change of size and state). Raw materials for manufacture are delivered in bulk by pipeline, railroad car, barge, etc, or may be produced in-plant, often as by-products of other reactions. Manufacturers may produce several pesticide active ingredients at a single plant, and such a plant usually consists of several separate but interconnectable production areas or subplants. A subplant contains the equipment necessary for carrying out all the unit processes and operations, such as reaction, distillation, filtration, and mixing, which are necessary to synthesize a product from raw materials. Subplant hardware may include mills, screens, hoppers, tanks, reactors, absorption columns, cooling towers, stills, filters, centrifuges, dryers, etc. Process monitoring, sampling, and analysis are performed to determine temperatures, pressures, flowrates, densities, and composition changes in order to control the chemical reactions.

(2) Pesticide Formulators

Pesticide formulating establishments are generally smaller than manufacturing establishments. The average formulator employs 32 workers, 18 of whom are employed in production [9]. Employees may include engineers, chemists, operators, and laborers. There are major variations among formulators both in size and in operating practices. Seventy-one

percent of all formulating establishments employ less than 20 employees [9]. Cnly 6% of all establishments employ more than 100 employees [9], but these larger plants dominate total production. Formulators with 100 or more employees account for 56% of production, whereas formulators with less than 20 employees account for only 12.5% of all production [9].

A formulating operation is generally less complex than a manufacturing operation. Usually, a formulator receives concentrated active ingredients from a manufacturer or a customer and dilutes them with various nonpesticidal materials known as "inerts." The term inert refers to the effect of the substance on the target organism relative to the effect of the pesticide on that organism. However, as with any chemical, a toxic effect can be obtained with any inert at some dose, and many so-called inerts are more toxic to man than the term would lead one to believe. The formulating process may also include physical or chemical treatment to yield particular product forms: dust, powder, wettable powder, granule, pellet, emulsifiable concentrate, or aerosol. Further processing may be done by wholesalers, retailers, repackagers, or end users. With a few exceptions, formulating is a simple process varying in type according to the desired end product [8(pp 1,11,12,7)].

The preparation of dust and powder pesticide formulations entails dispensing from hoppers, screening for size, and mixing with flour, silica, sulfur, lime, gypsum, talc, or clay in a hammer mill, roller mill, or other type of mill. Granule-formulating consists of dispensing and sizing the inert,

eg, clay, vermiculite, ground corncobs, diatomaceous earth, dissolving or melting the pesticide in a tank, and then spraying the pesticide onto the inert in a mixer [8(pp 1,11,12,34,35)].

Liquid formulations are generally prepared by placing both a pesticide and a solvent into a mixing tank provided with some type of agitator. The resultant liquid may be filtered or decanted to remove insoluble material.

The care exercised in substance handling and control during formulation varies greatly from the relatively sophisticated procedures and equipment found in a major facility where both manufacturing and formulating occur [8(pp 52-62)] to less complicated setups where, for example, second-hand equipment is used [8(pp 11-18)]. Chapters IV and V provide additional details on the manufacturing and formulating processes, work practices, and control processes.

(c) Industry Statistics

The Standard Industrial Classification Codes (SIC's) for establishments primarily involved in the manufacture and formulation of pesticides are:

(1) SIC 2869 - Manufacture of pesticide and other organic chemicals, not formulas; and

(2) SIC 2879 - Agriculture chemical manufacturers and formulators--including insecticides, herbicides, agricultural chemicals, household insecticides, and agricultural chemicals not otherwise classified.

The most recent Bureau of Census statistics (Table XIV-2) for these two industries show that there were 19 manufacturing

establishments and 388 formulating establishments in 1972 identified as primarily producing pesticides. Eighty-one percent of the value of shipment from all manufacturing and formulating establishments originated at these facilities. The remainder was produced at 6,679 other establishments, which produce pesticides as secondary products. These establishments are in a number of industries such as Industrial Organic Chemicals (SIC 286), Polishes and Sanitation Goods (SIC 2842), Plastic Materials and Resins (SIC 2821), Pharmaceutical Preparation (SIC 2834), and Industrial Inorganic Chemicals (SIC 281).

The geographic distribution of pesticide production in the US is shown in Table XIV-3. The greatest amounts of pesticides are produced in the South, followed by the North Central region. The South also has the largest number of formulating establishments (Table XIV-2), reflecting the region's high agricultural production. The value of shipment data in Tables XIV-3 and XIV-4 reveal that although the Western region has approximately 25% of the primary formulating establishments, they appear to be smaller than those found in other parts of the US, due to the low value of shipment for this region (11%). Conversely, the formulating establishments found in the North Central region are relatively larger than those found in other parts of the country. This region shipped 41% of dollar value from just 25% of US formulating establishments.

(d) Estimates of Worker Population

Table XIV-4 shows that approximately 8,700 production workers are employed in establishments identified as primary

manufacturers and formulators of pesticides. Of this total number of production workers, 1,900 work in manufacturing plants and 6,800 work in formulating plants.

In Table XIV-5, the distribution of formulating plant workers is shown by size of the formulating plant. Approximately 1,000 workers are employed in plants that have less than 20 employees, and another 2,100 workers are employed in plants that have between 20 and 95 workers.

To estimate the number of production workers exposed in other establishments producing pesticides as secondary products is difficult. However, in these industries there are over 350,000 additional production employees who have the potential for exposure because they work at a plant that produces pesticides [9].

Pesticide Properties and Worker Exposure Routes

The particle size, volatility, and solubility of materials present during the manufacture and formulation of pesticides significantly affect environmental concentrations and accessibility to the possible routes of entry of potentially exposed workers. The following descriptions are of specific chemical and physical factors involved in the major routes of pesticide exposure.

(a) Inhalation Exposure

The inhalation of pesticide dusts, vapors, mists, and gases may present a significant occupational hazard. Dust hazards are created by dry formulations that involve granules,

wettable powders, baits, and soluble powders. For example, captan, carbaryl, and chlorda are usually formulated in powder form, whereas chlorpyrifos and diazinon are often formulated in granular form. Exposure to pesticide vapor can occur in the production of fumigants such as ethylene dibromide, acrylonitrile, and trichloroethylene, and some fumigants, such as phosgene and ethylene oxide, are gases at standard temperature and pressure (STP). Although the carriers used in formulating pesticide dusts are described as "inerts" with respect to pesticide potency, several may present health hazards; for example, talc may contain asbestiform fibers.

In pesticide operations, dusts are generated by the mechanical agitation of solid materials as in packaging and milling. Hammer-mill grinding has been shown to produce powders with average particle sizes up to 75μ. Air-mill grinding creates micronized particles of 2-5μ[10].

Retention of particles within the lungs depends on many factors: size, shape, hygroscopicity, density, reactivity, and nasal or oral inhalation [11]. Hayes [11] speculated that in the absence of specific information on a particulate, it can be assumed that about 25% of inhaled material would be exhaled, about 50% would be deposited in the upper respiratory passages and subsequently swallowed, and about 25% would be deposited in the lower respiratory passages.

Several studies have been conducted to measure worker respiratory exposure during pesticide manufacture and formulation. Comer et al [12] reported that formulating plant

workers had higher potential for body-front exposure than did spraymen. Tests were conducted in three formulating plants during formulation of 4 and 5% carbaryl dusts. The workers studied were at bagging or mixing stations, areas of greatest potential contamination. Considerable variation was observed in the range of exposure values for each work activity. One of the factors observed as causing occasional high values of exposure was malfunctioning of the bag-filler spout mechanism, resulting in excess billowing dust. The formulation plant workers' mean respiratory exposure was 1.1 mg/hour of work activity. The amount of pesticide entering the body via the respiratory route was estimated from the contamination of special filter pads used in place of the usual outer absorbent filter pads which cover the filter cartridges of the respirators worn. To compare values, tests were also conducted on spraymen operating tractor-drawn airblast equipment as they applied 0.045-0.6% carbaryl spray to fruit orchards. For spraymen the mean exposure value was 0.09 mg/hour. It is reasonable to assume that the higher amounts inhaled by formulators reflect their frontal exposure to concentrated dry carbaryl dust, and that spraymen inhaled and consequently absorbed lesser amounts due to the larger size of the spray droplets.

In another study, Jegier [13] measured respiratory exposure of formulation plant workers during the formulation of 25% azinphos-methyl wettable powder. Air samples were taken in the breathing zone of the plant workers during the formulation process. Respiratory exposure was determined directly from the

quantities of azinphos-methyl deposited on filter pads attached to double-filter respirators. The concentrations in the air sample ranged from 1.07 to 9.64 mg/cu m. The filter pads held amounts from 0.72 to 8.24 mg/day. The highest concentrations were recorded in the blending area of the plant.

Hayes [14] reviewed the relationship between physical forms and the exposure hazards of the three major types of pesticide formulations (gases, dusts, and sprays). He found that materials in the form of a gas, vapor, or very fine particulate were inhaled more efficiently than are larger particles, and he concluded that the most hazardous form for respiratory intake was a gas, while dusts and then sprays followed in decreasing order of hazard potential. This effect of particle or aerosol size on exposure was further studied in agricultural spraymen by Wolfe et al [15] who concluded that the smaller aerosol particles (20-100μ diameter) generated by a concentrate-spray apparatus compared with those produced by a conventional spray machine (over 150μ diameter) resulted in increased potential exposure through the respiratory tract. The mean respiratory exposure by the concentrate-spray technique was 0.05 mg/hour while by the conventional dilute-spray method the mean was 0.02 mg/hour. The concentration of the pesticide in the preparation being sprayed was also mentioned as a factor to be considered in the assessment of potential exposure.

Pesticides have a wide range of vapor pressures, varying from nonvolatile materials, such as DDT and dieldrin, to compounds with extremely high vapor pressures, such as methyl

bromide. The volatility of the material affects the environmental concentration and, thus, is a significant factor in respiratory exposure.

Dichlorvos (DDVP), an OP compound, is an example of a highly volatile pesticide. Because of this property, DDVP is frequently formulated with wax or other suitable materials from which it is slowly released. Its high vapor pressure may cause large concentrations to accumulate in the air of the workplace during manufacturing and formulating operations. Menz et al [16] measured the exposure of factory workers to DDVP in the production and processing of a DDVP-releasing aerosol product. Measurements were taken in the vaporizer production and packaging room for 8 months, and DDVP levels ranging up to 3 mg/cu m were detected. Although the various measurements revealed considerable fluctuations, on the average, the employees were exposed to DDVP concentrations of).7 mg/cu m on each working day of the experimental period.

Many incidents of poisoning due to respiratory exposure to a variety of pesticides have been reported in the literature and are discussed later in this chapter. These incidents demonstrate the need to protect workers from this exposure mode. The experience with methomyl [17] shows that problems due to toxicity can be avoided by controlling the form of pesticides and by reducing the inhalation hazard. Methomyl is a highly toxic carbamate pesticide which was introduced in California in 1969 as a water-dispersible powder of 90% concentration. Shortly thereafter, a number of poisoning cases occurred in which

methomyl was implicated as the causative agent. These cases included acute intoxications in formulation plant workers and farm laborers from inhalation of the powder. Subsequently, a liquid formulation of methomyl was introduced to avoid exposures that resulted from handling the light pesticide powder, and this resulted in fewer methomyl poisoning incidents.

(b) Dermal Exposure and Absorption

Workers frequently experience dermal exposure to pesticides with subsequent absorption through the skin. Exposure studies of formulators [12] and agricultural workers [13] have shown that dermal exposure to pesticides occurs frequently if proper precautions are not taken. Wolfe and Armstrong [18] reported the dermal exposures of workers to DDT in two formulating plants. Highest exposures occurred at the bagging station where the mean dermal exposure was calculated to be 524.5 mg/hour. Comer et al [12] observed that carbaryl formulating plant workers received a mean dermal exposure of 73.9 mg/hour of work activity. Jegier [13] reported dermal exposure to azinphos-methyl by formulation workers in the blending area based on quantities of the pesticide collected on cellulose pads attached to workers' foreheads. The exposure levels ranged from 39.2 to 167.2 mg/day with a mean of 80.9 mg/day.

The rate at which a particular compound is absorbed through the skin is determined by the nature of the compound itself, by the condition of the skin, and by external factors such as temperature [11]. One major factor is the solubility of the pesticide. Lipid-soluble compounds, eg, parathion, DDT,

aldrin, and toxaphene, are absorbed more readily than water-soluble materials [11]. The most rapid and complete absorption through the skin occurs with chemicals which have some solubility in both water and lipid [19].

Skin absorption for any given compound is also proportional to the skin area exposed, and dependent upon the region exposed. Furthermore, different portions of the human skin absorb chemical substances at different rates. Maibach and coworkers [20] used 14C-labeled parathion to compare absorptive capacities of different parts of the human body. The results (see Table III-1) indicate that under experimental conditions the greatest rate of absorption and the most complete uptake in the male was through the scrotal skin where approximately 100% of the applied dose was absorbed. The possibility of pesticide being completely absorbed through the scrotal skin emphasizes the need for increased concern for protection of this area. Another area of the body where absorption was higher than expected was the head and neck, where 32-47% of the applied parathion dose was absorbed compared with approximately 9% for the forearm.

A major factor that may alter both the degree and rate of skin absorption is the condition of the skin barrier itself [11]. The skin barrier may be injured, thereby leading to increased absorption, by a number of factors including washing with organic solvents, irradiation, and thermal or chemical burns [11].

TABLE III-1

ABSORPTION OF PARATHION FROM
VARIOUS PARTS OF THE BODY

	Urinary 14C Excretion Expressed as Percent Applied Dose	Ratio to Forearm
Forearm	8.6 ± 5.6	1.0
Palm	11.8 ± 4.0	1.3
Abdomen	18.5 ± 11.5	2.1
Back of the Hand	21.0 ± 8.1	2.4
Scalp	32.2 ± 6.1	3.7
Forehead	36.2 ± 12.6	4.2
Ear Canal	46.5 ± 17.7	5.4
Axilla	64.0 ± 32.5	7.4
Scrotum	101.6 ± 18.8	11.8

Adapted from reference 20

Dermatitis and eczema also decrease the effectiveness of the skin barrier and increase uptake [21]. Increased circulation of blood through the dermis of the skin by physical movement and sweating also enhance the intake from the surface of the skin [11]. Very fine powders tend to be absorbed more readily than coarser powders rubbed on the skin in exactly the same way. Hayes [11] found that very finely ground technical dieldrin was absorbed as readily as dieldrin applied to the skin in solution; in contrast,

coarsely ground dieldrin powder was not as well absorbed. The particle sizes of the powders were not specified.

Solvents, binders, and inert materials used in pesticide manufacture and formulation also have an effect on pesticide dermal absorption and toxicity. For example, Brown [21] recorded significant changes in the LD50's of two experimental samples of carbamates (composition proprietary) in CFE strain rats with varying solvents, because of differences in dermal absorption. The different solvents tested included acetone, N-methylpyrrolidone, and xylene. The first sample of carbamate in acetone at a concentration of 20% resulted in an LD50 of >1,000 mg/kg compared with one of 100-200 mg/kg when the carbamate was dissolved in N-methylpyrrolidone at a concentration of 25%. A different sample of carbamate in acetone at a concentration of 20% yielded an LD50 of >100 mg/kg in males and 100 mg/kg in females, compared with ones of 10 mg/kg in males and <3 mg/kg in females obtained when a 5% solution in xylene was used. Increase in absorption may involve a solvent that by its own ready absorption helps to carry the toxicant through the skin. Injury to the skin by these agents may also increase dermal absorption.

Adherence of pesticides to the skin was demonstrated by Fredriksson [22] who studied the decontamination of human skin exposed to parathion. Using 32P-labeled parathion, applied to the skin of four volunteers, he demonstrated that a soap and water wash for 30 seconds removed only 36-48% of the remaining parathion if the wash was delayed for 6 hours. A wash with

alcohol, in which parathion is soluble, still allowed 10% of the dose to remain after the same amount of time.

In summary, for many pesticides, especially the OC, OP and carbamate insecticides, exposure via the dermal route is one of the most important sources of exposure. Extra care should be taken to protect all areas of the skin from exposure to percutaneously absorbable compounds, and employees should be well aware of the hazards of dermal absorption of pesticides.

(c) Gastrointestinal Absorption

Oral exposures occur through accidental splashing of liquid pesticides into the mouth, by smoking or eating with pesticide-contaminated hands, by rubbing the mouth area with contaminated hands, and by swallowing inhaled material that may have entered the upper respiratory tract or have been swept up the trachea by ciliary action into the pharynx. However, such oral exposures are difficult to measure or quantify.

The esters of such acids as 2, 4-D and 2, 4, 5-T would be expected to be hydrolyzed within the intestinal tracts of mammals, so that the free acids would be the entities to be absorbed through the walls of the tract. Both 2, 4-D and 2, 4, 5-T are weak acids, with pK's at 25C of 3.31 and 3.14, respectively, and are soluble in lipids and lipid solvents. Such compounds would be expected to be essentially nonionized in stomachs, where the pH is between 1.0 and 2.0, and to be well absorbed, therefore, from the stomach and the first portion of the duodenum [23,24]. As the pH of the contents of the intestinal tract rises after the influx of the succus entericus

and the pancreatic secretions, these compounds would become more ionized and, consequently, less lipid soluble and less well absorbed. Absorption would occur by the process described by Palay and Karlin [25] of pinocytosis into the mucosal cell, collection in the endoplasmic reticulum, and delivery into the lymphatic system. This same mechanism has reportedly applied to methoxychlor [26], another material soluble in lipids and lipid solvents. On the other hand, paraquat, a compound highly soluble in water (67 g in 100 ml), is usually highly ionized, with two positive charges per molecule. Accordingly, it is poorly absorbed from the intestinal tract [27].

(d) Ocular Exposure

The concentration of toxicant that contacts the exposed surface of the eyes due to spills or splashes may be greater than that reached in the body as a whole, so that local effects may be produced on the eye or its accessory structure (conjunctivae, eyelids, etc) in the absence of observable systemic effects. Upholt et al [28] discussed this phenomenon in a study of volunteers exposed to tetraethyl pyrophosphate in which no systemic illness was found, but in which miosis appeared.

Maddy and Topper [29] described many examples of eye injuries due to pesticides in California during 1975. One occurred to an employee who was working with a dust collector when some excess captafol powder was blown out causing the powder to enter through the side vents of his goggles, resulting in eye irritation. Another exposure occurred when a chemist observing a formulating procedure without wearing goggles was exposed to

chlorothalonil dust. He suffered from conjunctivitis and photophobia for 3 days and did not regain normal vision for a week [29].

General Toxicologic Effects of Pesticides

Pesticides have caused diverse toxic effects on various human and animal organs and organ systems including the liver, kidneys, skin, lungs, brain, nervous system, and eyes. Certain pesticides appear to be carcinogenic in humans and others have produced tumors of vital organs in test animals. They have also caused structural and functional defects in unborn experimental animals and mutagenic changes in hereditary characteristics in in vivo and in vitro test systems.

The many types of chemical compounds used as pesticides can be grouped on the basis of chemical structure into generic classes such as chlorinated hydrocarbons, OP's, carbamates, and chlorophenoxy acid esters and salts. While there are significant variations in the toxic effects of the individual pesticides within each structural class, common effects have been observed.

(a) Organochlorine Insecticides

DDT, aldrin, dieldrin, lindane, chlordane, toxaphene, and mirex are some of the most important OC pesticides in terms of production and use [7]. These compounds are all nonpolar substances and thus are soluble in lipids and organic solvents and are relatively resistant to metabolism or degradation. Consequently, these compounds have a strong tendency to penetrate cell membranes and to be stored in the body fat. Chronic,

long-term exposure to these compounds usually presents a more serious problem than acute exposure [30].

OC pesticides primarily tend to damage the liver and kidneys [2,31]. Several of these pesticides, including DDT [31], aldrin [2], dieldrin [2], and mirex [4,5], have produced benign and malignant tumors in the livers of chronically exposed experimental animals, particularly mice. The hazards from skin absorption are small when the material is dry or in powdered form. On the other hand, when dissolved in oil or organic solvents, the materials are well absorbed through the skin and constitute a considerable hazard. Behavioral changes, disturbances of sensory and equilibratory functions, involuntary activity of skeletal muscles, and depression of vital centers have also been attributed to exposure to OC insecticides, including DDT, aldrin, dieldrin, and Kepone [31-34].

(b) Organophosphorus Insecticides

There are a large number of OP insecticides in use. They include phosphates, phosphonates, phosphoramidates, pyrophosphates, thiopyrophosphates, and phosphorothioates.

In contrast to the OC insecticides, the OP compounds present a high hazard of acute intoxication which varies considerably from compound to compound. Parathion and fensulfothion are very toxic, with oral LD50's in rats of about 2 mg/kg [35]. Malathion is one of the least toxic compounds, with an oral LD50 in rats of 1,400 mg/kg [35]. These substances exert their toxic effects through their ability to inhibit cholinesterases (ChE's). OP compounds containing a P=S nucleus,

such as parathion, must be metabolically activated by exchanging an oxygen atom for the sulfur. Animals, man, and insects all perform this activation. In mammals, the activation is done by microsomal oxidases of the intestinal wall and liver. Other OP compounds do not require metabolic activation. The inhibition of ChE by active forms is essentially irreversible and renders their toxic actions persistent until the inhibited enzymes are replaced by newly produced ones. Repetition of a small dose may finally result in serious intoxication even though each single dose may inhibit only a small percent of the ChE activity. The symptoms result from the accumulation of excessive quantities of acetylcholine at peripheral, ganglionic, and central nerve endings and from an elevated concentration of acetylcholine in the blood plasma and interstitial fluids. Poisoning with reversible inhibitors, such as tetraethyl diphosphate (TEPP), is naturally more amenable to therapy.

Increased bronchial secretions, salivation, sweating, bradycardia, miosis, muscular weakness, hyperglycemia, low blood pressure, anxiety, headache, neurosis, slurred speech, disorientation, and convulsions are signs and symptoms that characterize poisoning by this group of compounds [36]. Respiratory failure is the most usual cause of death from a single, high dose. Such failure results from a combination of blockage of the respiratory tract from excessive secretion from glands of the mouth and respiratory tract, by possible bronchoconstriction, and by paralysis of the respiratory areas of the brain stem [37,38].

The degree of acute intoxication by most OP compounds may be gauged readily by the measurement of the extent of inhibition of acetylcholinesterase (AChE) in red blood cells (RBC's) or of the nonspecific ChE present in plasma. Axonal degeneration followed by degeneration of myelin sheath cells in peripheral nerves, and even in some cases, of degeneration of tracts within the spinal cord, has been observed. Such effects resulted from prolonged exposure to such OP compounds as tri-o-cresyl phosphate [39], mipafox [40], and leptophos [41]. Some evidence has accumulated that the chronic depression of AChE activity by OP compounds may be associated with behavioral changes [42-44], but there is some doubt of the scientific validity of these conclusions [45-47]. Based on analysis of available human and animal data pertaining to behavioral changes attributed to OP pesticides, it appears that insufficient criteria exist for assessing the significance of relatively subtle, apparently reversible, alterations in brain function on the health of exposed workers. However, there is cause for concern and additional research is recommended in this area.

(c) Carbamate Insecticides

These insecticides, which include carbaryl, methomyl, and propoxur, have more recently come into wide use. They are also ChE inhibitors and produce symptoms in humans similar to that of the OP insecticides. Unlike some OP compounds, the carbamates do not require activation by microsomal enzymes to inhibit ChE. The inhibition of ChE by carbamates is more readily reversible than that produced by most OP compounds [36]. Overexposure may result

in local effects, such as constriction of the pupil of the eye, sweating on a localized area of skin, secretion of fluid by glandular mucosa, etc. After absorption into the blood, the compound will contact first the ChE of the plasma and the erythrocytes and will inhibit one or both of them. Detoxification and dissociation of the inhibitor from the enzyme begins promptly, and the concentration of active enzyme in the blood rapidly assumes normal values while ChE in the central nervous system (CNS) or in effector organs may still be depressed. In this case, measurement of blood ChE activities would yield normal values and might lead the physician to conclude falsely that the patient had not been poisoned by a ChE inhibitor. Even though a blood sample may be taken at a time when its ChE activity is still depressed, dissociation of a carbamate inhibitor from the enzyme will proceed by hydrolysis after the blood sample has been collected. When carbamates are the compounds of interest, it is important that blood samples be examined for ChE activity as soon after collection as possible and that a rapid sampling and analytic method be used involving no, or minimal, dilution of the blood. However, due to the rapid reversal of carbamate-induced ChE inhibition, NIOSH does not recommend routine monitoring for persons exposed only to carbamate insecticides.

(d) Inorganic Arsenicals

Lead arsenate, Paris green, and sodium arsenite are insecticides that have been used for many years. Arsenic compounds are invariably dangerous cellular poisons [48]. They

exert their effects by reaction with sulfhydryl groups of important enzymes and are capable of affecting most organs in the body. Symptoms of acute intoxication include nausea, vomiting, diarrhea, intense pains or cramps in the intestine as well as in the stomach and esophagus, rapid fall of blood pressure to shock levels, convulsions, coma, and death. Symptoms and signs of chronic arsenic poisoning are characteristic gastrointestinal pains and diarrhea, injury and degeneration of the kidneys, edema, fatigue, and loss of appetite. Characteristic skin changes, which may appear also in mucous membranes, are flushing, edema, acne, and thickening and scaling of the epidermis. Hair loss, detachment of fingernails and toenails, and sometimes fatal exfoliative dermatitis may occur. Inorganic arsenic has been shown conclusively to produce skin cancer by prolonged use as a therapeutic agent [49]. Increased incidences of skin cancers, respiratory cancers, and leukemias have been observed in pesticide workers exposed to inorganic arsenicals [50].

(e) Nitrophenolic Herbicides

Nitrophenolic herbicides, such as the dinitrophenols and dinitro-o-cresol (DNOC), are highly toxic to humans and animals [36]. Most nitrophenols and nitrocresols are well absorbed from the gastrointestinal tract, through the skin, and from the lungs when very fine droplets are inhaled [36]. They irritate the skin and usually produce a yellow stain whenever contact occurs. Like other phenols, they are toxic to the liver, kidneys, and nervous system. The basic mechanism of toxicity is probably the uncoupling of oxidative phosphorylation [36]. Increased

oxidative metabolism depletes body carbohydrate and fat stores and leads to hyperpyrexia, tachycardia, and dehydration [36]. Symptoms of poisoning from these compounds are more severe when the ambient temperature is high [36]. Direct action on the brain causes cerebral edema which is manifested clinically as a toxic psychosis and sometimes as convulsions. Liver parenchyma and renal tubules show degenerative changes; albuminuria, pyuria, hematuria, and increased blood urea nitrogen (BUN) are often prominent signs of renal injury. Agranulocytosis has occurred following large doses of dinitrophenol [36]. Dinitrophenols have also been implicated in the formation of cataracts [51].

(f) Chlorophenoxy Herbicides

The chlorophenoxy acids, salts, and esters are irritating to skin, eyes, and respiratory and gastrointestinal linings. They are absorbed through the gut wall, the lung, and the skin. These acids are not significantly fat storable, and excretion occurs within hours or at the most within days, primarily in the urine [36]. They are regarded as being fairly nontoxic, although three cases of peripheral neuropathy were reported in workers after exposures to 2,4-D [52]. In a few individuals, local depigmentation has apparently resulted from prolonged and repeated dermal contact with these substances [36]. Some chlorophenoxy compounds have caused severe cases of dermatitis or chloracne in workers, although in some cases contaminants were the responsible agents [53].

Chlorinated dibenzodioxins (TCDD) are contaminants of 2,4,5-T. Neurotoxic effects and chloracne have been found in

workers exposed to TCDD-contaminated 2,4,5-T. Experimental animals exposed to TCDD may suffer teratogenic and mutagenic effects [54].

(g) Dipyridyls

Paraquat and diquat are the best known compounds of this class of herbicides. The dipyridyl compounds can bind to and injure the epithelial tissues of the skin, nails, eyes, nose, mouth, and respiratory and gastrointestinal tracts. Concentrated solutions cause inflammation and sometimes necrosis and ulceration of mucosal linings [36].

Autopsy cases of accidental or suicidal poisonings from paraquat show evidence of lung, liver, and kidney damage. Some cases had myocarditis, and one case showed transient neurologic signs. Most striking was the widespread cellular proliferation in the lungs [55]. Indications of diffuse toxic pneumonitis appear from 72 hours to 14 days after ingestion of paraquat. The pulmonary lesion has a complex histopathology, beginning with intra-alveolar edema and hemorrhage, followed by the proliferation of fibrous connective tissue. This fibrous connective tissue proliferation is often progressive and generalized and frequently results in death in 1-3 weeks [36].

(h) Urea, Uracil, and Triazine Herbicides

Monuron, bromacil, atrazine, and simazine are some of the better known herbicides in these categories. They have low oral acute LD50's, generally being above 1,000 mg/kg [55].

Most injuries reported with these herbicides involve skin irritation after prolonged contact. Some of these chemicals have

been implicated in injuries to the nervous system, liver, and kidneys, and have been known to cause increased permeability of capillaries at very high dosage levels in small laboratory animals, sheep, and cattle. Anemia and altered adrenal function have been detected in animals given extreme doses of atrazine [56]. These effects have not been observed in persons exposed occupationally or by accidental ingestion [36].

The herbicide amitrole, although not classified as a triazine, is structurally similar. This compound also has a very low acute oral toxicity in rats and mice, with a range from 15,000 to 25,000 mg/kg [55]. However, amitrole is a potent antithyroid agent, and significant effects on thyroid function have been observed at feeding levels as low as 2 ppm [57]. Amitrole has induced adenomas and adenocarcinomas in rats given 100 ppm in the diet for 2 years [58] and is also the chemical suspected of inducing an increased incidence of cancer in Swedish railway workers [59].

(i) Dithiocarbamates

There are three main groups in this class of fungicides. The first group contains the dimethyl derivatives including thiram, ziram, ferbam, and vapam. The second group is composed of the diethyl derivatives such as ethyl selenac, ethyl zimate, ethyl tellurac, and ethyl cadmate. And finally, there is the group of ethylene (bis) dithiocarbamate derivatives, which includes the pesticides zineb and maneb.

Many of the dimethyldithiocarbamate compounds are irritants and sensitizers [36]. The toxicity of these compounds

probably resembles that of disulfiram (Antabuse), which is used to condition individuals against beverage alcohol. They are metabolized in a manner similar to that of disulfiram. Disulfiram metabolites are powerful inhibitors of multiple sulfhydryl enzymes in the liver [60,61] and the CNS. Animal experiments indicate that thiram is more toxic than medicinal disulfiram [36]. Preliminary results reported by NIOSH [62] indicate that a serious toxic synergism exists between disulfiram and ethylene dibromide (EDB). In rats fed 0.05% disulfiram in the diet, mortality was 3/48 for males and 3/48 for females. Rats exposed to 20 ppm EDB by inhalation experienced mortality of 15/40 for males and 9/48 for females. However, rats exposed to both 0.05% disulfiram in the diet and 20 ppm EDB in air experienced mortality of 45/48 for males and 47/48 for females. All exposure periods were 13 months, and cause of death included an increased incidence of various tumors, including hemangiosarcomas of the liver, spleen, and kidney. Mortality for controls was 0/48 for males and 3/48 for females.

The toxic effects of these compounds can be categorized as those following absorption of the toxicant alone, and as those which result when the dithiocarbamate is followed by alcohol. Peripheral neuropathy and psychotic reactions have occurred in alcohol-abstinent individuals on high disulfiram regimens. Disulfiram followed by alcohol is characterized by flushing, excessive sweating, weakness, upper respiratory congestion, labored breathing, and in some cases, respiratory depression that has been life-threatening. High dietary intake of ferbam and

zineb has produced functional and anatomical damage to the CNS in rats [36].

In a screening study done by Bionetics for The National Cancer Institute (NCI) [4], a number of these pesticides were tested for their carcinogenic effects in mice. Elevated tumor incidences were observed in the mice fed ethyl selenac and bis (2-hydroxyethyl) dithiocarbamic acid potassium salt [4], whereas no significant increase in tumors was seen with zineb, maneb, ferbam, ethyl zimate, methyl zimate, methyl selenac, and ethyl cadmate. The authors also concluded that additional evaluation of ethyl tellurac and sodium diethyldithiocarbamate was needed. Ethylene thiourea (ETU) caused elevated tumor incidence when administered orally [4]. ETU is an oxidation product of the ethylene bisdithiocarbamate fungicides. Many compounds of this class, including zineb and maneb, are skin irritants and have caused dermatitis [63].

(j) Organomercurials

Organic mercury compounds are used as fungicides for seed, bulb, and corn treatment, and include phenyl mercury acetate, N-ethylmercuri-1,2,3,6-tetrahydro-3,6-endomethano-3,4,5,6,7,7-hexachlorophthalimide (EMMI), N-methylmercuri-1,2,3,6-tetrahydro-3,6-endomethano-3,4,5,6,7,7-hexachlorophthalimide (MEMMI), and 2-methoxyethylmercuric chloride.

Because these pesticides contain mercury, they should be regarded as highly dangerous. Compounds of mercury may be absorbed through the skin, the gastrointestinal tract, and the lungs. If high concentrations of ionizable mercury reach the

small intestine, severe abdominal pain and bloody diarrhea will result, with possible sudden death due to shock and circulatory collapse [64,65]. In general, the signs and symptoms of aryl and methoxyethyl mercury poisoning resemble those observed for inorganic mercury compounds [66]. Alkylmercury compounds also affect the nervous system, and the signs and symptoms include tremors, slurred speech, motor weakness, and abnormal reflexes [64]. The health effects of poisonings by mercurials are further described in NIOSH's criteria document on inorganic mercury compounds [67].

(k) Organotins

Trialkyl and triaryltin compounds are used as rodent repellants, molluscicides, fungicides, insecticides, and bactericides. Examples include triphenyltin acetate, bistributyltin oxide, tricyclohexyltin hydroxide, and triphenyltin hydroxide.

Adverse effects produced by occupational exposure to pesticide products containing triphenyltin acetate include irritation of the skin, conjunctivae, and respiratory tract, and liver damage. Signs and symptoms of poisoning by organotin compounds include general malaise, violent headaches, nausea, vomiting, diarrhea, and epigastric pains. These effects are described in detail in NIOSH's criteria document for organotin compounds [68].

(l) Miscellaneous Pesticides

Fumigants in common use include halogenated compounds such as methyl bromide and sulfuryl fluoride, cyanide compounds such

as acrylonitrile and hydrogen cyanide, and aluminum phosphide, a generator of phosphine. They are all highly toxic substances, especially by inhalation. They are intended for fumigation where there should be no exposure to humans. The halogenated aliphatic fumigants include liver, kidney, cardiac, and CNS intoxicants. Hydrogen cyanide is a rapidly acting poison which inhibits cytochrome oxidase, an enzyme necessary for the oxidative metabolism of all cells [36]. NIOSH has recommended that acrylonitrile be handled in the workplace as a suspect human carcinogen [69] and the Occupational Safety and Health Administration (OSHA) has regulated acrylonitrile as a carcinogen. This recommendation was based on data from animal experiments and on an epidemiologic study of workers handling acrylonitrile in a textile plant.

The coumarins and the indandiones are used as rodenticides. Warfarin is one of the best known coumarins [55]. These substances antagonize the action of Vitamin K to promote the hepatic production of prothrombin and several other clotting factors [55,70].

Another rodenticide of interest is 1-nitrophenyl-3(3-pyridylmethyl)-urea (Vacor). Unlike the coumarin-indandione rodenticides, Vacor has no anticoagulant action [36]; its exact mechanism of toxicity is unknown. Human poisonings have occurred only after deliberate ingestions, with varying symptoms depending on the dose and individual susceptibility. Ingestion is followed 4-48 hours later by nausea, vomiting, abdominal cramps, and mental confusion. These

may be followed by aching and tremors of the extremities, peripheral neuropathy, muscular weakness, and anorexia. Late and persistent manifestations of poisoning are postural hypotension and diabetes mellitus [36].

The fluoroacetates are another type of rodenticide. Sodium fluoroacetate is a powerful inhibitor of the tricarboxylic acid cycle and produces death by interfering with the operation of this important metabolic mechanism [36,55,71]. By forming fluorocitrate, it competitively and tightly occupies the receptive site of the enzyme aconitase and thus blocks the remainder of the tricarboxylic acid cycle [72]. It may cause cardiac ventricular fibrillation or convulsions, depending in large part on the species to which it is introduced. Sodium fluoroacetate is an extremely dangerous acute intoxicant.

Herbicides such as the acetanilides, acetamides, carbanilates, and anilides have recently been developed. These herbicides exhibit low systemic toxicity in laboratory animals, but irritate the skin, eyes, and mucous membranes. Propachlor and alachlor appear to have sensitizing properties; severe skin reactions have occurred in sensitive individuals [36].

Human Health Effects

While the United States has escaped major incidents of mass acute fatal poisoning, other countries have not [73]. Table XIV-6 lists the major cases of mass poisonings experienced throughout the world that clearly show the potential for great tragedy when pesticides are handled improperly. A variety of

toxic effects have been observed in workers exposed to pesticides during their manufacture and formulation. Workers who have been affected include formulators, mixers and loaders, cleaners and repairmen of pesticide handling machinery, warehouse workers, and truck loaders. The recent cases of occupational poisonings by Kepone, leptophos, and DBCP in the United States clearly demonstrate the variety of toxic effects that pesticides can manifest and the need for good engineering controls and work practices during the production and formulation of these pesticides.

In assessing how widespread this problem is in the workplace, the most reliable source of information comes from California. California's State Workmen's Compensation Law requires the reporting of injuries from occupational exposures. This requirement has led to the development of a statewide data base indicating the kinds and severity of injuries from pesticide exposure. In 1973, a total of 1,451 cases of occupational disease attributed to pesticides and agricultural chemicals was reported in California [17]. Of these, 156 occurred in manufacturing establishments. The breakdown of these cases by class of compound, specific agent, and industry category is shown in Table XIV-7. There were 1,343 reported occupational illnesses resulting from exposures to pesticides in California in 1975 [74]. Of these, 546 were concluded to be systemic, 436 involved skin injuries, 314 involved eye injuries, and 47 involved both the skin and the eyes. Fifty-four of the 1,343 cases involved pesticide manufacturing and formulating workers [29]. Of these

54 cases, 39 had systemic effects, 5 involved skin effects, 8 had eye changes, and 2 involved eye and skin injuries. The pesticide mevinphos was responsible for 25 of the 39 systemic illnesses. Other pesticides believed to have caused injuries include parathion, methomyl, penoxalin, chloropicrin, carbaryl, captafol, and malathion.

While statistical data on injuries and illnesses from occupational exposures to pesticides are available from California, similar data are not available on a national basis. Unavailability of these data has limited attempts to characterize the potential danger of individual pesticides and to identify those pesticides which present a higher risk to workers due to toxicologic properties and to overexposure because of poor work practices and controls. Accordingly, NIOSH has published a guideline for reporting occupational disease [75] which, if implemented, would provide better data on occupational health problems, including those associated with pesticides, than are currently available.

Much of the information now available on the human toxicity of pesticides comes from reports of accidental or suicidal exposures. They provide valuable accounts of the types of effects which result from pesticide exposure. In addition to case reports, some epidemiologic studies have been reported that provide information on the effects of chronic exposures in workers handling pesticides. Review of case reports and epidemiologic studies reveals the variety of effects which are seen as a result of pesticide exposure. Human exposures to

pesticides affected the skin, eyes, and nervous, reproductive, hepatic, renal, respiratory, hematopoietic, and cardiovascular systems. Moreover, available studies have implicated the inorganic arsenical pesticides [48,50,76], benzene [77-80], acrylonitrile [69,81], creosote [82], certain hexavalent chromium salts [83], and amitrole [59] as human carcinogens. The following discussion of human health effects is arranged according to the different target organs.

(a) Neurologic Effects

Neurotoxicity is a well-documented toxic effect of pesticides in humans, often in association with the OP, carbamate, and OC pesticides [55]. Two major incidents of occupational neurotoxicity associated with pesticides involve the OC pesticide Kepone and OP pesticide leptophos.

Kepone is a chlorinated hydrocarbon insecticide used domestically as an ant and roach poison. It is related to the pesticides mirex, DDT, aldrin, and dieldrin, all of which have been restricted by the EPA. Kepone was produced in Hopewell, Virginia, in a converted garage which began operation in 1973; the plant produced only Kepone [34,84].

In July 1975, a Hopewell physician submitted an employee's blood sample for analysis to the Center for Disease Control in Atlanta. The analysis revealed a Kepone blood level of 7.5 ppm (CW Heath, Jr, written communication, January 1976). Subsequently, workers at the Hopewell plant were discovered to have a variety of ailments which led to a detailed study and investigation of 133 employees out of the total of 148 then

current and previous employees [34].

The workers suffered complex neurologic disorders characterized by insidious onset of tremors, chest pains, weight loss, mental changes, arthralgia, skin rash, opsoclonia, muscle weakness, loss of coordination, and slurred speech. Seventy-six of the 133 (57%) had experienced tremors following exposure to Kepone. The findings indicated that Kepone produces neurologic disorders involving the brain, the peripheral nerves and muscles, and the liver [34]. In addition to the neurologic findings, sperm counts showed cligospermia with no motile forms (CW Heath, Jr, written communication, January 1976). NCI has released the results of a study indicating that Kepone is carcinogenic in the mouse and the rat [85].

The testimony and photographs submitted at the Senate Hearings of April 21, 1976 [84] show that not even minimal health standards were applied at the Hopewell plant. Kepone exposure was not in the least controlled, and extremely poor housekeeping practices existed at the plant. Consequently, workers were exposed to the massive amounts of Kepone that led to the reported intoxications.

A recent study [86] has indicated that cholestyramine shows promise as a detoxification agent for workers exposed to Kepone. In 22 Kepone-exposed workers administered 16 grams of cholestyramine per day for 5 months, mean blood half-life of kepone was reduced from 165 to 80 days and the mean fat half-life was reduced from 125 to 64 days.

Another dramatic example of occupational poisoning

involved leptophos, an OP insecticide. Leptophos was produced in Texas for export until January 1976. In June 1975, 12 cases of serious neurologic disorders in employees were identified by a medical consultant to the manufacturer. These cases were not reported to NIOSH until September 1976 [87].

Following notification of the neurologic disorders in September 1976, NIOSH began a study in December 1976 of all present and former employees. NIOSH contacted the majority of the 301 then current and former employees and informed them of the availability of medical examinations. Between January and April 1977, 155 persons reported for comprehensive examinations that evaluated general physical status, neurological status, and measures of neuromuscular, ophthalmological, psychological, and biochemical function. A reproductive history survey was also conducted [41].

A substantial number of those examined had slight to serious neurologic, electromyographic(EMG), electroneurographic (ENG), and psychologic performance abnormalities. Many of those studied showed sensation abnormalities of the hands and feet. Results of the psychologic performance tests for those exposed, when compared with the results for unexposed controls, suggested an impairment of psychomotor performance. A few of those studied showed significant EMG abnormalities. Most of the abnormalities involved three muscles: the extensor digitorum brevis, abductor hallucis, and gastrocnemius. Fifty-seven workers had abnormal ENG results. Out-of-range latency measurements for the sensory nerves (median, ulnar, and sural) were found for 17, 13, and 7%

of the participants, respectively. Of the 29 workers with abnormal latency findings, 8 showed abnormal findings for both the median and ulnar nerves. The number of out-of-range values for muscle action potentials and nerve conduction velocities of motor nerves was greater than expected. Results from chest X-ray and blood and urine tests revealed no unusual findings. No statistically significant differences were found in the reproductive history survey [41].

NIOSH medical officers believe that the signs seen in these workers are compatible with OP poisoning, even though a number of other chemicals were used in the manufacture of leptophos including toluene, a suspected neurotoxic solvent. Also, during the period of 1971-75, the plant also manufactured a resin called Klyrvel which required use of n-hexane, a solvent that has been associated with severe neurologic disorders [41]. NICSH concluded that the health of the workers involved was adversely affected by conditions that could have been prevented by more careful medical surveillance, work practices, and engineering controls.

The incidents with Kepone and leptophos directed national attention to the hazards of pesticide exposure in manufacturing and formulating workplaces, and to the problem of occupational neurotoxicity. The literature reveals many other reports of neurotoxic effects by pesticides, and some of these reports are presented in the following paragraphs, according to chemical composition.

(1) Organophosphorus Insecticides

The neurologic effects resulting from exposure to OP insecticides can be classified as effects either directly related to ChE inhibition or delayed neurotoxic actions.

(A) Cholinesterase-Mediated Effects

Most OP poisonings involve effects that are directly related to ChE inhibition. The mechanism of toxicity involves the inhibition of AChE at cholinergic nerve synapses with resulting accumulation of acetylcholine at these sites. This leads initially to junctional transmission and later to inhibition of synaptic transmission as the postjunctional membrane develops a state of persistent depolarization [37]. A more detailed discussion of the mechanism of ChE inhibition is provided in the NIOSH criteria documents and recommended standards for the OP insecticides malathion [88], parathion [89], and methyl parathion [90].

Most poisonings involving ChE inhibition result from acute exposures. Due to their rapid metabolism and excretion, accumulation of OP insecticides in the body does not occur [55]. Small repeated exposures, however, can result in progressive inhibition of ChE which, if it continues, can reach a level at which signs and symptoms will occur similar to those produced by a single high dose. The manifestations of poisoning resulting from accumulated acetylcholine in nerve tissue and effector organs can be classified as muscarinic, nicotinic, and CNS effects. Muscarinic effects involve smooth muscle, the heart, and exocrine glands. They include respiratory tightness, sweating, nausea, vomiting, abdominal cramps, and pupil

constriction (miosis). The nicotinic effects involve muscular fatigue and weakness, twitching, fasciculations, and cramps. The CNS effects include tension, anxiety, headache, emotional instability, confusion, ataxia, slurred speech, convulsions, and respiratory and circulatory depression [55,37].

The onset of these systemic effects varies with the compound, the route, and the degree of exposure. According to Vale and Scott [37], the interval between exposure and symptoms may be as short as a few minutes, is usually less than 12 hours, and rarely exceeds 24 hours. In most cases, unless exposure causes death, neurologic effects dependent upon inhibition of ChE's are reversible. Local and less severe effects usually last less than a day. Miosis often disappears in less than a week, and most other symptoms diminish over the next 6 to 18 days [91].

Diagnosis of OP poisoning is usually based upon symptomatology and blood ChE levels [92]. Although symptoms are not directly related to blood enzyme activities, the activity of the enzymes in the blood usually provides a rough approximation of the activity in the nervous system [92,93]. Measurements of blood ChE levels are therefore a useful indication of the extent of poisoning. The following case studies present an overview of the signs and symptoms seen in OP acute poisonings.

Vale and Scott [37] reported an OP pesticide poisoning in a 51-year-old female formulation worker. The woman was exposed to demeton-S-methyl while repackaging it in a poorly ventilated room. She first noticed that her pupils were very small, while combing her hair during the afternoon break. Within a few

minutes she had a violent abdominal cramp, vomited, sweated profusely, developed severe diarrhea, and fainted. The woman was hospitalized and developed characteristic nicotinic twitching of abdominal and limb muscles. Following treatment with atropine and diazepam, her condition improved and her RBC ChE activity returned to its preexposure level within 10 weeks.

Grigorowa [94] investigated poisonings in a German plant producing methyl parathion. The first series of examinations, in February of 1959, involved 47 workers. Of the 47 studied, 18 (39.3%) reported mild symptoms, including lack of appetite, gastric distress, visual disturbances, sleeplessness, fatigue, nervousness, and slight headaches. Severity of the symptoms did not appear to be related to length of employment, which ranged from a few months to 6 years. Although plasma ChE activity was apparently measured, no values were reported. The following July, 35 workers were examined and 29 reported more severe symptoms than those reported in February. The author stated that signs and symptoms indicative of CNS involvement were frequent and included headaches, dizziness, nausea, insomnia, fatigue, visual disturbances, increased perspiration, shooting pains in the heart, loss of appetite, vomiting, stomach pains, fibrillar muscular twitching of the eyelids, and numbness of the legs, arms, or fingers. Time of onset for these specific symptoms was not provided. Of the 29 symptomatic workers, 27 had plasma ChE activities that showed activity inhibition of 11-68.4% compared with the February measurements. In 21 workers this inhibition was greater than 30%. It is not clear from the article whether

all cf the 35 workers examined in July were members of the group of 47 workers examined in February.

The author included a brief description of two cases of poisoning seen in the plant during the summer of 1959. One case involved a 48-year-old worker with 5 years' work experience. The man developed a headache and became weak and dizzy while filling bags with methyl parathion dust. He lost consciousness for a few minutes at home after work. Identical symptoms recurred when work was resumed. He reported that his left hand had been numb for a few days, that his right hand had little feeling, and that he had experienced frequent twitching of the eyelids. When examined in February, he had complained of insomnia. When measured in the summer, plasma ChE activity was 64.4% of the February value.

Another case involved a 27-year-old worker with 1 year's experience. He had no complaints during the February examination, but when examined in July, he complained of severe headaches, loss of appetite, nausea, watering of the eyes, and insomnia. For 8 weeks, he had experienced arm and leg numbness with arm impairment sometimes involving only two or three fingers. In the July examination, his plasma ChE activity was 60.2% of the February value.

Petty [95] reported two cases of OP insecticide poisoning. The first involved an employee of an agricultural experiment station who participated for three spring seasons in spraying trees and plants with various pesticides, including parathion, EPN, DDT, dieldrin, and lead arsenate. He did not handle

pesticides during the fall and winter months. The first two springs the patient experienced symptoms of nausea, cramping, aches, and pains which decreased during the fall and winter. During the third spring he noted symptoms similar to those he experienced previously along with nervousness, and difficulty in balancing. Eventually he was found unconscious and was hospitalized, where he was weak, lacked sensation in the hands and feet, and had paresthesias of the extremities. Plasma and RBC ChE's were 34 and 37% of normal, respectively. Although blood ChE activities returned to normal, his symptoms persisted with varying severity during the next 2 years.

The second case involved a physician who applied insecticides to his lawn and shrubbery every Sunday. Initially he used DDT and other insecticides, but later used malathion exclusively. He sprayed a 6% malathion solution with a garden hose attachment and was often thoroughly soaked with spray after the application. After several months of such spraying, he was tired, irritable and experienced paresthesias of the face and oral cavity. The next spring, soon after applying a 50% malathion solution to plants in his yard and living room, he experienced marked weakness, tremors, and headaches. He collapsed and was hospitalized. During his hospital stay he improved, but facial sensation was still decreased, gait was unsteady, and muscles were weak. The following year the patient was still experiencing marked muscle weakness, fatigue, and loss of appetite.

Both individuals included in this study had repeated

exposures to various pesticides. Although depression of blood ChE levels was measured in the first man, no such evidence was available for the other individual. The author does mention that there may be no relationship between the OP exposure and the peripheral or cranial nerve damage.

Low doses of OP insecticides are thought to depress ChE activity without causing the symptoms of acute poisoning [90]. In such asymptomatic individuals, determination of subclinical alterations was made by measurement of blood ChE's, by electromyography, and by electroencephalography.

The literature reveals differing opinions on the most sensitive means for determining the effects of subclinical exposure to pesticides [96,97]. In a study of 36 workers exposed to OC and OP pesticides at a Dutch chemical company [96], 16 workers were tested and found to have an abnormal electromyogram. These EMG responses were quantified by measuring the maximal voltage of the muscle action potentials elicited by optimal stimulation of the motor nerve to the adductor pollicis muscle in the forearm. Abnormal EMG responses were often observed, although blood ChE activity levels were normal. This was observed often in workers whose primary exposure was to OP and carbamate compounds and rarely in workers who mainly handled CC pesticides. The authors stated that measurements of blood ChE levels can be misleading in determination of overexposure to many compounds, especially when the exposure is of a chronic nature. They suggested observation of subjective symptoms, such as headache and nausea, or EMG recording, as preferred indices of

overexposure in pesticide workers.

Jager et al [97] also used EMG's to study workers engaged in the manufacture and formulation of OP and OC pesticides. The OP compounds involved were dimethyl vinylphosphates; the OC compounds were unspecified. EMG and whole blood ChE activity were measured. Of the 36 workers exposed to OP pesticides, 17 had abnormal EMG readings compared with only 1 abnormal EMG reading in 28 workers exposed only to OC pesticides. The abnormal EMG's of pesticide workers were similar to the EMG's of the patients with myasthenia gravis, a condition characterized by muscle weakness and fatigability. The difference between the EMG patterns of healthy individuals and myasthenic patients is that the action potential spikes in a train have about the same amplitudes in normal subjects, whereas in myasthenic patients the action potential spikes, after the first discharge in a train, decrease in voltage progressively and rapidly. In pesticide workers, signs of progressive impairment of EMG appeared over a workweek and then disappeared over the weekend. The researchers did not find, however, a correlation between abnormal EMG readings and depression of the activity of ChE's in whole blood.

Other means have been employed to detect effects of OP pesticide exposure. Rayner et al [98] suggested that hyporeflexia may be a sensitive indicator of chronic OP pesticide exposure. They studied Japanese orchid farmers selected on the basis of high OP usage. They were also exposed to fungicides and OC's. The force of the Achilles tendon reflex was measured objectively with a machine designed for that purpose and showed a

depression of the reflex as compared with controls (P=0.001) and confirmed preliminary observations of hyporeflexia in the exposed agricultural workers. At the present time no completely satisfactory criteria exist for determining the significance of slight alterations in brain electrical activity, as determined by electroencephalography, or in neuromuscular function, as determined by electromyography, on the health of exposed workers.

(B) Delayed Neurotoxic Effects

Delayed neurotoxic effects have also been attributed to the OP insecticides. While substantial data are available on delayed neurotoxic effects in experimental animals from exposure to pesticides [99-101], few human cases have been reported. It has been suggested that delayed neurotoxicity is the result of some action other than ChE inhibition, such as axon degeneration followed by demyelination of tracts in the spinal cord or of peripheral nerves [39]. Demyelination was the probable explanation for the paralyses seen in "Ginger Jake" poisoning caused by tri-o-cresylphosphate (TOCP) [39,102]. Such effects have been reported also for the OP pesticides, mipafox [40] and leptophos [41].

Bidstrup et al [40] reported three cases of delayed paralysis in research chemists involved in the production of mipafox. These individuals had previously experienced symptoms of mild poisoning while exposed to other OP compounds. In the first case, the initial signs and symptoms were vomiting, muscular weakness, and eye problems. Following treatment for her acute poisoning, the patient was released. Three weeks later the

patient was rehospitalized with flaccid paralysis of both legs. The muscles of the right hand were also weakened, and paralysis progressed over the next few weeks. During this time, blood ChE activity levels were greatly reduced. Cranial nerves were normal. EMG studies showed reduced interference patterns. All muscles were tender, and twitchings of leg and face muscles occurred. The patient's condition improved slowly, although leg muscles below the knee remained paralyzed. Six months later the patient was still in a wheelchair, although toe and ankle movements were increasing. Nine months after initial exposure to mipafox, EMG's showed evidence of a lower motor neuron lesion. This patient was able to leave the hospital after about 2 years, but still has, some 26 years after the incident, weak leg muscles so that walking is difficult and must be assisted by a cane or other supporting device (JH Wills, written communication, May 1978).

A coworker of case one initially experienced respiratory difficulties and eye irritation. A week and a half later the patient experienced weakness and loss of tone in the muscles of both lower limbs, particularly below the knee. An EMG showed a reduced interference pattern but no sign of lower-motor neuron degeneration. The patient's condition improved gradually, but after discharge, he had bilateral foot drop, which was more pronounced on the right side. Six months later the patient still experienced tiredness, and after walking 200-300 yards, his foot became "floppy."

The third affected worker was much less severely poisoned

than the other two and never required hospitalization. The authors [40] believed that in the two most severely affected cases, prolonged failure of impulse transmission at the motor end-plate initially contributed to the paralysis. However, subsequent EMG's in case one were indicative of peripheral neuritis with no evidence of neuromuscular block. For that reason the authors felt that demyelination was the most likely cause of the persistent symptoms.

(2) Carbamates

The carbamate insecticides are also ChE inhibitors [17] and the symptomatology in affected humans is similar to that produced by OP insecticides. However, inhibition of ChE's by carbamates is readily reversible [17].

Cases involving methomyl include a mill operator and two laborers in a methomyl formulating plant who experienced episodes of acute intoxication within a 2-week exposure period. A foreman in another formulating plant reported being nauseated, dizzy, and feeling "drunk" after working with methomyl. In another case, a 17-year-old farm laborer who applied methomyl to an orange grove became ill shortly after work, lost consciousness, and was hospitalized for 1 day. The adverse effects associated with methomyl included nausea, vomiting, dizziness, weakness, and respiratory abnormalities [17].

A case of poisoning by aldicarb involved the foreman of a manufacturing plant who ran a mechanical bagging machine for 1 day. Several hours after exposure, the foreman experienced nausea, dizziness, depression, weakness, and tightening of chest

muscles. RBC ChE was inhibited to 43% of normal. Three hours after the initial ChE measurement, the RBC ChE level was again measured and showed recovery; however, the individual still complained of tightness of the chest. By the next day he had recovered, and returned to work [103].

Tobin [104] reported on the anti-cholinesterase effects following exposure to carbofuran. A survey of workers in a carbofuran manufacturing plant revealed the following symptoms in decreasing frequency: vague feelings of malaise, excessive sweating, lightheadedness, nausea, blurring of vision, hypersalivation, and vomiting. No one reported chest tightness, muscular twitching, convulsion, or loss of consciousness. The author included two case studies which involved formulation workers who used a power concrete mixer to prepare a 10% granular product of carbofuran and complained of profuse perspiration, weakness, nausea, and blurred vision. One worker completely recovered 2 hours after exposure ceased, the other felt weak and nauseated 1 hour after exposure ceased but required no treatment.

A study of possible effects on CNS function after subchronic exposure to the insecticide carbaryl was performed by Wills et al [105]. For this study, male volunteers between 25 and 57 years of age were selected from inmates of a New York State prison. The carbaryl was administered orally in gelatin capsules. Dose regimens included: no dose, 0.06 mg/kg/day carbaryl for 6 weeks, and 0.12 mg/kg/day carbaryl also for 6 weeks. Indications of possible carbaryl-related subjective neurologic effects were found in interviews of the subjects

during and after the 6-week period of the study. Despite the apparent dose-relatedness of these neurologic symptoms, no decrease in blood ChE activity levels was seen except for a very slight depression observed in one individual in the low-dose group on day 3 of the test. Most of the subjective symptoms reported by the high-dose group did not appear until the 4th or 6th week of the study.

(3) Organochlorine Pesticides

While neurotoxic effects of OP and carbamate pesticides are fairly well documented, less information is available for other pesticide classes. In addition to Kepone, other OC pesticides have been associated with human neurotoxicity [32,33,106-110]. Although the precise mechanism of their neurotoxicity is unknown, the action of OC pesticides does differ from that of the OP and carbamate compounds [55].

Kazantzis et al [106] reported on a formulating plant worker who developed epileptiform convulsions after a short period of heavy exposure to aldrin. The man had been working with aldrin for little more than a week. His job was to transport paper bags of aldrin and fuller's earth (an inert filler), open the bags, and then empty them by hand into a mechanical mixer. He had to lean over into the exhaust hood of the mixer to do this. He wore overalls and a cotton wool pad as a mask. He had two convulsive attacks with loss of consciousness. Analysis indicated a high body fat concentration of dieldrin, the principal metabolite of aldrin. Electroencephalograms (EEG's) showed irregular alpha rhythms.

Later, nine fellow workers were also examined. Two of these nine workers described symptoms characteristic of aldrin poisoning. The two men worked on the micronizer and both experienced involuntary jerking of the limbs, irritability, and vomiting. EEG's revealed irregular alpha rhythms. Improvement was seen in both men following cessation of exposure. One of these men returned to work and subsequently experienced several attacks of unconsciousness. Blood and fat analyses showed high dieldrin content. Another worker, who was not among those previously examined, also lost consciousness and convulsed while working with the 50% aldrin mixture. An EEG contained abnormalities similar to those of the other three men with aldrin intoxication.

Nelson [107] reported convulsions in 3 of 35 workers exposed to 25% aldrin concentrate. The workers also complained of nausea, vomiting, vertigo, loss of weight, malaise, and headache; recovery in all cases was complete.

Bell [108] reported the case of a man overexposed to aldrin while repackaging 5-pound bags of the substance. Ventilation during this work was poor, and no attempt was made to prevent skin contamination. On the evening of the 2nd day of working under these conditions he had a convulsive seizure. An EEG examination revealed abnormalities, and a biopsy of fat 2 weeks later revealed 40 ppm dieldrin; the patient recovered fully.

Avar and Czegledi-Janko [109] studied 15 men exposed to aldrin in a fertilizer plant for varying periods of up to 5 years. The men were examined during the last month of their exposure. In three men with poisoning symptoms, EEG's contained

changes typical of convulsive states, and all had had convulsive fits. Blood dieldrin concentrations for these three individuals ranged between 0.3 to 0.19 ppm. Seven months after the last known exposure, both EEG findings and dieldrin concentration returned to background level, and clinical symptoms ceased. In others examined during the last month of exposure, signs and symptoms of poisoning were present when the concentration of blood dieldrin was greater than 0.10 ppm and were absent when it was less than 0.05 ppm.

Hoogendam et al [32,33] reported on a 9-year health survey of 300 workers in plants manufacturing OC pesticides. The pesticides involved were aldrin, dieldrin, and endrin. Although during that period no fatalities or permanent injuries were found, 17 of the workers experienced convulsive intoxications. Of these, 5 had more than one convulsion, and 2 had more than one convulsion on a single day. In several of the cases, convulsions were preceded by myoclonic jerks [32], but usually without any prodromal symptoms. Specific EEG anomalies including bilateral synchronous spike and wave complexes thought to be associated with alterations in brain stem function were observed [33]. Clinical and neurologic recovery after removal from exposure was rapid and complete in all cases.

Derbes et al [110] reported the case of a 23-year-old woman with 2 years' experience working in a pesticide factory who spilled an unknown amount of a suspension containing 25% chlordane, 26% DDT, 39% Velsicol AR 50, and 10% triton-X on the front of her clothing. Forty minutes later she became confused

and rather suddenly began having generalized seizures. She died in the ambulance on the way to a doctor's office suggesting massive exposure. Autopsy revealed nonspecific pathologic changes in the brain, lungs, and kidneys.

(4) Other Pesticides

Neurologic effects have been reported following exposure to a variety of other pesticides including methyl bromide [111,112], 2,4,5-T [53], monosodium acid methane arsenate [113], organomercury compounds [114], and diphenyl [115]. Greenberg [111] observed CNS damage in a worker who had been exposed to methyl bromide while fumigating cocoa beans. The worker first lost and then regained the ability to walk. Two months later, however, he suffered a recurrence of toxic manifestations with violent seizures and altered brain electrical conductivity, as shown by electroencephalography. Three years later the EEG pattern had improved, but there was still some lack of muscular coordination, and intelligence and personality tests showed that he was at the borderline of mental retardation. He appeared to have suffered mild to moderate permanent brain damage.

Hine [112] reviewed 10 cases of methyl bromide poisoning, 5 of which are discussed below. The review included three case reports of fatal poisoning. The first involved a worker who ate his lunch in an area adjacent to a boxcar full of rice which had been fumigated with methyl bromide the night before. That evening he developed sudden tonic-clonic seizures and lost consciousness. His wife drove him to the emergency room of a

hospital where he was given resuscitative measures. Upon examination he showed tremors, fasciculation of the muscles, cyanosis, and irregular gasping respirations. He died 20 minutes after the initial seizure. The second case involved a man who had fumigated almonds on the night prior to becoming ill. Four hours after leaving work he experienced difficulty in breathing, chest pain, chills, and excessive sweating. He was discovered later undergoing tonic-clonic convulsions and did not regain consciousness before death.

Poisoning was also seen in a man who worked up to 10 hours/day sacking rice, hauling it out of box cars, and piling it in a warehouse. He was unaware that the rice had been fumigated with methyl bromide in the car. He became ill on day 4 and his initial signs and symptoms included a cough and a sore chest. Six days later he had general muscular discomfort, mild disorientation, and difficulty in breathing. He was hospitalized and later died.

Hine [112] also reviewed two cases of nonfatal methyl bromide poisoning. One case involved a woman employed on an almond-sorting belt for 2 months. Fumigation was done in a warehouse 50 feet from her work station. The door to the warehose was open allowing the fumigant to drift down the conveyor belt. No fumigation had been done 48 hours prior to the pericd of her first reported illness. Her first symptoms were coolness of the chest and burning of the nose and throat. A second exposure occurred the following night after she had been at work 2 hours. She became ill and was unable to move her arms.

The next day she was confused, had headaches, nausea, and loss of leg control. She was hospitalized and began to hallucinate. Six months later she still had hallucinations, headaches, limb discomfort, and general body soreness. Laboratory examination showed persistent leukopenia and a moderately abnormal EMG. Thirty months after the initial symptoms, the patient was still not at work due to malaise, decreased reaction time, muscle pains, and depression.

Another man became ill within 1 week after he had cleaned dead worms out of a series of rooms that had been fumigated with methyl bromide 10 days earlier. The five rooms cleaned were 100 sq ft in size with small doors, no windows, and no permanent exhaust systems; however, air hoses had been rigged in an attempt to remove vapors. He worked inside the rooms about half a day for 4 days, did not wear a mask while working, and was not advised of the hazard. The man's first symptom was extreme nausea. He was taken to a hospital, where he lost consciousness for 4 hours. After regaining consciousness, his symptoms included nausea, weakness, numbness, dizziness, paranoia, and disorientation which persisted for 24 hours. No objective evidence of neurologic disease was found.

Other neurotoxins associated with pesticides are the chlorinated dibenzodioxins (especially 2,3,7,8-tetrachloro-dibenzo-p-dioxin, TCDD) that contaminate several pesticides, including the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the wood preservative pentachlorophenol (PCP), and the bactericide hexachlorophene. Jirasek et al [53] reported

TCDD-related neuropathy among factory workers in Czechoslovakia producing 2,4,5-T and PCP. The majority of 55 workers suffered what were stated to be severe neurasthenia and depressive syndrome. In 17 subjects, signs of peripheral neuropathy, especially in the lower extremities, were confirmed by electromyographic examinations.

De Palma [113] cited three cases of arsine intoxication in a chemical plant where monosodium acid methane arsenate (MSMA) was being produced. Two of the cases apparently involving the nervous system are described below. During the reaction of methyl chloride with sodium arsenite, a paddle became detached in the vat and the operation was stopped before completion of the reaction. The tank was drained, but a solid residue remained on the vat floor. An aluminum ladder was used to descend into the tank. The first man entered the tank without a mask or respirator to assess the damage. After a minute or two, he noticed bubbling at the foot of the ladder and bent over to get a closer look. He felt a sudden chill and left the vat. He soon experienced a burning sensation in his feet, which later involved his whole body. He then experienced nausea, vomiting, and abdominal pains. He was anuric, irritable, confused, and agitated when finally hospitalized. He was released after 54 days with pain, numbness, burning and hypoesthesia of his feet, ankle and leg weakness, and occasional finger numbness. The peripheral neuropathy was decreased, but still persisted 6 months later.

Another worker went halfway down the ladder and proceeded

to wash down the tank floor with a hose while steam was piped up through the center drain. He was in contact with rising steam for about 15 minutes. Shortly after this task he noticed that his urine was unusually dark. He became jaundiced and was hospitalized. During this period he experienced fever, paranoid delusions, anemia, and progressive weakness; he lost 50 pounds and could not walk more than 20 feet without having to rest. A program of physical rehabilitation was initiated. His condition improved and he was discharged. Six months later he still had partial loss of sensation on both feet and neuralgia in his toes and heels.

Ahlmark [114] documented five cases of neurotoxicity from methylmercury compounds in occupationally exposed individuals. The first case involved a man employed for 3.5 years in a factory where methylmercury was manufactured. He was specifically occupied with the extraction of methylmercury iodide and also with releasing the excess pressure in the bottles in which the methylmercury iodide had formed. The author noted that the man was known to be extremely careful in his work and that he observed all the protective precautions in detail. His first symptoms included giddiness, a "funny" feeling in his fingers, and numbness of the fingertips. These symptoms were followed by indistinct speech, numbness of the tongue, and trembling hands. His condition continued to deteriorate until he could not stand and his speech was barely understandable. After about 4 months he began to improve, but 9 months later he still suffered from muscular incoordination and could not eat properly.

Three of the five cases involved packers of seed dressings containing methylmercury. All experienced tingling in the hands. One of the cases progressed until the individual had difficulty walking, balancing, and speaking. The first individual died; the other two individuals had no other symptoms of poisoning.

The fifth case involved a man who had impregnated wood with methylmercury. His first symptoms included numbness in his hands and forearms, incoordination, dizziness, and an unsteady walk. The symptoms gradually increased, his speech was impaired, and he became blind. He died less than 1 month after hospitalization.

Seppalainen and Hakkinen [115] described a neurophysiologic study of 24 out of 31 workers exposed to the fungicide diphenyl in a Finnish paper mill where wrapping paper was impregnated with the diphenyl. The group included those exposed to the greatest amounts and/or who had symptoms or signs suggestive of poisoning, including headaches, gastroinestinal complaints, general fatigue and numbness, and aching of the limbs. Ten of the 24 workers had abnormal EEG's with mainly diffuse, slow wave abnormalities. During a 2-year observation period no actual improvement was seen. Some workers also had electroneuromyography (ENMG) abnormalities and 7 also exhibited fibrillations in some muscles. In addition, nerve conduction velocity, especially that of slower motor fibers, was reduced in several cases. The author did note that exposure to diphenyl highly exceeded the TLV of 1 mg/cu m, though no quantitative results were reported.

(b) Behavioral Effects

Neurotoxic responses to pesticides may also be manifested

as behavioral or psychologic alterations [43,44,116,117]. Such manifestations are often subtle and difficult to measure.

The behavioral effects of chronic but clinically nontoxic exposure to OP pesticides were reported by Korsak and Sato [43]. Thirty-two individuals were divided into two groups of low and high chronic occupational exposure based on a logarithmic index derived from yearly and daily exposure combined with age. The volunteers were tested using a series of neuropsychologic tests and a computer-based electroencephalographic technique. Blood was drawn for pesticide residue analysis and for plasma ChE activity determinations. The results indicated that extent of chronic exposure does have definite quantifiable effects upon apparently asymptomatic individuals. Length of exposure to high levels of OP pesticides was significantly related to deficits in performance on Part B of the Trial Making Test and on the Bender Visual Motor Gestalt Test. Both tests have been reported to be indicators of brain dysfunction. However, insufficient criteria exist to permit correlation of subtle, reversible alterations in brain function with the health of the worker. There were no significant relationships between length of exposure and deficits in performance on the Tactual Performance Tests.

Metcalf and Holmes [116] used EEG techniques, psychiatric interviews, visual and auditory evoked response tests, and physical examinations to measure response to pesticides. They found more cases of nervousness, changes in memory and sexual activity, problems in sleeping, and easy fatigability in persons exposed to OP pesticides than in the control group.

Dille and Smith [117] reported that two pilots employed in spraying OP pesticides suffered acute poisoning symptoms and later showed symptoms of severe depression and anxiety. The abnormal EEG of one pilot persisted for 6 months. The authors attributed the psychiatric symptoms to the effects of chronic exposure to OP pesticides.

Gershon and Shaw [44] discussed the psychiatric sequelae seen in four workers following chronic exposure to OP insecticides. The individuals involved were a scientific field officer employed in checking the efficacy of OP insecticides, including parathion and malathion, a greenhouse technician exposed to parathion, malathion, and other pesticides, a horticultural technical officer exposed during spraying of an unspecified pesticide, and a farmer exposed to malathion and other insecticides. Each had experienced symptoms of acute OP poisoning at various times prior to the detection of psychologic alterations. Depressive and schizophrenic reactions were observed in the four individuals. The authors [44] hypothesized that these effects were quite possibly caused by the action of OP compounds on brain ChE. However, other investigators have questioned whether the reactions described had any direct relation to ChE inhibition by OP compounds [45,46].

(c) Reproductive System Effects

Recently, several reports have appeared of chemically induced, occupationally related infertility in males. Four of five members of a farm worker crew who had intensive occupational exposure to a wide variety of pesticides complained of impotence,

according to one report [118]. The pesticides used included OP and CC compounds, triazines, carbamates, dipyridyls, and dithiccarbamates. All complained of difficulty in achieving and maintaining an erection. Normal function returned between 2 months and 1 year after cessation of exposure in all the workers. There was no neurologic deficit in any of them and no loss of libido. Oligospermia was reported in some workers poisoned by Kepone (CW Heath, Jr, written communication, January 1976).

Most recently, reports have associated 1,2-dibromo-3-chloropropane (DBCP) with infertility among workers [119]. Since the early 1950's, the fumigant DBCP has been used worldwide to control parasitic worms that attack the roots of various fruit, vegetable, and cotton plants. In July 1977, Whorton et al [119,120] investigated worker infertility in 145 employees at a major chemical company. Approximately 45% of the workers tested had sperm counts less than 40 million/ml. For this study, the authors considered normal sperm counts to be 40 million/ml or greater [120]. There also appeared to be a direct relationship between exposure duration and sperm count. Workers with sperm counts of 1 million/ml or less had been exposed for at least 3 years. No worker whose sperm count exceeded 40 million/ml had been exposed for more than 3 months. Others exposed who were not azoospermic had reduced sperm motility and increased abnormal sperm forms. Similar tests conducted by two other major chemical companies on their dibromochloropropane (DBCP)-exposed employees revealed sperm counts of less than 20 million/ml in 55% of one group and in 18% in another group [120].

One factor under consideration is the significance of duration and intensity of exposure. Although all severely affected individuals were, or had been, production workers for at least 3 years, the shortest time of exposure associated with oligospermia was only 1 year [119]. Interestingly enough, DBCP concentrations in the workplace air of affected workers were below the recommended 1 ppm airborne levels. Levels of 0.3-0.4 ppm were measured at one plant during May and July of 1977 [120]. Another important question yet to be answered completely is whether the infertility is reversible. Some return of low sperm counts toward normal after discontinuance of exposure to DBCP has been reported recently [121].

(d) Hepatic Effects

Effects on the liver have been reported for a number of pesticides including aldrin/dieldrin and endrin [122,123], 2,4-D [124], copper sulfate [125], and organotin compounds [126,127]. The reported effects range from stimulation of hepatic microsomal enzyme activity [122,123] to severe pathologic damage [126,127].

Toxicologic studies in animals have indicated that DDT and most other chlorinated hydrocarbon insecticides have caused liver damage if the dosage was sufficiently high and the exposure prolonged [2]. Actual reports of liver damage in humans have not been found, however. This may be due to the fact that early or moderate liver damage is difficult to detect in humans without histopathologic studies.

Microsomal enzyme induction by endrin has been observed. Hayes and Curley [122] performed tests on men employed in a

factory where endrin, aldrin, dieldrin, and certain OP compounds were manufactured. Workers engaged in the manufacture of endrin had increased activity of hepatic microsomal enzymes. Hunter and Robinson [123] assessed hepatic microsomal enzyme activity of workers engaged in the manufacture of pesticides by measuring changes in the urinary excretion of D-glucaric acid. Men employed in the manufacture of endrin alone had greater D-glucaric acid excretions than workers exposed to either aldrin or dieldrin. Low 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels found in endrin workers also suggest enzyme induction, since DDE is metabolized by microsomal enzymes. A significant inverse relationship between the blood DDE level and urinary D-glucaric acid excretion is further evidence that these two changes are related to enzyme induction.

Bashirov [124] examined 50 individuals engaged in the manufacture of the amine salt and butyl ester of 2,4-D. Liver function was evaluated by a broad range of tests. The same analyses were conducted in 20 control subjects with no known exposure. Various liver dysfunctions were found among the exposed group including a decrease in urinary hippuric acid, changes in albumin formation, a decrease in the albumo-globulin coefficient, a decrease in the prothrombin index, a decrease in blood sugar levels, changes in the characteristic glycemic curve, and bilirubinemia. These were more pronounced in workers with longer exposures to these herbicides.

In the methyl bromide poisoning cases reported by Hine [112], some liver damage was reported. A forklift operator

responsible for transferring boxes of almonds in and out of the fumigation chambers of a nut-processing facility developed jaundice 3 months after the start of his fourth season of employment. Liver function tests were abnormal and he had clay-colored stools and dark urine. He changed jobs, and 2 years later, liver function studies were normal. Two other workers, who died, had signs of liver changes. One had moderate fatty infiltration of the liver on autopsy, and the other had an elevated serum glutamic oxaloacetic transaminase (SGOT) during his terminal illness.

Pimentel and Menezes [125] reported on three individuals who were exposed while spraying vineyards with a solution of copper sulfate neutralized with hydrated lime (Bordeaux Mixture) for prevention of mildew. Exposure varied, but in one case was as long as 12 years. All three individuals showed similar hepatic changes at autopsy or biopsy, including proliferation and diffuse swelling of Kupffer cells and formation of well-defined histiocytic or sarcoid-type granulomas containing copper.

Liver damage has been attributed to the fungicide, Brestan-60, which is composed of 60% triphenyltin acetate, 15% maneb, and 25% water. Horacek and Demcik [126] described liver damage in a Czechoslovakian spray-plane pilot who had been exposed to the fungicide Brestan-60 and other pesticides. One pilot developed indigestion and severe diarrhea after working with Brestan-60 for an unstated time. He continued to work for several days while experiencing severe heartburn and dryness of the mouth which was not relieved by drinking large amounts of

fluid. After about 1 week, his vision was affected to the extent that he could only make out the outlines of nearby objects. About 2 weeks after the onset of the initial symptoms, he had an enlarged and very tender liver and was subsequently hospitalized. Liver damage was confirmed by biopsy and microscopic examination, which showed increased collagen, moderate round cell infiltration, and slight portal and periportal fibrosis in the edges of the affected portal biliary areas; also, there was evidence of hepatocyte regeneration. Elevated serum glutamic pyruvic transaminase (SGPT) values returned to normal following dietary and insulin treatment for diabetes and vitamin and steroid therapy to improve the liver condition. Eleven months later, biopsy revealed active regeneration of the damaged liver parenchyma, and apart from a slight clinical enlargement of the liver, recovery was complete.

Another case of liver damage due to Brestan was reported [127] for a Yugoslavian formulator who had spilled a solution of the fungicide on his hands and chest while loading a plane. Redness of the skin on his chest and abdomen appeared within 3 hours and was followed the next day by the appearance of vesicles the size of wheat grains. He complained of dizziness, headache, epigastric pain, nausea, and fatigue. Upon hospitalization, his SGOT and SGPT values were elevated. Within 1 month, his SGOT value had increased to 150 units (U) and his SGPT to 575 units, respectively; and he complained of pain in the right hypochondrium, ie, over the liver. The normal ranges for these values are 8-33U/ml for SGOT and 1-36U/ml for SGPT [56]. Two

months after exposure, clinical examination revealed tenderness of the liver and enlargement to two fingers' breadth below the costal margin, resulting in a diagnosis of liver damage. At this time SGOT and SGPT values were 94 units and 196 units, respectively. Continued deterioration over the next 2 years led to a diagnosis of chronic hepatitis.

(e) Renal Effects

Effects on the kidney have also been observed in pesticide workers [128-133]. These effects range from depression of creatinine clearance and phosphate reabsorption [131] to severe tubular degeneration [128].

Davay [128] reported kidney damage in a worker exposed to methyl bromide during its manufacture. The worker was exposed during a shift when methanol and liquid bromine were being refluxed. He worked for 3 hours within a radius of about 12 feet from the sampling and filling valves near the receiving vessels. Findings revealed that methyl bromide apparently escaped from an open sampling valve which the worker noticed and closed. Three hours later he developed nausea and vomiting. Within 50 minutes, the vomiting increased in frequency and severity until the worker lost consciousness and went into convulsions. Following hospitalization, urinalysis revealed albuminuria indicating the possibility of cloudy swelling and/or tubular degeneration of the kidneys. In spite of hemodialysis and peritoneal dialysis, the worker's condition deteriorated and his renal function did not improve. Death occurred on day 18 from respiratory failure as a result of severe injury to the CNS and kidneys.

Strunge [129] reported renal damage following exposure to a mercury fungicide, methoxymethyl mercury silicate, in a 60-year-old man who had worked for 5 years as a bagger and cleaner for a firm that disinfected grain. He used no precautions and smoked and ate at his workplace. He was admitted to the hospital with edema of the genitals and lower limbs. Cholesterol, urinary protein, erythrocyte sedimentation rate, and alpha-2-globulin were elevated, and serum protein and albumin were low. Renal biopsy before treatment showed slight fibrinoid changes in the basement membrane and Bowman's capsule involving all glomeruli. He responded to steroid treatment and was discharged with diagnosis of nephrotic syndrome probably secondary to the mercury fungicide.

Morgan and Roan [130] reported a study of renal function in 65 persons occupationally exposed to pesticides. The group included 24 formulators and applicators of agricultural pesticides, 18 pest control operators, and 23 controls. The agricultural pesticides included DDT, toxaphene, parathion, phosdrin, and a variety of other OP compounds. These workers had an average of 10 years exposure. Among the pest control operators, the average experience was 7 years with exposure to lindane, chlordane, dieldrin, and other carbamate and OP compounds. Controls had no more than the ordinary household exposure to pesticides. Prior to this study, 14 of the formulators had been poisoned by pesticides, 12 requiring some hospitalization. None of these workers had been symptomatic during the 6 months preceding the test.

No differences were noted in creatinine clearance, tubular reabsorption of phosphate, amino acid nitrogen, osmolality, or free water clearance among the exposed groups. Plasma uric acid was lower in the agricultural group, and uric acid clearance was lower and resorption higher in the pest control group. No correlations were found between any of the variables and duration of employment.

Begley et al [131] cited a study of 18 workers exposed to pentachlorophenol (PCP) at a wood treatment plant. Blood and urine samples were taken from each worker on the morning of the last workday prior to a 20-day vacation, and on the mornings of the 3rd, 6th, 13th, and 20th days of the vacation. PCP concentrations in the blood averaged 5.1 ppm before vacation, falling to 2.2 ppm by the end of the vacation. Blood concentrations correlated with observed renal function measurements, which were initially abnormal but later returned to normal values. Creatinine clearance and phosphorus reabsorption values were depressed before vacation but showed significant improvement during vacation, suggesting that PCP exposure reduced glomerular filtration rate and depressed tubular function. Recovery followed a nonexposure period.

In 1966, Mann et al [132] studied kidney function in 70 spraymen and formulators occupationally exposed to various unspecified pesticides. Kidney function tests included phosphate reabsorption, urinary titratable acid, ammonium excretion after loading, and concentrating ability. Significant decreases were found in the renal function of pesticide workers compared with

those of an unexposed control group. The authors stated that chronic exposure to pesticides can result in multiple, potentially irreversible, renal tubular dysfunctions which increase with duration of exposure.

In studies with human volunteers [105] at a New York State prison, daily doses of 0.012 mg/kg carbaryl for up to 6 weeks were found to be associated with a lowering of the urinary amino acid/creatinine ratio which lessened after discontinuance of the daily doses of carbaryl for 15 weeks. This change suggested that carbaryl in this daily dose decreased kidney reabsorption of amino acids.

Tocci and associates [133] found indication of changes in kidney and liver function in persons occupationally exposed to unspecified pesticides. Changes in the functions of these organs were detected by measuring the SGOT, serum alkaline phosphatase (SAP), and creatinine concentrations. Sixteen percent of the study group showed evidence of damage to renal tubules.

(f) Dermatologic Effects

In 1975, 436 cases of skin injury due to exposure to pesticides were reported [29] in California. The pesticides implicated included: malathion, diazinon, omite, paraquat, chlordane, and difolatan. Dermatitis was a major cause of occupationally related visits to physicians. Exposure to pesticides resulted in primary irritation [53,134-138] or sensitization [139-141]. Primary irritants caused dermatitis by direct action on the skin.

A particularly severe skin problem associated with

pesticide exposure is chloracne, which is characterized by acne-like eruptions [134]. Following exposure, a delay of 6-8 weeks typically occurs before the disease is manifested, and once established, recovery may take years. This skin disease among employees of pesticide producers was first observed during the 1950's in workers at a German herbicide manufacturing plant and was caused by TCDD, a contaminant of some chlorophenol pesticides.

Twenty-nine subjects in a 2,4-D and 2,4,5-T manufacturing plant in Newark, New Jersey, developed chloracne [135]. In addition to chloracne, many of the workers also showed hyperpigmentation and increased skin fragility suggestive of the superficial lesions of porphyria cutanea tarda symptomatica. Poland et al [136] reexamined all of the employees of the same factory several years later after the level of TCDD in the 2, 4,5-trichlorophenol had been reduced from 10-25 mg/kg to less than 1 mg/kg. They found chloracne in 13 of 73 workers.

The International Agency for Research on Cancer (IARC) reported a 1949 accident which affected 288 people at the 2,4,5-T producing plant of the Monsanto Chemical Company in Nitro, West Virginia [54]. Signs and symptoms included chloracne, melanosis, muscular aches and pain, fatigue, nervousness, and intolerance to cold.

In 1976, an explosion in a chemical factory in Seveso, Italy, resulted in the release of a vapor cloud of 2,4,5-T and TCDD [137]. As a result of this explosion, an area inhabited by some 2,000 people was contaminated. The presence of TCDD was not

immediately known, so evacuation of the affected area did not begin for over 2 weeks. The first signs of skin problems were seen in local children several days after the explosion. Animal deaths were also reported. As time progressed, more than 500 people were treated for poisoning. An unspecified number of these individuals developed chloracne as a result of the TCDD exposure.

In addition to TCDD-contaminated pesticides, several other kinds of pesticides and their intermediates have been associated with chloracne. Taylor et al [138] reported 41 workers who developed chloracne as a result of exposure to 3,4,3',4'-tetrachloroazoxybenzene (TCAB) during the manufacture of the herbicide 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione. In most of the workers, the chloracne appeared during their first 2 months of employment, but varied overall from 1 week to 8 months. Family members of four workers also developed chloracne, probably due to contaminated work clothes or tools being carried home.

Deeken [134] reported six cases of chloracne from occupational exposure to 2,6-dichlorobenzonitrile (dichlobenil). Those involved were exposed either during dumping of dichlobenil powder into a slurry during formulation or during bagging of the final product. The lag-time between initial exposure to the compound and the development of the acneiform eruptions varied, ranging up to 5 months. The eruption generally consisted of several hundred open pinpoint comedones. No acne cysts were observed. As long as exposure continued, response to the usual

forms of treatment was poor. Once contact with dichlobenil was stopped, improvement occurred.

A variety of other skin reactions have been reported in response to pesticides [142-145]. Kazen et al [142] studied a termite control operator who had habitually used his left hand to protect his face while he sprayed chlordane and aldrin with his right hand. The man subsequently developed a severe dermatitis of the left hand.

Brown [143] reported "skin flushes" or sudden reddening, usually of face and forearm, in 17 employees of a triazapentadiene manufacturing plant. The flushes were reported to last from an hour to a couple of days and usually occurred after the consumption of alcohol.

Radimer and colleagues [144] presented four cases of epidermal necrolysis in individuals whose homes were fumigated with a mixture containing acrylonitrile and carbon tetrachloride. The first evidence of skin disease appeared 11-21 days after their initial exposure to the fumigant. The skin condition was characterized by patches of intense tender erythema that rapidly progressed to huge blisters that opened. Three of the four patients were adults and they died from shock and/or gastrcintestinal bleeding.

Bisby and Simpson [145] reported a case of dichlorvos poisoning manifested by an acute skin reaction in a male pest control operator who had worked with pesticides for more than 9 years. One month earlier, he noticed a slight burning sensation of the skin after contact with dichlorvos. The skin was washed

with no further ill effect. The poisoning episode occurred during the routine spraying of a 1% solution of dichlorvos. Some of the chemical leaked onto the operator's shoulder and slight local irritation occurred. The operator stopped, put on clean overalls, placed a plastic sheet between the spray unit and his back, and completed his work, all without washing. During the day he noticed increasing local irritation and burning of the contaminated area, and at the end of his shift, excessive tiredness. Three days later he had extensive areas of erythema and bullae typical of acute contact dermatitis. ChE activity levels were extremely low indicating severe systemic OP poisoning. There were no other signs of clinical abnormalities. Exposure to pesticides was avoided, recovery was uneventful, and the patient returned to work after a month of rest.

Several pesticides have caused skin hypersensitization, an allergic reaction, in humans [139-141]. Patch tests are often done to determine whether a suspected compound is the sensitizer.

Edmundson and Davies [139] reported occupational dermatitis in four workers exposed to naled while cutting chrysanthemum plants. The women involved had been doing this type of work for from 1 month to 9 years. On the day of the incident, the field in which they were working had been sprayed with a mixture of naled, captan, and dicofol, and some of the plants were still wet with the solution at the time of cutting. While in the field, all four women experienced burning and itching of the face, neck, and arms, and later, welts and/or rashes. Contact sensitization type dermatitis was the diagnosis.

Patch tests carried out 2 weeks later showed a positive reaction only for naled in each woman.

Spencer [140] reported three farmers who developed acute dermatitis after spilling the herbicide allidochlor, a derivative of 2-chloroacetamide, on their shoes or clothing. All three developed a violaceous eruption with bullae on contact areas. Patch testing a few months later on two of the individuals resulted in a 4+ (strong) response for allidochlor, and was negative for other pesticides that they had used.

Milby and Epstein [141] described the experimental testing of the sensitivity of volunteers to malathion. Eighty-seven men were divided into four groups. The malathion used was 95% pure, analytical standard grade malathion in ethanol. In Group I the skin was first irritated by a 3-second freeze with dichlorodifluoromethane to enhance sensitization, and then 10% malathion was applied. In Group 2, 10% malathion was applied to a nonirritated skin site. Groups 3 and 4 were first irritated with dichlorodifluoromethane and then exposed to 1.0 and 0.1% malathion, respectively. In 30 days, all subjects were retested with a nonirritating 1% concentration of malathion in ethanol at a new site. The findings indicated that a single exposure to 10% malathion readily induced contact sensitization in almost half of the subjects, and the average intensity of the reactions was great. Weaker solutions had much less tendency to sensitize, even when applied to irritated skin. To explore the degree of sensitivity, a group of five highly malathion-sensitive subjects were tested with weak solutions of malathion in water and

acetone. All gave strong reactions (bullae) at a 1 ppm concentration in acetone, and positive responses were also evoked by applying a commercial preparation of 0.9% malathion in water.

A subsequent study of two groups who used malathion in their occupations (157 mosquito abatement workers and 43 poultry workers) revealed that about 3% reacted to a 1% malathion patch test. Several of these individuals gave histories of previous episodes of dermatitis which had defied diagnosis.

The herbicide monuron has also been associated with contact dermatitis [146]. Two episodes are included in EPA's Pesticide Episode Review System data.

(g) Ophthalmologic Effects

In 1975, 314 eye injuries were reported in California due to pesticide exposure [74]. Most eye injuries were acute, characterized by damage to the conjunctivae, cornea, and associated structures because of the corrosive properties of pesticide active ingredients or formulations [29,143]. A variety of pesticides have been associated with such injuries, including triazapentadienes, weed oil, and difolatan.

Brown [143] reported three workers in a plant manufacturing triazapentadienes who experienced intense eye irritation 6-12 hours following their workshift. Two of the three were admitted to a hospital. One had a large area of corneal de-epithelialization, and the other had superficial punctate keratitis. The injuries were traced to escaping vapors of ethanol, isopropyl alcohol, and formidines from a leaking valve in the recycling part of the plant.

Maddy and Topper [29] described eight cases of eye injury in persons working in pesticide manufacturing and formulating plants. In one case, an employee was exposed to weed oil when the nozzle he was using to fill a can broke off and sprayed his face, chest, and legs. He was treated for chemical burns to the eyes. Another case involved an employee working with a dust collector. Some difolatan powder blew through the side vents of his goggles and caused chemical irritation in his left eye. A third case of eye injury involved an employee emptying a drum of lime sulfur. While he was setting the drum down, the top opened and some liquid contacted his face and eyes. Although he was wearing safety glasses, his eyes were nevertheless injured.

Cataracts are often considered to be a condition of old age. However, pesticides have been implicated in the production of lens and corneal cataracts. The pesticide compounds 2, 4-dinitrophenol (DNP), used both as an herbicide and as a fungicide, and dinitro-o-cresol, used as an insecticide, were implicated in the 1930's as causing cataracts after large doses were ingested for weight control purposes [51].

(h) Respiratory System Effects

Inhalation is an important route of exposure for pesticides. After inhalation, pesticides gain access to the bloodstream, and systemic toxicity can result. In some cases, inhalation of pesticides can also result in local damage to the respiratory system itself, ranging from localized burning in the mouth and throat [147] to pulmonary fibrosis [125,148].

Pimentel and Menezes [125] reported three cases of

vineyard sprayer's lung. The three men were all exposed while spraying vineyards with a solution of copper sulfate neutralized with hydrated lime (Bordeaux Mixture) for the prevention of mildew. Case 1 was exposed on the job for 3 years, Case 2 for 12 years, and Case 3 for an unspecified length of time. Case 1 had symptoms for 3 years prior to hospitalization. He was dyspneic and cyanotic when hospitalized. A chest X-ray showed diffuse bilateral reticular and micronodular shadows. Pulmonary function studies showed a restrictive ventilatory defect. The patient died of bilateral spontaneous pneumothorax. Autopsy showed bilateral diffuse pulmonary fibrosis with emphysema in lower lobes. Histology indicated numerous histiocytic granulomas and fibro-hyaline nodular scars. These lesions contained abundant inclusions of copper. Case 2, an alcoholic, was hospitalized for a febrile syndrome. A chest X-ray showed reticular and micronodular shadows. The liver was irregular and hard. The patient's condition deteriorated progressively until he died. Autopsy showed numerous blue nodules and extensive fibrosis in both lungs. Histologically, there were numerous histiocytic granulomas and extensive nodular scars. The lesions contained considerable amounts of copper. Case 3 was hospitalized for weakness, joint and muscular pains, and loss of appetite. A chest X-ray indicated increased lung markings and possible pneumonia with pleural reaction. The liver was enlarged. Biopsies of both the liver and lung were made. Histology showed histiocytic granulomas that were in an advanced condition of sclerosis and hyalinization in some areas. These lesions

contained abundant inclusions of copper.

Warraki [147] reported two cases of acute bronchopneumonia in agricultural workers exposed to toxaphene sprays. The first man reported he had been heavily exposed to toxaphene for 2 months. A chest X-ray revealed marked bilateral hilar lymphadenopathy with fine miliary opacities heavily distributed over both lungs. Pulmonary function tests demonstrated a vital capacity 36.2% and a maximum breathing capacity 19% of predicted normal. The second man had been heavily exposed to toxaphene spray for the first time 1 month before entering the hospital. A chest X-ray showed coarse miliary shadows in both lungs with maximum distribution in the middle zone. Pulmonary function studies demonstrated a vital capacity of 22% of predicted normal. Both men showed dramatic improvement with avoidance of exposure and treatment with corticosteroids.

Weiner [148] reported a case of bronchial asthma attributed to exposure to an OP pesticide in a 41-year-old chemical formulation worker with a history of rhinitis and 21 years' exposure to sulfur powder, who had recently begun packaging mevinphos. When a leak developed in a can he was using as a filler, he removed his mask and gloves to fix the leak. During this time he inhaled a large quantity of the material and spilled some on his hands. Thirty minutes later he was hospitalized with labored breathing, cyanosis, and miosis. Moist rales were present throughout the lungs. The man responded to atropine treatment. Three weeks later while working with unspecified powders, he had coughing, wheezing, and labored breathing. Over

the next 3 years he had repeated bouts of pneumonia, and pulmonary function studies showed decreased forced vital capacity on two occasions. The authors speculated that the observed susceptibility to respiratory effects occurs only in subjects predisposed to allergy.

Paraquat has caused pulmonary edema, severe irritation of mucous membranes, and acute renal failure, usually after accidental or suicidal ingestion. If the immediate effects do not produce death within a week, the patient may either recover fully or die in the delayed stage of the poisoning. This is characterized by rapid development of pulmonary fibrosis, with the appearance of granular opacities on radiographs of the chest. The patients become dyspneic from the combined effects of decreased pulmonary capacity of the lungs. Death from respiratory failure and anoxemia occurs 2-4 weeks after the poisoning [149].

Such progression of pulmonary fibrosis was observed by Davidson and Macpherson [150] in two cases of paraquat poisoning due to accidental ingestion of Gramoxone W. In the first case, pulmonary function was normal until day 7 following exposure. On day 7 a chest radiograph showed fine granular opacities. Thereafter, lung function tests showed rapid deterioration. Open biopsy on day 9 showed interstitial edema, fibroblastic activity, and fibrosis. Following death on day 17, an autopsy showed the lungs to be enlarged and almost solid throughout. Interstitial tissue showed considerable fibrous thickening, disorganizing the lung structure. In places, air spaces were lined by a thick

membrane resembling hyaline membrane disease. Reactive hyperplasia was seen in many areas. In the second individual, ill-defined opacities were evident on day 6 following exposure. The patient experienced respiratory distress and lung function showed rapid deterioration. After death on day 25, autopsy showed complete obliteration of the air spaces by fibroblastic proliferation and inflammatory cells. The alveolar walls and interstitial tissue were broadened by fibrous tissue. Both upper lobes showed gross emphysema.

Barthel [151] cited data on three of five cases of lung fibrosis found in pest control workers. The workers had many years of experience during which they were exposed to several pesticides including OP compounds, OC's, and arsenates. X-ray examination of the lungs revealed disseminated foci mainly localized in the lung periphery and diffuse spotted and striped lung shadows with signs of diffuse emphysema.

(i) Hematopoietic Effects

Pesticide exposure has been associated with hematologic alterations [152-159]. Toxic materials may affect the components of the blood by influencing their production, rate of peripheral destruction, or distribution [55].

Furie and Trubowitz [152] reported a case of chlordane exposure followed by the development of megaloblastic anemia. The individual mixed a chlordane solution and then poured it around the inside and outside of his house/office for termite control; during this operation, chlordane was spilled on his hands. Actual total skin contact was estimated to be about 3-5

hours during a 2-month period. Chlordane fumes were very strong in his office where he spent approximately 300 hours during a pericd of 6 months. After that time, he experienced severe shortness of breath, fatigue, and tachycardia. Hematologic analyses revealed anemia with the presence of megaloblasts. Examination of the bone marrow confirmed hypercellularity. A diagnosis of megaloblastic anemia was made. As the patient's hemoglobin levels increased after a series of blood transfusions, his condition gradually improved.

Sanchez-Medal and coworkers [153] noted 20 cases of aplastic anemia during 8 years at Hospital de Enfermedades de la Nutricion in Mexico City. In 16 of the 20 cases, pesticides appeared to be the only possible offending agents since all patients had repeated contact during the 6 months preceding clinical onset of their disease. The insecticides involved were DDT alone, or DDT in association with lindane, dieldrin, or DDVP. Clinical and laboratory findings in these cases did not differ from those in aplastic anemia due to other causes. Also reported was the case of a 13-year-old boy hospitalized with aplastic anemia which was circumstantially related to DDT. The boy's home had been repeatedly sprayed with DDT for 2 years, and in the 4 months preceding his hospital admission DDT was sprayed every other day. The patient was recovering when he was accidentally reexposed in his hospital room when a volunteer sprayed a 10% DDT spray. He had an anaphylactic reaction within 1 hour of reexposure, and his blood dyscrasia worsened. He died 30 hours later.

The American Medical Association registry on blood dyscrasias reported 44 cases of aplastic anemia associated with pesticides through 1963 [154]. Of these cases, 13 were related to lindane, and in 7 cases, lindane was the sole agent; 19 cases were related to DDT, and in 3, DDT was the sole agent; 12 cases were related to chlordane, and of these, chlordane was the sole agent in 4 instances.

Palva et al [155] reported the case of a 64-year-old farmer who was spraying 2-methyl-4-chlorophenoxyacetic acid (MCPA) with a manual sprayer that leaked, so that his clothing became soaked with the herbicide. Two weeks later he had spontaneous hematomas and manifested lethargy. He was pancytopenic 2 months later. He responded to steroid treatment within 2 months and was free from symptoms in 5 months.

Samuels and Milby [156] conducted a further study on a lindane exposed population first studied by Milby et al [157]. The population included 79 individuals who had been exposed daily to lindane for a period of weeks to years. Seventy-one of these 79 were employed in lindane processing plants. The other 8 individuals included the residents of two households in which lindane vaporizing devices were operated for pest control purposes. Milby et al [157] found that the concentration of lindane in the blood reflected recent lindane absorption. The mean concentration of lindane in the blood appeared to be a valid indicator of relative exposure intensities; however, levels did not appear to increase with increasing duration of exposure. Samuels and Milby [156] found isolated instances of leukopenia,

leukocytosis, granulocytopenia, eosinophilia, monocytosis, and thrombocytopenia in the same population. Pancytopenia with reticulocytopenia was not observed. There did not appear to be a correlation between abnormal findings and either duration or intensity of exposure. Monocytosis appeared to decline after the 5th year of employment in 57.7% of the cases.

West [158] reported the case of a girl with an atypical blood count and anemia. In addition, four other members of her family had mild anemia. All recovered when a lindane vaporizer which had been operating for 1.5 years was removed from the home.

Davignon et al [159] reported a 3-year study of three groups: 441 apple-growers who had worked with insecticides, 170 subjects who did not actually use insecticides but lived in or near orchards, and 162 controls with no known contact with insecticides. No difference was observed in average RBC counts and hemoglobin concentration in the three groups. However, the average leukocyte count was significantly lower for the apple-growers and those living near orchards than for controls, although levels in both groups were within the range of the normal population.

(j) Cardiovascular Effects

Few reports are available on the effect of pesticides on the cardiovascular system. Health surveys of workers with intense occupational exposure to DDT have not detected cardiovascular changes [160].

Butzingeiger [161] examined 180 vinedressers and cellarmen who had used arsenic insecticides and who had expressed symptoms

consistent with chronic arsenic intoxication. Of the 180 examined, 41 (22.8%) showed evidence of vascular disorders in the extremities. Of the 15 cases described in detail, cold hands or feet, or both, were common and apparently preceded the development of gangrene on the toes or fingers in six cases.

Electrocardiogram (ECG) findings of vinegrowers with chronic arsenic intoxication were reported by Butzengeiger [162]. Of 192 ECG's, 107 (55.7%) were normal, 30 (15.6%) showed slight changes which alone were insufficient for definite diagnosis of cardiac damage, and 55 (28.7%) revealed definite changes. In 19 of the 55 altered ECG's, changes were attributed to age, arteriosclerosis, or intercurrent disease. In the remaining 36, arsenic poisoning was considered responsible. ECG abnormalities included Q-T prolongation and flattened T-wave. Follow-up studies revealed a decline in ECG abnormalities along with the symptoms suggestive of diminution of arsenic intoxication.

Overexposure to OP insecticides has been associated with blood coagulation and vascular changes [163]. In one male over 50 years of age, two cerebrovascular incidents followed overexposure to disulfoton (GE Quinby, MD, written communication, May 1978).

Blood pressure elevation has been sporadically reported [164-166] but no well-controlled study has been done of the relationship to pesticide exposure. Sandifer et al [164] reported on a long-term study of pesticide-exposed workers in which systolic blood pressure was elevated among a cohort of formulators and pest control operators as compared with matched

controls. This elevation was correlated with blood DDT and DDE levels. There were no differences between exposed and controls in smoking, family history of hypertension or diabetes, and educational level.

Richardson et al [165] studied 23 pesticide formulators exposed to various pesticides including chlorinated hydrocarbons, eg, DDT, OP compounds, eg, parathion, and carbamates, eg, carbaryl. The 23 exposed formulators and 20 controls were tested for catecholamine and cortisol metabolism. Blood pressure was measured in all. Epinephrine and norepinephrine concentrations in plasma and urine were lower in the exposed group, while urinary metanephrine concentrations were similar in both groups. Mean systolic blood pressure was higher in the exposed group and correlated significantly with blood DDT levels.

Morton and coworkers [166] examined the effect of pesticide exposure on blood pressure. The blood pressures of 153 pesticide workers from 28 different pesticide manufacturing and formulating plants in Oregon and of 76 controls were measured. Sixty-nine of the workers were from one phenoxy herbicide plant. The controls were medical center employees. No difference was found in mean systolic and diastolic blood pressure among the three groups, but 38% of the phenoxy herbicide workers had systolic blood pressure greater than 150 and/or diastolic blood pressure greater than 90, compared with 29% of all other pesticide workers and 30% of controls. There was a higher frequency of family history of hypertension in the herbicide group, suggesting that the workplace had little influence on the frequency of hypertension

in the workers studied.

(k) Carcinogenic Effects

Relating specific causative agents to cancer in humans is a difficult task and requires properly designed epidemiologic studies. These studies involve the correlation of an increase in cancer incidences as compared with expected incidence among occupationally exposed workers. Such correlations are difficult because workers typically have repeated exposures to multiple substances in the workplace. Furthermore, workers are often transient, which makes these studies much more difficult.

Arsenical pesticides have been shown to cause human cancer. Roth [76] described 47 German vinegrowers chronically poisoned by occupational exposure to arsenical insecticides in the vineyards and by arsenic-contaminated wine. Cancer was listed as the cause of death in 30 of the 47 cases (64%), and malignancies were observed in an additional three cases. In the 33 subjects with malignancies, a total of 75 tumors was found including tumors of the lung, skin, liver, larynx, bile duct, esophagus, and tongue. Bronchial cancer was listed as the cause of death in 16 cases, and 6 of these individuals also had from 1 to 4 skin cancers. In the 2 cases where death was attributed to cancer of both lungs, both subjects also had skin cancer and 1 had cancer of the larynx. The individual who died of cancer of the bile duct also had a bronchial carcinoma. Of the 6 individuals with liver sarcomas, 1 also had 2 skin cancers. And, of the 5 cases with esophageal cancer, 1 also had cancer of the tongue and another had 3 skin cancers. In the 3 individuals who did not die from

their malignancies, 18 skin cancers were found. In 8 cases, "arsenic cirrhosis" was listed as a cause of death and was observed in an additional 25 cases.

In another study, Baetjer and coworkers [50] discussed the mortality of retired workers with exposure to arsenical pesticides during the manufacture of calcium, sodium, and lead arsenate. Of 22 deaths among the retirees, 17 were due to cancer. The expected cancer deaths for the group was 4.43, based on the general Baltimore population. By site, the ratio of observed to expected cancer deaths was 10/1.49 (6.71) for respiratory cancer, 3/1 (3.0) for lymphatic and hematologic cancers (lymphosarcomas), and 4/2.69 (1.49) for all other neoplasms. A death rate analysis was also conducted, and once again, cancer mortality was found to be significantly increased.

In a study by Axelson and Sundell [59], an excess of all cancer types was found in Swedish railway workers who primarily used amitrole (a triazole derivative), 2,4-D, and 2,4,5-T. The elevated incidence of cancer seemed primarily associated with exposure to amitrole and combinations of amitrole with other pesticides. This compound has also been shown to be carcinogenic in animals [58].

NIOSH has recommended that acrylonitrile be handled in the workplace as a probable occupational carcinogen [69]. This recommendation is based on the results of animal studies and a recent preliminary epidemiologic study of 470 textile workers exposed to acrylonitrile during polymerization which indicated an excess incidence of lung and colon cancer among workers with

potential acrylonitrile exposure. A total of 16 cancer cases occurred between 1969 and 1975 among the cohort first exposed between 1950 and 1955; only 5.8 cancer cases would have been expected, based on company rates (excluding the cohort). However, as is commonly the case, workers may have been exposed to a variety of other chemicals which may have acted on the biologic system synergistically. Also, the results of the study are preliminary, approximately one-third of the cohort having been studied [81].

The NIOSH criteria document on coal tar products [82] included an evaluation of the biologic and health effects of creosote. Overexposure to creosote caused burns, conjunctivitis, depression, headaches, vertigo, transitory confusion, and nausea [82]. Squamous-cell carcinomas were also reported for a creosote factory worker and a painter who scoured with creosote [82]. Skin tumors were found in cresote-treated mice, rats, and dogs, and lung tumors were found in mice. NIOSH has recommended that creosote be handled in the workplace as a probable carcinogen [82].

Benzene has been associated with a variety of hematologic abnormalities including leukemia. Forni and Vigliani [77] estimated that at least 150 benzene-related leukemia cases have been reported. In addition to these case reports, several epidemiologic studies have also associated benzene with cancer. McMichael [78] studied a cohort of rubber workers with solvent exposure. The results suggested an increased risk of death from lymphatic leukemia in those exposed to solvents. The authors

demonstrated a dose-response of both duration and intensity of solvent exposure with lymphatic leukemia.

Infante et al [79,80] reported results of an epidemiologic study of Pliofilm workers exposed to benzene. The study identified a statistically significant excess incidence of leukemia in benzene exposed workers compared to a nonexposed control population. A fivefold excess of total leukemia and tenfold excess of myelomonocytic leukemia were found. NIOSH has recommended that benzene be handled in the workplace as a probable carcinogen and OSHA has also regulated the compound as an occupational carcinogen.

Certain compounds of hexavalent chromium have also been implicated as carcinogens on the basis of human and animal studies. Chromium compounds implicated in these studies have lead NIOSH to infer that all hexavalent chromate salts of alkaline earth metals are probable human carcinogens and this would include the pesticides zinc mercury chromate and copper zinc chromate [83].

Barthel [167] investigated the tumor incidence in 316 long-term exposed pesticide workers in the Newbrandenburg district of Germany. There were 30 cases of tumors of which 11 were bronchial carcinomas. The incidence of bronchial carcinoma in the group was 20 times that expected in an age-specific general population. Frequency at all other cancer sites did not differ significantly from expected. Exposure to pesticides ranged from 6 to 23 years and included phenoxyacetic derivatives, OC's, OP's, organic nitro derivatives, and some arsenic

compounds. Since the workers were exposed to various chemical compounds simultaneously or alternately, the carcinogenic effect could not be associated with any one specific pesticide.

Although the literature contains numerous reports of pesticide poisonings, many of these resulted from exposure modes that are not usually experienced in the workplace. In those cases where the exposure took place during the manufacture or formulation of pesticides, exposures were seldom limited to a single substance. Evaluation of the hazard involved in the manufacture of pesticides is further complicated by the scarcity of successful epidemiologic studies. Epidemiologic studies are hampered by the difficulties involved in identifying a suitable cohort of employees and control group. In view of the limitations encountered in both poisoning cases and epidemiologic studies, it is important to use animal test results to predict and, in some cases, to reinforce effects in humans.

Effects in Experimental Animals

Toxicologic evaluations of pesticides have for many years focused on common laboratory animals as the experimental model for man's physiological, biochemical, metabolic, and pathological response to these chemicals. These evaluations have produced a large body of information on the local and systemic effects of pesticides in animals. In recent years there has been an emphasis on obtaining better information on chronic exposure effects. This emphasis has led to the finding that some pesticides are animal carcinogens. In addition, other

irreversible effects such as teratogenicity, mutagenicity, and reproductive disorders have been observed in animals. In the following sections, various aspects of acute and chronic pesticide toxicity will be discussed.

(a) The Acute Toxicity of Pesticides

While acute toxicity determinations have been conducted in various animal species including the rat, mouse, hamster, guinea pig, rabbit, cat, dog, and monkey, such measurements are most commonly made in the rat and the mouse. The large body of toxicity data which exists for these two species makes it possible to evaluate the toxicity of a pesticide relative to other pesticides.

A number of genetic and environmental factors contribute to the variability of acute toxicity. One major factor is the difference in the susceptibility of the exposed individuals of any species, including man. While individual variability among animals and man is not predictable, other variables are. Animals of the same strain but of different sexes often provide marked differences in the acute LD50's when tested. Comparisons of the oral toxicity of 85 pesticides by Gaines revealed male-female sex ratios of the LD50's ranging from 0.21 to 4.62 [99]. Variability also occurs between different routes of exposure. Gaines determined that the ratio of dermal-oral LD50's for 57 compounds tested by both routes ranged from 0.2 to 21.0.

Old and young animals of one species may differ rather markedly in their response to pesticides as shown by differences in their acute LD50's. Brodeur and DuBois [168] tested 16 OP and

carbamate compounds in both adult and weanling rats and found that 15 were more toxic in weanling than in adult rats by ID50 ratios that varied from 1.25 to 4. Only schradan was more toxic to adults and by a ratio of 5 to 1. Increased susceptibility of newborn animals to toxic agents can be explained as resulting from the undeveloped state of the detoxication mechanisms of newborn animals [168]. Lu et al [169] found that DDT and dieldrin were 20 and 5 times as toxic, respectively, to adult as to newborn Wistar rats, whereas, 99.6% malathion was almost 30 times as toxic to newborn as to adult rats [169].

An effect of ambient temperature on toxicity has also been demonstrated. Increased toxicity with increased temperature has been found by Furman et al [170] for DNP.

Diet also modifies acute toxicity. The presence of fat in the diet has a tendency to increase the absorption of nonpolar pesticides and thus lower acute LD50 values. Both rats and mice demonstrated increased toxic effects from DDT on a 15% fat diet compared to a 5% fat diet. The protein content of diets can also modify the degree of acute toxicity [171]. Boyd and Krijnen [171] showed a marked increase in carbaryl toxicity in rats on a protein deficient diet. The oral LD50 of carbaryl was reduced from 575 mg/kg on a 27% protein diet to 84 mg/kg on a 3% protein diet.

The toxicity of a pesticide can be increased or decreased if it is present in animal or man in conjunction with another active compound which affects its metabolism. Potentiation is often the result of inhibition of detoxification mechanisms,

whereas antagonism is often the result of enzymatic detoxification [172-174]. Experiments have shown that certain pesticides dramatically potentiate the toxicity of other pesticides. Frawley et al [175] observed that the lethal doses for EPN and malathion when administered separately to dogs were 200 mg/kg and >4,000 mg/kg, respectively. However, when they administered the pesticides simultaneously, the dogs died at dose levels of 2 mg/kg EPN and 100 mg/kg malathion. This synergism extended to the inhibition of erythrocyte ChE. Subchronic feeding of both compounds to dogs produced low blood ChE inhibition at exposure levels one-half to one-thirtieth of those required for the separate compounds to exert similar effects. Similar experiments conducted with rats resulted in increased ChE inhibition but to a lesser extent than in dogs [175]. Other mechanisms for potentiation and antagonism also exist [172,173].

The presence of OC pesticides, such as dieldrin, has reduced the toxicity of several OP pesticides. Apparently the OC stimulates enzymatic detoxification of the OP [172,173].

Another factor affecting toxicity is fractionation. This concerns the time over which the total dose is administered or the administration of the total dose in smaller subunits. Normally, fractionated administration allows more time for the pesticide to be detoxified or excreted. Where the toxic agent is actually the metabolite of the pesticide, such decreased toxicity may not result [176].

Obviously, toxicologic mechanisms of pesticides are far more complicated than acute testing normally reveals.

Variability in toxicity according to sex, age, route of exposure, diet, environment, and multiple exposures certainly applies to humans as well as to experimental animals. Factors affecting toxicity of pesticides in the workplace should always be considered, especially in light of the range of susceptibility exhibited by most heterogeneous populations.

While the previous discussion of acute toxicity applies to a general understanding of the subject, some studies have elements deserving special consideration. Inhalation and dermal toxicity studies deserve elaboration in view of their relation to occupational exposures.

(1) Inhalation Toxicity Studies

Study of the toxicities of substances in animals by inhalation is of great significance to the field of industrial toxicology. Except for iv injection, inhalation is the most effective and rapid route for the entry of substances into the body and for production of toxic effects. Many of the problems of worker-induced systemic toxicity have resulted from inhalation exposure as described earlier.

Because chronic inhalation toxicology experiments are expensive and difficult to perform, relatively few long-term studies of this nature have been performed for pesticides, despite their importance. Such experiments require that animals be placed in special exposure chambers that have intake and exhaust systems as well as some mechanism for maintaining a consistent and measurable concentration of the pesticide. These experiments are difficult to conduct, especially for studies of

dusts. In many experiments, animals are often removed from the inhalation chamber and replaced on an established regimen during the experimental period.

Because of the experimental difficulties, a single discrete dose cannot be administered, and few experiments utilize the same period of exposure, making dose comparison difficult. The observed toxicity value may be different from that obtained orally because chemicals absorbed through the lungs partly bypass the liver, where most detoxification occurs. On the other hand, some compounds are actually activated by liver enzymes, eg, parathion, and in this case, the direct path to the liver resulting from oral administration could result in greater toxicity than would be expected through inhalation.

(2) Dermal Toxicity Studies

The term dermal toxicity includes the production of local effects on the skin and of systemic toxic effects by cutaneous absorption of pesticides.

Because of the importance of contamination of the skin in the industrial setting, many acute dermal toxicity experiments of pesticides have been conducted. Dermal exposure may be a particularly hazardous aspect of occupational contact with pesticides because workers may not be aware that certain compounds have a remarkable ability to penetrate the skin. Gaines [177] has found a closer relation between the dermal toxicities of some pesticides in rats and the occurrence of occupational poisoning than between oral toxicities and occupational poisoning. He reported that dieldrin has an oral

LD50 in the same order of magnitude as lindane and DDT, 46, 88, and 113 mg/kg, respectively. However, at that time Hayes [178] had reported 100 cases of dieldrin-related occupational poisoning. Gaines was not aware of any occupational poisonings associated with DDT or lindane, but he speculated that this phenomenon was due to dieldrin's dermal LD50 of 90 mg/kg as compared with those of lindane (1,000 mg/kg) and DDT (2,510 mg/kg) [177].

Local skin effects produced by pesticides are also of major concern and include local irritation and the development of hypersensitivity (allergic reactions) to the substances. The irritancy or direct dermal toxicity of compounds is commonly evaluated by applying the compound to the shaved skin of the rabbit. Allergenicity or hypersensitivity frequently is evaluated by applications to the shaved skin of guinea pigs.

(b) Chronic Toxicity

Studies of chronic toxicity in animals have not been reported in the open literature for the majority of registered pesticides. Studies that have been reported have resulted in the observation of irreversible toxic effects which have not been detected by acute toxicity studies. The organ most frequently damaged is the liver, the major site of detoxication of chemical substances. The CNS, the peripheral nerves, the kidneys, and other organs may also be irreversibly affected. The major irreversible effects observed in experimental animals and in in vitro test systems include carcinogenesis, mutagenesis, teratogenesis and other reproductive effects, and neurotoxicity.

While testing for mutagenesis and teratogenesis does not necessarily involve chronic exposure, such effects are severe enough to warrant discussion here. Mutagenesis testing itself may require a multigenerational study to detect affected individuals. All of these will be discussed in the following sections. From an occupational point of view, dermal and inhalational exposures in chronic animal studies are more applicable to the actual workplace situation than is oral exposure.

(1) Carcinogenesis

Much concern for the health of workers in the pesticide industry centers around the possibility that they may be developing cancer as a result of their exposure to compounds in the workplace. As discussed earlier, exposure to arsenical pesticides has been associated with increased risk of developing skin cancer, leukemia, and lung cancer in pesticide workers [48]. Exposure to vinyl chloride, a pesticide intermediate, has resulted in the induction of liver cancer [179]. The finding of similar tumors in experimental animals [180] and in workers exposed to vinyl chloride has lent credence to the belief that animal toxicity experiments are significant in judging whether induction of human cancer by a given chemical is likely.

Inorganic arsenic compounds, acrylonitrile, benzene, amitrole, certain hexavalent chromium compounds, and creosote, all of which have been reviewed earlier in the document, are considered by NIOSH to be probable carcinogens based on evidence from both humans and experimental animals. The following is a

review of 113 additional pesticides with respect to carcinogens based on evidence from both humans and experimental animals. Of the 113 compounds reviewed, 26 (see Table XIV-8) are considered suspected occupational carcinogens based on evidence found in animals and presented herein. Carcinogenicity data are reviewed also for 27 pesticides for which the evidence for or against carcinogenicity in test animals is inconclusive. These compounds require further testing before they can be termed suspected occupational carcinogens or placed in the final group of pesticides for which data from experiments with animals have their carcinogenic potential. Of the 113 compounds reviewed, 26 (see Table XIV-8) are NIOSH has placed 60 pesticides in this final category. Remaining unreviewed are approximately 1,300 pesticides for which data were not available.

(A) Suspected Occupational Carcinogens

NIOSH recommends that the following pesticides should be handled in the workplace as suspected occupational carcinogens. Pesticides are included in this classification if laboratory studies indicate a statistically significant relationship between tumor development and pesticide administration in one or more mammalian species.

(i) Aldrin/Dieldrin

After reviewing the available world literature, NIOSH has determined that aldrin and dieldrin should be handled in the workplace as suspected occupational carcinogens [2]. The International Agency for Research on Cancer (IARC) reviewed the literature on the carcinogenicity of these two

compounds in 1974 and found no evidence for the induction of cancer in animals by aldrin, but did link aldrin with its metabolite dieldrin. Dieldrin was concluded to be a hepatccarcinogen in mice [181]. In 1972, EPA concluded that dieldrin was a carcinogen in the mouse [182] and restricted its usage based partially on that conclusion (Federal Register 39:7246, October 18, 1974).

(ii) Bis(2-chloroethyl) Ether

Bis(2-cholorethyl) ether was administered by Bionetics Research Labs [4] daily by stomach tube to two groups of 18 male and 18 female B6C3F1 and B6AKF1 mice at a rate of 100 mg/kg for 21 days. This was part of a screening study in which 106 pesticides were tested for carcinogenicity for NCI. The chemical was subsequently administered at a concentration of 300 ppm in the diet for 80 weeks. Hepatoma incidence was 14 tumors/16 male B6C3F1 treated mice and 9 tumors/17 male B6AKF1 treated mice compared with 8 tumors/79 and 5 tumors/90 male control mice of the respective strains. The incidence in females was 4 hepatomas/18 and 0/18 treated mice compared with 0 tumors/87 and 1 tumor/82 in the controls of the respective strains. Hepatoma incidence was significant at a level of P=0.01 [4].

In the same study [4], subcutaneous injections of 215 mg/kg were administered once per animal to the same strains. Reticulum cell sarcomas occurred more frequently in the treated mice than in controls (P=0.01). In B6C3F1 males, the incidence of sarcomas was 4/15, while B6AKF1 males had an incidence of 2/14

compared with 8/27 and 0/8 in controls, respectively. Females had 1/17 and 1/18 in treated animals and 1/9 and 5/17 in controls, respectively [4]. The IARC reviewed the literature in 1975 and found bis(2-chloroethyl) ether to be an animal carcinogen [183].

(iii) Bis(2-hydroxyethyl)dithiocarbamic Acid, Potassium Salt

In the Bionetics study reported in 1968 [4], 18 male and 18 female B6C3F1 and B6AKF1 mice were administered 464 mg/kg bis(2-hydroxyethyl)dithiocarbamic acid, potassium salt, by oral intubation at 7 days of age. The same amount was given daily until the mice were 28 days old, at which time the compound was mixed with the ground feed at 1,112 ppm; the diet continued for 80 weeks. The total number of mice that developed tumors was significant at a level of P=0.01; the occurrence of hepatomas among the tumor types was also significant at the same level. In male B6C3F1 treated mice, 16 tumors developed in 14/16 (88%) mice; 13 were hepatomas. In male B6C3F1 controls, 23 tumors developed in 22/79 (28%) mice, 8 of which were hepatomas. The tumor incidence in B6C3F1 females was 13/18 (72%) (12 hepatomas) compared with 8/87 (9%) (0 hepatomas) in controls. In 13/17 (76%) B6AKF1 male survivors, 14 tumors were found (13 were hepatomas). In B6AKF1 male controls, 17 tumors (5 hepatomas) were found in 16/90 (18%) mice. The number of female B6AKF1 mice which developed tumors was 7/16 (44%), and 3/7 were hepatomas. In control mice of the same sex and strain, 9 tumors were found in 7/82 (9%) mice; one was a hepatoma [4].

Ethylene thiourea (ETU) is an impurity and degradation product of ethylenebisdithiocarbamic acid (EBDC) fungicides [184,185] including bis(2-hydroxyethyl)dithiocarbamic acid, potassium salt. ETU is also the principal product of the in vivo metabolism [186] and in vitro degradation [187-189] of EBDC fungicides. Crops sprayed with EBDC compounds have contained ETU in subsequent field studies [190-192]. Because ETU has induced cancer in mice and rats [4,193] and is a metabolite of EBDC fungicides, all EBDC fungicides should be handled as suspected carcinogens in the workplace. These include the calcium, diammonium, disodium (nabam), magnesium (maneb), potassium ammonium, and zinc (zineb) salts and all coordination products of these salts.

(iv) 2-(p-tert-Butylphenoxy)-isopropyl-2-chloroethyl Sulfite

The miticide 2-(p-tert-butylphenoxy)-isopropyl-2-chloroethyl sulfite was tested in mice and used as a positive control in a large screening study for NCI [4]. Groups of 18 male and 18 female B6C3F1 and B6AKF1 mice were administered 464 mg/kg 2-(p-tert-butylphenoxy)-isopropyl-2-chloroethyl sulfite by oral intubation from 7 to 28 days, followed by a diet of 1,112 ppm for 78 or 81 weeks. The number of hepatomas and the total number of mice observed with tumors were significant at levels of P=0.05 and P=0.01, respectively. Seven of the 16 B6C3F1 male survivors had tumors compared with 22/79 in male controls; 8/17 females of the same strain developed tumors as compared with 8/87 in female controls. Five out of 7 tumors in B6C3F1 males were

hepatomas, and 8/23 were hepatomas in male controls. One out of 8 tumcrs in female B6C3F1 mice was a hepatoma; no hepatomas were found in the controls. In the B6AKF1 strain, 2/17 males had tumors (one was a hepatoma), compared with 16/90 male controls (5 hepatcmas among 17 tumors). Four of the 16 female B6AKF1 survivors had tumors other than hepatomas. In B6AKF1 female controls, one hepatoma was found among 9 tumors in 7/82 mice [4].

The miticide was also tested in rats and dogs [194]. In rats fed 400 ppm, 7/90 (8%) developed tumors: 2 liver carcinomas and 5 bile duct adenomas. None of the 193 controls produced similar lesions. Dogs fed 500 or 828-1,420 ppm for 3.5 years were studied. All 14 animals surviving 811 or more days developed cancer of the biliary system while no tumors appeared in the controls [195]. The IARC has reviewed the literature on this compound and has concluded that it is an animal carcinogen [196].

(v) Captan

The fungicide captan was studied by NCI for carcinogenicity in rats and mice [197]. Both sexes of rats received dietary doses of 2,525 or 6,050 ppm. Both sexes of mice received 8,000 or 16,000 ppm. Thyroid and adrenal gland tumors were found in female rats; however, these endocrine tumors were believed to have been spontaneous and not related to treatment. In the treated mice, incidences of polypoid carcinoma of the duodenum were statistically significant both in male mice (P=C.C33), with incidences of 0/68 (0%) in the controls, 1/43 (2%) in the low- dcse group, and 3/46 (7%) in the high-dose

group, and in female mice (P=0.022), with incidences of 0/68 (0%) in controls, 0/49 (0%) in the low-dose group, and 3/48 (6%) in the high-dose group. When the incidences of various adenomatous polyps were combined with those of polypoid carcinoma, the figures for male mice increased substantially in significance (P=0.008) 0/68 (0%) in the controls, 3/43 (7%) in the low-dose group, and 5/46 (11%) in the high-dose group. NCI concluded that under the conditions of this bioassay, tumors in the duodenum of mice were associated with administration of captan, but there was no convincing evidence that the tumors observed in rats were related to treatment [197].

(vi) Carbon Tetrachloride

NCI tested a group of male rats which received subcutaneous injections twice weekly of 1.3 ml/kg of a 50% solution of carbon tetrachloride in corn oil. Of these, 4/12 Wistar rats, 8/13 Osborne-Mendel rats, and 12/15 Japanese rats, which had survived 70 weeks or more, developed hepatocellular carcinomas. No tumors were induced in the 12 rats of each strain in the control group. It was concluded that carbon tetrachloride can induce carcinomas of the liver in rats [198]. NCI has also used carbon tetrachloride as a positive control chemical in bioassay testing [199].

Della Porta et al, in an NCI study [200], administered 30 weekly doses of 0.0625-0.125 ml/kg carbon tetrachloride to 10 male and 10 female hamsters by intubation. Liver-cell carcinomas developed in five animals of each sex that survived 10 or more weeks after the cessation of treatment. No controls were

reported.

As a result of a review of all available data on animal bioassays, NIOSH recommended that occupational exposure should be limited to 2 ppm as a ceiling based on a 1-hour sampling time and 45 liter sample, which should materially reduce the risk of cancer from occupational exposure to carbon tetrachloride [201].

(vii) Chloramben

In an NCI study [202], Osborne-Mendel rats and B6C3F1 mice received either 10,000 or 20,000 ppm of chloramben. The pathologists determined that chloramben did not induce tumors in the rats although some abnormal symptoms appeared. The incidence of hepatocellular carcinoma in both male mice [9/69 (13%) in controls, 16/48 (33%) in the low-dose group, and 14/48 (29%) in the high-dose group, $P<0.029$] and female mice [2/67 (30%) in controls, 7/48 (15%) in the low-dose group, and 10/50 (20%) in the high-dose group, $P=0.004$] was higher than that in the controls. However, spontaneous hepatocellular carcinoma is not uncommon in this strain of mouse, particularly in males. Therefore, the pathologists concluded that the hepatocellular carcinomas seen in treated male mice were not treatment related. However, it was also the pathologists' opinion that the tumor incidence in female mice had a significant relationship to the treatment with chloramben [202].

(viii) Chlordane

In an NCI bioassay [203], groups of 50 mice of each sex received chlordane in the diet in concentrations of 29.9 or 56.2 ppm (male), and 30.1 or 63.8 ppm (female).

Hepatccellular carcinoma showed a highly significant dose-related trend in mice. Incidence rates for males were: 2/18 (11%) in controls, 16/48 (33%) in the low-dose group, and 43/49 (88%) in the high-dose group ($P<0.001$). For females, the incidence rates were: 0/19 (0%) in the control group, 3/47 (6%) in the low-dose group, and 34/39 (88%) in the high-dose group ($P<0.0001$). EPA has concluded that chlordane is carcinogenic in mice and has restricted its usage based partially on that conclusion (Federal Register 41:7552-85, February 19, 1976).

(ix) Chlorobenzilate

Chlorobenzilate was tested by Bionetics Research Labs [4] in 18 male and 18 female B6C3F1 and B6AKF1 mice for NCI. The mice were given a single dose of 215 mg/kg chlorobenzilate by stomach tube at 7 days of age. When the animals were 4 weeks old, the compound was administered at 603 ppm in the diet for 83 weeks. Total tumor incidence and that of hepatoma were both increased significantly ($P=0.01$). In B6C3F1 mice, 11/17 males developed 13 tumors, 9 of which were hepatomas, compared with 22/79 control males with tumors, 8 of which were hepatomas. In females, 2/18 developed 2 nonhepatomatous tumors compared with 8 nonhepatomatous tumors in 8/87 controls. In B6AKF1 mice, 8/17 males developed 8 tumors, 7 of which were hepatomas. Among control males, 16/90 developed 17 tumors, 5 of which were hepatomas. In treated females, 3/18 developed 3 tumors but no hepatomas; in control females 7/82 developed 9 tumors, with 1 hepatoma [4].

In an NCI bioassay [204], chlorobenzilate was fed to B6C3F1

mice and Osborne-Mendel rats. Average levels in the diet were 4,231 and 7,846 ppm for male mice and 3,200 and 5,908 ppm for female mice, during the 78 weeks of feeding. Dosed mice had significantly higher incidences of hepatocellular carcinomas than did control mice: 4/19 (21%) in control males compared with 32/48 (67%) in low-dose males (P=0.001) and with 22/45 (49%) in high-dose males, (P=0.034); 0/20 (0%) in control females compared with 11/49 (22%) in low-dose females (P=0.016) and with 13/50 (26%) in high-dose females (P=0.007). Cortical adenomas in rats were discounted after comparison with historical controls [204].

(x) Chloroform

A study conducted by NCI [199] showed that chloroform administered by gavage produced liver tumors in B6C3F1 mice and kidney tumors in Osborne-Mendel rats. Groups of 50 male and 50 female mice (35 days old) were given two dose levels of chloroform in corn oil five times/week for 78 weeks and sacrificed after 92 to 93 weeks. The average dose levels were 138 and 277 mg/kg for males and 238 and 477 mg/kg for females. Except for a reduced survival in females given the higher dose, the survival was comparable for test and control groups. At the end of the experiment, 44/45 (98%) of the males and 39/41 (95%) of the females given the high dose had hepatocellular carcinomas; 18/50 (36%) of the males and 36/45 (80%) of the females at the lower dose developed liver carcinomas. For male controls, the liver tumor incidence was 1/18 (6%) while no tumors occurred in 20 female controls. The incidence of tumors in test animals was significantly different (P<0.001) from that in controls [199].

In this same study, groups of rats were started on test at 52 days of age. Two dose levels of chloroform were given by gavage for 78 weeks, and the animals were sacrificed 111 weeks after the start of the experiment. The dose levels for males were 90 and 180 mg/kg, and females were given average levels of 100 and 200 mg/kg. The kidney epithelial tumor incidence in male rats was significantly increased (P=0.0016) over controls. No tumors were observed in 99 controls; 4/50 (8%) and 12/50 (24%) were noted in low- and high-dose level test groups, respectively. A decreased survival rate was noted in all test rats [198]. NIOSH recommended that occupational exposure to chloroform should be limited to 2 ppm as a ceiling based on a 1-hour sampling time and 45 liter sample, which should materially reduce the risk of cancer from occupational exposure to chloroform [205].

(xi) DBCP

In 1972, the NCI undertook studies of the possible carcinogenicity of the fumigant DBCP [206]. The final report [206] revealed that male B6C3F1 mice received 160 or 80 mg/kg/day of DBCP by gavage for 11 weeks, 200 or 100 mg/kg/day for 14 weeks thereafter, and 260 or 130 mg/kg/day for an additional 22 or 33 weeks, respectively. The time-weighted average daily doses were 219 mg/kg for the high-dose group and 113 mg/kg for the low-dose group. The female mice of the same strain received 120 or 60 mg/kg/day during the first 11 weeks and thereafter received the same doses as the males. The time-weighted average doses for the females were 209 mg/kg and 109 mg/kg. Osborne-Mendel rats of both sexes were given

identical doses: 12 or 24 mg/kg for the first 9 weeks, 15 mg/kg for 69 weeks (males) or 64 weeks (females) thereafter for the low-dose rats, followed by a 5-week observation period for the low-dose group males, and 30 mg/kg for 55 weeks for the high-dose group. For both male and female rats, the time-weighted average daily doses, 5 days/ week, were 15 mg/kg for the low-dose group and 29 mg/kg for the high-dose one.

The incidences of squamous cell carcinoma of the stomach in male mice were 43/46 (93%) in the low-dose group and 47/49 (96%) in the high-dose group compared with 0/20 in controls ($P<0.001$). In female mice, squamous cell carcinomas of the stomach occurred in 0/20 (0%) controls, 50/50 (100%) low-dose animals, and 47/48 (98%) high-dose animals ($P<0.001$).

The incidences of stomach cancer in male rats were 0/20 (0%) in the control group, 47/50 (94%) in the low-dose group, and 47/50 (94%) in the high-dose group ($P<0.001$). In female rats, the incidences were 0/20 (0%) in the control group, 38/50 (76%) in the low-dose group, and 29/49 (59%) in the high-dose group ($P<0.001$). Adenocarcinoma of the breast appeared in 2/20 (10%) controls, 24/50 (48%) low-dose females, and 31/50 (62%) high-dose female rats ($P<0.001$).

Based on this study, OSHA has determined that DBCP poses a carcinogenic risk to workers. OSHA has promulgated a permanent standard for occupational exposure to DBCP that sets permissible exposure limits of 1 ppb as an 8-hour time-weighted average and 10 ppb as a ceiling (Federal Register 43:11514, March 17, 1978).

(xii) DDT, o,p'-DDD, and p,p'-DDD

After a thorough review of the available world literature, NIOSH has determined that DDT should be handled in the workplace as a suspected occupational carcinogen [31]. The DDT metabolites o,p'-DDD and p,p'-DDD have also caused cancer in mice [31]. In 1974, IARC also reviewed available literature and found that DDT caused liver cancer in mice [207]. Similarly, EPA concluded in 1975 that tumors were produced in mice experimentally exposed to DDT, and EPA restricted the usage of DDT based partially on that conclusion [208].

(xiii) EDB

The fumigant ethylene dibromide (EDB) was assayed for carcinogenicity in a study for NCI and preliminary results were published [209]. The final bioassay report is scheduled for publication in 1978. According to the preliminary results reported by Powers et al [209], EDB was given to rats at 80 or 40 mg/kg and to mice at 120 or 60 mg/kg by daily intubation for 54 or 62 weeks, except when toxicity forced the total discontinuation of administration or reduction of the maximum tolerated dose to that of one-half the maximum tolerated dose during the experiment. The tumors were squamous-cell carcinomas which originated in the forestomach, invaded locally, and metastasized throughout the abdominal cavity. In rats, an average of 83% of the males developed tumors vs 70% of the females, and tumor incidence was greater at the lower dose than at the higher dose at the termination of the experiment after 54 weeks (98 vs 68% in males and 82 vs 58% in females,

respectively). The control populations did not develop squamous-cell carcinomas of the stomach. The fraction of mice that developed squamous-cell carcinomas was 74% in males and 72% in females by the termination of the experiment at 90 weeks. The data from this single study indicate that EDB is a carcinogen after daily introduction of about one-half the maximum tolerated dose into the stomach of rats and mice for up to 62 weeks [209]. NIOSH has recommended a standard for occupational exposure to ethylene dibromide that would limit exposure to 1.0 mg/cu m as a ceiling based on a 15 minute sampling period [210].

(xiv) Heptachlor

Groups of 50 mice were administered heptachlor in feed. Time-weighted doses averaged 6.1 and 13.8 ppm for male mice and 9 and 18 ppm for female mice. Hepatocellular carcinoma showed a highly significant dose-related trend in males: in the control group 5/19 (26%), 11/46 (24%) in the low-dose group, and 34/47 (92%) in the high-dose group, ($P<0.001$) and in females: 2/10 (20%) in the control group, 3/47 (6%) in the low-dose group, and 30/42 (71%) in the high-dose group, ($P<0.0001$) [211]. Heptachlor was not significantly carcinogenic in rats. EPA has concluded that heptachlor is carcinogenic in mice (Federal Register 41:7552-85, February 19, 1976) and has restricted its use partially based on that conclusion.

(xv) Kepone

In an NCI bioassay study [85], Kepone was fed at average concentrations of 8 and 24 ppm to male rats,

18 and 26 ppm to female rats, 20 and 23 ppm to male mice, and 20 and 40 ppm to female mice. Clinical signs of toxicity were observed in both species, including generalized tremors and dermatologic changes. A significant increase ($P<.05$) was found in the incidence of hepatocellular carcinomas in high-dose level rats and in mice at both dose levels of Kepone. The incidences in the high-dose groups were 3/44 (7%) and 10/45 (22%) for male and female rats, compared with 0% in 105 and 100 controls, respectively, for both sexes; and 43/49 (88%) and 23/49 (47%) for male and female mice, compared with 8/49 (16%) and 0/40 (0%) for male and female controls. For the low-dose mice the incidences were 39/48 (81%) for males and 26/50 (52%) for females. Also, the length of time until detection of the first hepatocellular carcinoma observed at death was shorter for treated than control mice and appeared inversely related to the dose for both sexes and species [85]. NIOSH has recommended that the workplace environmental level for Kepone should be limited to 1 mg/cu m as a time-weighted average concentration [212].

(xvi) Mirex

In the screening test performed by Bionetics Research Labs and reported in 1968 [4], mirex was administered both orally and subcutaneously in two groups of 18 male and 18 female B6C3F1 and B6AKF1 mice. In the first study, 10 mg/kg mirex was fed to the animals by oral intubation from day 7 to day 28, after which the compound was administered in the diet at 26 ppm. Feeding was continued for 59 weeks in the male mice of both strains and for 70 and 69 weeks in female B6C3F1 and

B6AKF1 mice, respectively. The number of mice that developed tumors was significant (P=0.01), and the occurrence of hepatomas was also significant (P=0.01) among the tumor types observed. Incidence in male B6C3F1 mice was 7/18 (39%; 6 were hepatomas) compared with 23 tumors in 22/79 (28%) controls (8 were hepatomas). In female mice of the same strain, incidence was 8/16 (50%; all hepatomas) compared with 8/87 (9%; no hepatomas) in controls. Six tumors, 5 of which were hepatomas, were observed in 5/15 (33%) male B6AKF1 mice, compared with 17 tumors (including 5 hepatomas) in 16/90 (18%) male controls. Tumor incidence in female B6AKF1 mice was 10/16 (62%; all were hepatomas). In corresponding controls, 9 tumors, one of which was a hepatoma, were found in 7/82 mice (9%) [4].

In the second test, the same number of male and female B6C3F1 and B6AKF1 mice received a subcutaneous injection of 1,000 mg/kg mirex on the 28th day; the experiment was terminated at the same time as the first study. The results showed that the total number of mice that developed tumors was statistically significant at P=0.01, and of the total tumors observed, reticulum cell sarcomas and hepatomas occurred at a significant level (P=0.01). In B6C3F1 male mice, 9 tumors were found in 8/18 (44%) mice (including 6 reticulum cell sarcomas and 2 hepatomas), compared with 31 tumors in 27/141 (19%) male controls (including 8 reticulum cell sarcomas and 9 hepatomas). Incidence in female B6C3F1 treated mice was 0/17 (0%) compared with 9/154 (6%; 1 reticulum cell sarcoma) in untreated females of the same strain. Seven tumors (including 1 reticulum cell sarcoma and 4 hepatomas)

were found in 6/17 (35%) treated B6AKF1 males; the incidence in controls was 8/161 (5%; including 1 hepatoma). In B6AKF1 female mice, 3 reticulum cell sarcomas and 1 hepatoma were among the 5/18 (28%) tumors observed. In female controls, 5 reticulum cell sarcomas were found among 18 tumors in 17/157 (11%) mice [4].

The hepatocarcinogenicity of mirex has been shown in rats at 50 and 100 ppm in a chronic feeding experiment reported by Ulland et al [5]. Liver lesions including neoplastic nodules and hepatocellular carcinomas were observed. In the 50 ppm group, 1/26 (4%) males and 0/26 (0%) females developed hepatocellular carcinomas. The incidences for the 100 ppm groups were 4/26 (15%) in males and 1/26 (4%) in females. While the incidence of hepatocellular carcinomas is not significantly increased compared with that of control animals, when neoplastic nodules are included, the incidence in high-dose males (7/26, 27%) is significantly different ($P<0.05$) from that in controls (0/20). Neither hepatic nodules nor hepatocellular carcinomas were observed in control rats [5].

(xvii) Nitrofen

The herbicide nitrofen was bioassayed for NCI [213] at time-weighted average doses of 2,300 and 3,656 ppm for male rats, 1,300 and 2,600 ppm for female rats, and 2,348 and 4,696 ppm for both male and female mice. The incidence of pancreatic carcinomas had a statistically significant ($P<0.001$) positive association with the concentration of nitrofen in the diet of female rats, 0/110 in the control group, 2/50 (4%) in the low-dose group, and 7/50 (14%) in the high-dose group. By week

45, 50% of high-dose males were dead. This prevented the evaluation of the carcinogenicity of nitrofen in male rats. In mice of both sexes, the incidence of hepatocellular carcinoma at both high- and low-dose levels was highly significant ($P<0.001$) when compared with the controls: 9/74 (12%) controls, 36/49 (73%) low dose, and 46/48 (96%) high dose, for males and 0/80 (0%) controls, 36/41 (88%) low dose, and 43/44 (98%) high dose, for females. The incidence of hemangiosarcoma had a statistically significant relationship with nitrofen concentration in the diet of high-dose male mice when compared with controls ($P=0.022$). Incidences of hemangiosarcoma in male mice were 0/74 (0%) controls, 1/44 (2%) low dose, and 4/48 (8%) high dose.

(xviii) 2-Nitropropane

Groups of Sprague-Dawley rats and New Zealand White rabbits were exposed to commercial grade 2-nitropropane in an inhalation study performed for NIOSH [214]. Fifty male rats and 15 male rabbits were exposed to 207 ppm 2-nitropropane for 7 hours/day, 5 days/week. A second group of identical composition was exposed to 27 ppm on the same schedule, and a third untreated group served as controls. Ten rats from each group were killed after exposure periods of 2 days, 10 days, 1 month, 3 months, and 6 months. Liver neoplasms, identified as hepatocellular carcinomas or hepatic adenomas, were observed in all 10 rats exposed to 207 ppm 2-nitropropane for 6 months. No tumors were observed in any other test or control rats or rabbits. However, in rats exposed to 207 ppm for 3 months,

hepatocellular hypertrophy, hyperplasia, and necrosis were observed. Rats exposed to 207 ppm 2-nitropropane for 1,3, and 6 months also showed increased liver weights [214].

In another inhalation study, five species of laboratory animals were exposed to acute and chronic levels of 2-nitropropane. Two animals of each species received treatment at the various exposure levels, which ranged from 9,000 ppm for 1 hour to 83 ppm for 26 weeks. Initial results showed nc histologic changes in monkeys, rabbits, guinea pigs, and rats exposed to 328 ppm, or less, for any period of time. Severe liver damage and slight to moderate heart and kidney damage were observed in two cats that died within 17 days of exposure to 328 ppm 2-nitropropane. A subsequent examination showed clear cell foci in two rats that had been exposed to concentrations of 300 ppm for 119 hours. These types of lesions were similar to those found in the above study and have been frequently observed in rats exposed to known hepatic carcinogens prior to the development of hepatocellular carcinomas [214].

While one animal study has indicated carcinogenicity of 2-nitropropane in rats, a complete evaluation has not been made. In light of this study, however, NIOSH believes that it would be prudent to handle 2-nitropropane as a suspected occupational carcinogen. NCI is currently bioassaying 2-nitropropane.

(xix) 1,1,2,2-Tetrachloroethane

An NCI bioassay of 1,1,2,2-tetrachloroethane [215] indicated that this compound is carcinogenic in mice. Groups of 50 male and 50 female B6C3F1

mice (35 days old) were given two dose levels of 1,1,2,2-tetrachloroethane in corn oil by gavage five times a week for 78 weeks and sacrificed after 90 weeks. The average levels were 142 and 282 mg/kg/day for both sexes. In males, hepatocellular carcinomas occurred in 3/36 (8%) in the control group, 13/50 (26%) in the low-dose group, and 44/49 (90%) in high-dose mice ($P<0.001$). In females, the same tumor was found in 1/40 (3%) in the control group, 30/48 (63%) in the low-dose group, and 43/47 (91%) in high-dose mice ($P<0.001$). While no statistically significant tumor rates occurred in rats and no conclusive evidence for carcinogenicity in rats was presented, 2 hepatocellular carcinomas and 1 neoplastic nodule (both rare in male rats) were found in 49 male rats, while none were found in control rats [215].

(xx) Tetrachloroethylene

A bioassay study by NCI [216] indicated that tetrachloroethylene is carcinogenic in mice. Male and female B6C3F1 mice in groups of 50 were administered tetrachloroethylene in corn oil by gavage 5 days/week for 78 weeks followed by observation for 12 additional weeks before sacrifice. Male mice received 536 or 1,072 mg/kg/day and females received 386 or 772 mg/kg/day. Hepatocellular carcinomas occurred in a significant number of both sexes. In males, the tumors were found in 2/20 (10%) control, 32/49 (65%) low-dose, and 27/49 (56%) high-dose mice ($P<0.001$). In females, the incidences were 0/20 (0%) control, 19/48 (40%) low-dose, and 19/48 (40%) high-dose mice ($P<0.001$). Rats dosed with 471-949

mg/kg/day experienced a high rate of early mortality, and consequently, tetrachloroethylene carcinogenicity could not be assessed [216].

(xxi) Tetrachlorvinphos

Using B6C3F1 mice and Osborne-Mendel rats in groups of 50 animals of each sex [217], tetrachlorvinphos was bioassayed by NCI for carcinogenicity. Mice were fed 8,000 or 16,000 ppm for 80 weeks and sacrificed after 92 weeks. Male mice developed a significant incidence of hepatocellular carciroma: 0/9 (0%) control, 36/50 (72%) low dose, and 40/50 (80%) high dose ($P<0.001$). Female mice experienced an increased incidence of neoplastic nodules: 1/48 (2%) control, 14/49 (29%) low dose ($P<0.001$), and 9/47 (19%) high dose ($P=0.007$). Rats received 4,250 or 8,500 ppm for 80 weeks with sacrifice after 111 weeks. Female rats developed C-cell adenoma of the thyroid in 1/46 (2%) in the control group, 2/50 (4%) in the low-dose group, and 7/46 (15%) in high-dose rats ($P=0.013$). Cortical adenoma of the adrenal was also significant in female rats: 0/50 (0%) in the control group, 2/49 (4%) in the low-dose group, and 5/50 (10%) in the high-dose group ($P=0.017$) [217].

(xxii) Trichloroethylene

Trichloroethylene was administered to rats and mice of both sexes by intubation 5 days/week at two dose levels for 78 weeks. Mice were necropsied after 90 weeks and rats after 110 weeks. Male mice receiving an average of 2,339 mg/kg/day had a 64% incidence of hepatocellular carcinomas ($P<0.001$) while those receiving an average of 1,169 mg/kg/day had

a 52% incidence (P=).004). In control males, 5% developed those tumors. Female mice receiving 1,739 mg/kg/day had a 23% incidence rate of hepatocellular carcinomas (P=0.008) while those at 869 mg/kg/day did not develop a significantly greater amount of tumors than controls. Rats did not develop any tumors at a significantly increased rate [218].

As a result of animal testing and metabolic similarity to such carcinogens as vinyl chloride, NIOSH has concluded that trichloroethylene has a carcinogenic potential in the workplace although not a particularly strong one [219]. NIOSH recommends that a level of 25 ppm can be uniformly achieved in industry by use of existing control technology and should be met. Worker exposure should continue to be reduced beyond this level as methodology develops.

(xxiii) Trifluralin

Trifluralin was assayed in another NCI study [220]. The average high and low dietary concentrations of the herbicide were 4,125 and 8,000 ppm for male rats, 4,125 and 7,917 ppm for female rats, 2,000 and 3,744 ppm for male mice, and 2,740 and 5,192 ppm for female mice. For female mice, the correlation between increased dosage and elevated incidence of hepatocellular carcinomas was significant (P<0.001), with incidences of 0/60 (0%) in controls, 12/47 (26%) in low-dose mice, and 21/44 (48%) in high-dose mice. Also significant was the relationship between dose and incidence of alveolar/bronchiolar adenomas in female mice, 0/59 (0%) in the controls, 6/43 (14%) in the low-dose group, and 3/30 (10%) in the high-dose group

(P=).036). Squamous-cell carcinomas of the stomach were observed in dosed female mice, but not in controls. Although incidences of these tumors were not statistically significant [0/60 (0%) in controls, 4/45 (9%) in the low- dose group, and 1/44 (2%) in the high-dose group] they are unusual lesions in B6C3F1 mice and were considered to be treatment related [220]. Significant evidence of carcinogenicity was not indicated in male mice and male and female rats.

(B) Pesticides for Which Available Test Data Are Inconclusive

The 27 pesticides discussed in this section have not yielded conclusive evidence to implicate them as cancer-suspect agents because of poor experimental design, lack of statistical analysis of the data, or conflicting data among separate studies. Also discussed in this section are certain compounds tested in the Bionetics Research Labs screening study performed for NCI and reported in 1968 [4]. Positive results reported for the compounds tested in that study but not supported by other confirming evidence are included below. Many of these compounds are now being bioassayed by NCI. Their test results are summarized in Table XIV-9. Additional well-designed experiments are recommended to develop the quality data necessary for their classification.

(i) Azobenzene

Groups of 18 male and 18 female B6C3F1 and B6AKF1 mice were administered 21.5 mg/kg azobenzene in 0.5% gelatin by stomach tube from days 7 to 28, after which the animals were fed 56 mg/kg azobenzene in the daily diet for 80 weeks. As reported by Bionetics Research Labs in 1968, incidence of hepatomas in treated male B6C3F1 mice was 8 tumors/18 mice compared with 8 tumors/79 controls, and in male B6AKF1 mice, the rates were 2/18 compared with 5/90 in controls (P=0.01). The incidence of hepatomas in female mice was similar to that in the controls [4].

(ii) Calcium Cyanamide

Calcium cyanamide was tested for NCI in 18 B6C3F1 and 18 B6AKF1 male and female mice and the results reported in 1968 [4]. The compound was administered at 100 mg/kg by oral intubation from days 7 to 28, after which it was mixed with the ground feed at 240 ppm until the mice were necropsied during week 82. Results showed that the occurrence of reticulum cell sarcomas was significant (P=0.01). Tumor incidence was 5 sarcomas/16 males and 3 sarcomas/18 females in B6C3F1 mice, compared with 5/79 and 4/87 for controls. In B6AKF1 mice, 2 hepatomas developed in 17 female mice and none were found in the 18 males necropsied, compared with 1/90 and 3/82 for male and female controls, respectively [4]. An NCI bioassay report is scheduled for release in 1978.

(iii) (2-Chloroethyl)triethyl-

ammonium Chloride

In a study for NCI reported in 1968 [4], (2-chloroethyl)triethylammonium chloride (CCC) was tested on B6C3F1 and B6AKF1 mice. Eighteen male and female mice of each strain received 21.5 mg/kg CCC by oral intubation on days 7-28, after which the compound was mixed with the ground feed at 65 ppm for 82 weeks. The number of hepatomas found at necropsy was significant at a P=0.01 level. In B6C3F1 mice, 5 hepatomas occurred in 18 males and 0 hepatomas in 18 females, compared with 8 hepatomas/79 and 0/87 in controls, respectively. Five hepatomas were found in 18 B6AKF1 male mice and 9 in 15 female mice that survived, compared with 5 hepatomas/90 and 1/82, respectively, in the control group [4].

(iv) Chloropicrin

Chloropicrin was tested by NCI in a bioassay study [221] using Osborne-Mendel rats and B6C3F1 mice. The compound was administered by gavage in corn oil 5 days/week during dosing periods. Because of early high mortality, rats were only dosed periodically during the study. Average doses for male rats were 25 or 26 mg/kg/day, and for female rats, they were 20 or 22 mg/kg/day. Mice were dosed at 33 or 66 mg/kg/day, 5 days/week for 78 weeks without interruption. All dosing of animals stopped at 78 weeks. Rats were observed for 32 more weeks and mice for 13 more weeks. The short survival time for rats did not permit an assessment of carcinogenicity for them.

While the mice did not have any statistically significant tumor incidences, two carcinomas and a papilloma did occur, which happened only rarely in historical controls. In summary, short survival of the rats prevented any carcinogenic effect in this species, and the mice did not demonstrate any significant tumor incidences [221].

(v) 2,4-D

In one carcinogenicity test, groups of male and female Osborne-Mendel rats were fed 2,4-D at rates of 0-1,250 mg/kg by Hansen et al [222]. The only statistically significant ($P<0.05$) increase in tumors (of no particular organ) occurred in high-dose males. The authors stated, "No target organ tumors were observed: the individual tumor types were randomly and widely distributed and of the type normally found in aging Osborne-Mendel rats....These tendencies do not reflect important pathologic differences."

In a study [4] for NCI, 2,4-D was tested in B6C3F1 and B6AKF1 mice. Only the isooctyl ester formulation significantly induced tumors. Eighteen male and 18 female mice of both strains received a subcutaneous injection of 2,4-D isooctyl ester at 21.5 mg/kg when 7 days of age. The observations continued through week 84 in the B6C3F1 strain and through weeks 86 and 81 in male B6AKF1 and female B6AKF1 mice, respectively. Results showed the total number of mice which developed tumors was significant ($P=0.01$), and the significant tumor type which occurred was reticulum cell sarcoma ($P=0.01$). In B6C3F1 males, tumor

incidence was 6/18 compared with 31 in 27/141 controls (3/18 reticulum cell sarcomas in treated vs 8/141 in untreated mice). Incidence was 2/18 in female B6C3F1 mice compared with 9/154 in control mice. Values for reticulum cell sarcomas were 0/18 in test and 1/154 in control mice of the same strain. In the B6AKF1 strain, the total number of mice which developed tumors was 2/18 in treated males and 8/161 in control males. The incidence of reticulum cell sarcomas was 0/18 and 0/161 in treated and untreated males, respectively. In B6AKF1 females, 6 tumors were found in 5/17 mice, 5 of which were reticulum cell sarcomas. In the B6AKF1 female controls, 18 tumors were found in 17/157 mice, 5 of which were reticulum cell sarcomas [4]. However, in another rat feeding study [223] and in a mouse feeding study [4] for NCI, significant tumor incidences were not observed when using 2,4-D and its isopropyl and butyl esters.

(vi) Dimethoate

Dimethoate, an OP insecticide, was given to rats orally at dose levels of 5-30 mg/kg and intramuscularly at 15 mg/kg. Malignancies developed in 9/71 orally treated rats: 2 malignant reticuloses, 4 spleen sarcomas, 1 colon sarcoma, and 2 liver carcinomas. In rats dosed intramuscularly, 6/30 rats developed cancer: 2 spleen sarcomas, 1 ovarian sarcoma, 1 unspecified sarcoma, 1 malignant reticulosis, and 1 liver carcinoma. Controls (36 oral, 35 intramuscular) developed no tumors. Mice topically administered dimethoate developed 4 leukoses and 1 mammary tumor (5/19

animals), but no controls were reported [224]. No probability statistics were presented. An NCI feeding study [225] tested dimethoate in rats and mice. Rats received average doses of 155 or 310 ppm (males) and 192 or 384 ppm (females). Mice were fed at rates of 250 or 500 ppm (both sexes). In spite of signs of typical dimethoate toxicity (tremors and hyperexcitability), low-dose rats and all treated mice survived long enough to allow an evaluation of carcinogenicity. No statistically significant increase in tumor incidence occurred in either sex or species. It was concluded that no carcinogenic effect resulted from dimethoate administration [225].

(vii) Dimethoxane

An experiment by Hoch-Ligeti et al [226] with dimethoxane administered as a 1% solution in the drinking water, a very large dose, resulted in the induction of malignant tumors, mostly hepatomas, in 14 of 25 male rats. One of 14 control animals developed cancer in the liver, kidney, spleen, and lungs. The small number of animals, the lack of experimental details, and the lack of statistical analysis for this single study do not provide a sufficient basis for a final conclusion.

(viii) 2,4-Dinitrotoluene

NCI [227] bioassayed 2,4-dinitrotoluene using groups of 50 Fischer 344 rats or 50 B6C3F1 mice of each sex. Dietary levels were 0.008 and 0.04% for mice and 0.008 and 0.02% for rats. Dosing occurred for 78 weeks followed by 13 additional weeks' observation for mice or 26 additional weeks'

observation for rats. While no malignant tumors developed at significant rates in rats, benign skin or subcutaneous fibromas did develop in 0/46 (0%) control, 7/49 (14%) low-dose, and 13/49 (27%) high-dose male rats (P=0.003), and benign mammary fibroadenomas occurred in 9/48 (19%) control, 12/49 (24%) low-dose, and 23/50 (46%) high-dose female rats (P=0.016). No tumors developed at significant rates in mice [227].

(ix) Diphenylacetonitrile

Eighteen male and female B6C3F1 and B6AKF1 mice were administered a single subcutaneous injection of diphenylacetonitrile at 464 mg/kg in a test performed for NCI by Bionetics Research Labs and reported in 1968 [4]. The mice were injected on day 28, and survivors were killed in week 77 of the study. The total number of tumors was significant (P=0.05), and of the total tumor types, reticulum cell sarcomas were significant at a level of P=0.01. Other tumor types were reported to be insignificant in occurrence. In B6C3F1 mice, 5/18 males developed tumors, 3 of which were sarcomas. Two reticulum cell sarcomas were present in the 3/16 female B6C3F1 mice which develcped tumors. Results for the B6C3F1 control group showed that 27/141 males developed 31 tumors, 8 of which were sarcomas, and that 9/154 females developed tumors, 1 of which was a sarcoma. In the B6AKF1 strain, 3/18 males developed tumors (1 was a sarcoma) compared with 8/161 control males (no sarcomas). Three out of 17 B6AKF1 female mice showed 4 tumors, 2 of which were reticulum cell sarcomas compared with 17/157 female control mice which developed 18 tumors, 5 of which were reticulum cell sarcomas [4].

(x) Endosulfan

Endosulfan was tested for NCI in 18 B6C3F1 and 18 B6AKF1 male and female mice by Bionetics Research Labs [4]. The two strains were administered 1.) mg/kg endosulfan by oral intubation on days 7-28, after which the compound was added to the ground feed at 3 ppm until the end of the study during week 77. Necropsies showed that the total number of tumors was significant (P=).)5). Tumors developed in 5/14 male and 3/10 female B6C3F1 mice, compared with 23 tumors in 22/79 male and 8 tumors in 87 female control mice. In strain B6AKF1, 6 tumors developed in 5/16 males and 3 in 16 females, compared with 17 tumors in 16/90 males and 9 tumors in 7/82 females in the control group. Pulmonary adenomas were also significant at P=0.05. Tumor occurrence of this type in B6C3F1 mice was 2 tumors/14 males and 1 tumor/10 females, compared with 5/79 males and 3/87 females in the control group. In the B6AKF1 strain, 4 pulmonary adenomas were found in 16 male mice and 1 in 16 female mice; values for B6AKF1 controls were 9 tumors/90 males and 3/82 females [4].

In a recent NCI bioassay [228], endosulfan was fed to rats at 223-952 ppm and to mice at 2.0-6.9 ppm. High early mortality occurred for male rats and mice, and no conclusions about carcinogenicity could be made for them. Endosulfan was not found to be carcinogenic for female rats and mice.

(xi) Endrin

Treon et al [229] tested endrin in 6 groups of 20 male and 20 female Carworth rats. A diet of), 1,

5, 25, 50, or 100 ppm endrin was administered for 2 years. Survivors at 80 weeks included 12/80 rats given 50 or 100 ppm, 23/40 given 25 ppm, 28/40 given 5 ppm, 31/40 given 1 ppm, and 28/40 in the control group. The incidence of neoplasms in treated rats was no greater than that among controls.

The tumorigenicity of endrin was tested in Osborne-Mendel rats by Deichmann et al [230]. Diets containing 2, 6, or 12 ppm endrin (technical grade 98%) were administered to 50 male and 50 female rats for lifespan. The mean survival time in endrin-treated rats ranged between 17.6 months in males given 12 ppm to 20.8 months in females given 2 ppm. The mean survival time in a control group was 19.7 months for male rats and 19.5 months for female rats. The proportion of tumor-bearing rats and the incidence of mammary tumors, lymphomas, and other tumors were similar in treated and control rats. No liver-cell tumors were reported. Endrin is being tested by NCI and results are scheduled for release in 1978.

(xii) Ethylan

As reported in the 1968 Bionetics study [4] for NCI, 18 male and 18 female B6C3F1 and B6AKF1 mice received 215 mg/kg ethylan by oral intubation. The mice were administered the compound in this manner from days 7 to 28, after which 815 ppm ethylan was mixed with the ground feed for the duration of the experiment. The number of hepatomas found at necropsy (during week 84 for both B6C3F1 sexes, during week 86 for B6AKF1 male, and during week 87 for B6AKF1 female mice) was significant at a level of P=0.01. Seven hepatomas were found in

16 treated B6C3F1 male mice, and 1 hepatoma was found in 17 treated B6C3F1 female mice, compared with 8 hepatomas/79 male and 0/87 female control animals of the same strain, respectively. In treated B6AKF1 mice, 1 hepatoma was found in both groups of 18 male and female mice necropsied, compared with 5/90 male and 1/82 female control mice [4].

(xiii) Ethylene Oxide

Eighty-six inbred female Swiss-Webster mice were exposed inadvertently to ethylene-oxide-treated ground corncob bedding for 150 days in an experiment not designed to study the effects of ethylene oxide. The mice lived in untreated bedding for their lifetime (a maximum of 900 days) following exposure. Tumors developed at various sites in 63 exposed mice; no tumors were reported in 83 female mice 100-600 days old, which were not exposed to treated bedding [231]. This "study" was not designed for the purpose reported here and few conclusions can be drawn from it.

No skin tumors were observed on 30 female 8-week-old ICR/Ha Swiss mice which had been painted with 0.1 ml of a 10% solution of ethylene oxide in acetone three times/week for life. The average length of survival was 493 days [232].

Negative results were also obtained in a study by Walpole [233]. Twelve rats were subcutaneously injected with 1 g/kg ethylene oxide in arachis oil for 94 days on a unspecified schedule. No sarcomas were observed throughout the animals' lifetimes.

Ethylene oxide has produced genetic defects in a variety of

test systems (see Table XIV-10). The most likely mechanism for the production of these defects (mutations) is the alkylation of cellular constituents, including deoxyribonucleic acid (DNA). Such reactions have been observed with both proteins and nucleic acids, and the resulting molecules could very likely possess abnormal functional properties in the living organism [234].

While limited animal tests have not clearly indicated carcinogenicity, the alkylating and mutagenic properties of ethylene oxide are sufficient bases for concern, and NIOSH has recommended that workers should not be exposed at concentrations greater than a 135 mg/cu m (75 ppm) ceiling determined during a 15 minute sampling period and a 90 mg/cu m (50 ppm) TWA for up to a 10-hour day or 40-hour workweek.

(xiv) HCCH (Technical BHC)

HCCH typically contains the following isomers of hexachlorocyclohexane: 55-70% alpha, 6-8% beta, 10-18% gamma, 3-4% delta, and a small amount of epsilon. Nagasaki and coworkers [235,236] found that HCCH produced hepatomas in 20/20 male mice fed 600 ppm but none at lower levels. No tumors developed in control mice. However, Goto et al [237] found liver nodules but no definite cancers in male mice receiving 600 ppm HCCH in their diet for 26 weeks. Similarly, Fitzhugh et al [238] did not find an excess of tumors in rats fed 10, 50, 100, and 800 ppm HCCH. No statistical analyses of the data were given.

(xv) IPC and CIPC

Van Esch et al [239] studied the

effect of isopropyl phenylcarbamate (IPC) and isopropyl chlorophenylcarbamate (CIPC) as tumor initiators on Swiss mice in conjunction with the promoters croton oil and Tween 60. IPC and CIPC were administered as a single 15 mg dose once by intubation, as 10 weekly 15 mg doses by intubation, or in the diet at 0.1% for 6 months. Five percent croton oil was applied dermally two times/week, and Tween 60 was applied six times/week, both for 6 months. IPC applied 10 times with croton oil produced significantly more tumors (papillomas) than croton oil by itself ($P<0.05$). No other IPC combinations produced results significantly different from controls. A single application of CIPC with croton oil resulted in a significant increase in the papilloma incidence in females ($P<0.05$). No other CIPC combinations were significant. The authors stated that IPC and CIPC have weak initiating activity; however, while indicating cancer initiation, this study does not show that the substances were carcinogenic, and further testing is recommended by NIOSH.

(xvi) Lindane (gamma-HCCH)

Carcinogenic studies of lindane have shown both negative and positive results. It was determined not to be carcinogenic in the NCI bioassay study in rats and mice [240]. Negative results were also reported by Nagasaki et al [241] for male mice fed 100-500 ppm lindane, and by Fitzhugh et al [238] for male and female rats fed 5-1,600 ppm. Positive results occurred in a study by Thorpe and Walker [242] with a 96% incidence of liver tumors in male mice fed 400 ppm lindane compared with 24% in controls ($P<0.05$), and with a 95% incidence

in treated females compared with 23% in controls ($P<0.01$). A study by Goto et al [237] showed liver nodules in male mice examined after 26 weeks on a 300 ppm lindane diet.

(xvii) Mexacarbate

Mexacarbate was tested on male and female B6C3F1 and B6AKF1 mice for NCI. Mexacarbate was administered to 18 animals of each sex and strain by oral intubation at a dose of 4.64 mg/kg on days 7-28, after which the compound was mixed with the ground feed at 11 ppm. Necropsies in week 81 showed that the formation of hepatomas was significant at $P=0.05$. Tumor incidence in B6C3F1 mice was 5 hepatomas/16 in males and 0 hepatomas/17 in females compared with 8/79 and 0/87, respectively, in the controls. In B6AKF1 mice, incidence of hepatomas was 2 tumors/17 and 0/17 in male and female mice, respectively, and 5/90 and 1/82 in the control mice [4]. A bioassay report by NCI is scheduled for release in 1978.

(xviii) PCNB

Pentachloronitrobenzene (PCNB) was also tested by Bionetics Research Labs for NCI [4]. Eighteen male and 18 female B6C3F1 and B6AKF1 mice were given single doses of 464 mg/kg PCNB by oral intubation when the animals were 7 days of age. The same amount was given daily until the mice were 4 weeks old, when they were administered a diet containing 1,206 ppm PCNB. The experiment lasted 78 weeks. The number of mice observed with all types of tumors was statistically significant ($P=0.01$), and hepatomas occurred at the same level of significance. Six tumors, including 2 hepatomas, developed in

5/18 (28%) B6C3F1 male mice compared with 23 tumors, including 8 hepatomas in 22/79 (28%) male controls. Tumor incidence in B6C3F1 females was 5/18 (28%) compared to 8/87 (9%) in female controls (4 were hepatomas in treated mice and no hepatomas were found in controls). Eleven B6AKF1 male mice out of 17 (65%) developed 13 tumors (10 were hepatomas); 17 tumors were found in 16/90 (18%) male controls, including 5 hepatomas. Two tumors, 1 of which was a hepatoma, were found in 2/17 (12%) female B6AKF1 mice compared with 9 tumors, 1 of which was a hepatoma, in 7/82 (9%) female controls. Incidences of other tumors were similar in treated and control animals [4].

A bioassay study [243] conducted by NCI, however, has not found indications of carcinogenicity. In this study, mice or rats were fed PCNB in the diet at levels up to 14,635 ppm. These groups of 50 animals each showed no rare or unusual tumors. No statistically significant positive associations between PCNB and incidence of neoplasms were shown for any test group [243].

(xix) Piperonyl Butoxide

The occurrence of reticulum cell sarcomas was shown to be significant at a probability level of P=0.05 in male and female B6C3F1 and B6AKF1 mice orally administered piperonyl butoxide in a test for NCI by Bionetics Research Labs [4]. Eighteen mice of each sex and strain received a 100 mg/kg dose of the compound by oral intubation on days 7-28, after which the compound was mixed with the ground feed at 300 ppm for the remainder of the study. Results from the necropsies performed in week 83 showed reticulum cell sarcoma occurrence at

a rate of 5 tumors/15 male and 2/18 female B6C3F1 mice, compared with 5/79 and 4/87 in male and female controls, respectively. In B6AKF1 mice, reticulum cell sarcomas numbered 0 in 18 males and 1 in 18 females, respectively, compared with 1/90 and 3/82 in controls [4]. A bioassay report by NCI is scheduled for release in 1978.

(xx) Piperonyl Sulfoxide

Piperonyl sulfoxide was tested by Bionetics Research Labs for NCI [4] on groups of 18 male and female B6C3F1 and B6AKF1 mice. The two strains received a 46.4 mg/kg subcutaneous injection of the compound on day 28. Reticulum cell sarcomas were shown to occur at a significant level (P=0.05) in both male and female B6C3F1 mice, necropsied during week 71 and week 72, respectively. Male and female B6AKF1 mice were killed in weeks 83 and 84 of the experiment, respectively, and showed the same extent of reticulum cell sarcoma formation. Tumor occurrence was 1 sarcoma/18 mice for both male and female B6C3F1 mice, compared with 8/141 and 1/154 in controls. In B6AKF1 mice, sarcomas were found in 1/18 and 3/18 male and female mice vs 1/161 and 5/157 for controls of the same strain. The development of reticulum cell sarcomas was also shown to be significant (P=0.01) in an oral test using the two strains of mice. Piperonyl sulfoxide was administered at 46.4 mg/kg by oral intubation on days 7-28, after which it was mixed with the ground feed at 111 ppm until the experiment was terminated during the same weeks cited above. Reticulum cell sarcomas were found at a rate of 8/18 and 1/18 in male and female

B6C3F1 mice, respectively, vs 5/79 and 4/87 in controls. In B6AKF1 mice, no tumors were found in the 18 males necropsied (1/90 for male controls), and 2 sarcomas were found in 17 female mice (3/82 for female controls) [4]. A bioassay report by NCI is scheduled for release in 1978.

(xxi) Sodium N,N-dimethyldithiocarbamate

Bionetics Research Labs [4] tested sodium N,N-dimethyldithiocarbamate (SDDC) in B6C3F1 and B6AKF1 mice. Eighteen male and female mice in each strain were administered 215 mg/kg SDDC by oral intubation on days 7 to 28, after which the compound was mixed with the ground feed at 692 ppm for the duration of the 78-week study. The total number of tumors was reported to be significant (P=0.05), and of the types of tumors found, pulmonary adenomas and hepatomas occurred at a significant level (P=0.05). In the B6C3F1 mice, 10/17 males had 11 tumors (including 3 pulmonary adenomas and 7 hepatomas). Twenty-two B6C3F1 male control mice of the 79 necropsied had 23 tumors (including 5 pulmonary adenomas and 8 hepatomas). Incidence in B6C3F1 control female mice was 8 tumors/87 mice, 3 of which were pulmonary adenomas. Six tumors occurred in 18 male B6AKF1 mice, 5 of which were pulmonary adenomas and none were hepatomas. In the male control group of this strain, 17 tumors (including 9 pulmonary adenomas and 5 hepatomas) were found in 16/90 mice. Two out of 18 female B6AKF1 mice had tumors, 1 of which was a pulmonary adenoma. In the B6AKF1 control group, 9 tumors (3 pulmonary adenomas and 1 hepatoma) were found in 7/82

female mice [4]. An NCI bioassay report is scheduled for release in 1978.

(xxii) Strobane

In the Bionetics study [4] performed for NCI, strobane was tested in male and female B6C3F1 and B6AKF1 mice. Eighteen mice of each sex and strain received 4.64 mg/kg strobane by oral intubation from day 7 to day 28, after which the compound was administered at 11 ppm in the ground feed for 80 weeks. Significant results included the total number of mice that developed tumors (P=0.01) and the incidences of hepatomas (P=0.01) and of reticulum cell sarcomas (P=0.05). Eight out of 15 (53%) male B6C3F1 mice developed tumors; 2 were hepatomas and 5 were reticulum cell sacromas. In the corresponding control group, 23 tumors were found in 22/79 (28%) mice; 8 were hepatomas and 5 were reticulum cell sarcomas. Tumor incidence in female B6C3F1 mice was 3/18 (17%) compared with 8/87 (9%) in controls; incidence of hepatomas was 0/18 (0%) and 0/87 (0%) and of reticulum cell sarcomas was 2/18 (11%) and 4/87 (5%) in female B6C3F1 treated and untreated mice, respectively. Eleven out of 18 (61%) male B6AKF1 mice developed tumors, all of which were hepatomas. In corresponding control mice, 17 tumors were found in 16/90 (84%) mice, 5 of which were hepatomas and 1 of which was a reticulum cell sarcoma. No tumors of any type were found in female B6AKF1 mice. Values for controls were 9 tumors, 1 of which was a hepatoma and 3 of which were reticulum cell sarcomas, in 7/82 (9%) female mice [4]. The IARC review of literature discussed only the Bionetics study [244].

(xxiii) 2,4,5-T

Mice were given drinking water containing 100 mg 2,4,5-T/l ad libitum for 2 months followed by 80 ppm of 2,4,5-T in the diet until death. Females showed a significant difference ($P<0.03$) in tumor rates, namely 13/25 (52%) for treated animals vs 9/44 (20%) for controls; neither males of this strain nor mice of another strain showed significant differences [245]. In the Bionetics Research Labs study [4], mice fed 2,4,5-T did not develop cancer to any significant extent; subcutaneous injection was likewise ineffective [4].

(xxiv) Thiourea

Thiourea was administered in varying concentrations in the diet to groups of 18 Osborne-Mendel rats for 104 weeks; 14 of the 29 survivors developed hepatic cell adenomas [246]. Tumor incidence was 3/5 (100 ppm), 4/8 (250 ppm), 2/8 (500 ppm), and 5/8 (1,000 ppm). Rats receiving a 2,500 ppm diet did not survive for more than 17 weeks. Untreated rats surviving 2 years showed a 1% spontaneous incidence of hepatic cell adenomas. Too few animals survived to provide adequate interpretation, and no statistical analysis was performed.

A 10% thiourea solution was administered intraperitoneally to a group of 12 rats 3 days/week for 6 months [247]. This group contained an unspecified number of each sex. On the 1st day, 3 ml were injected, 4 ml on the 2nd day, and 4 ml on the 3rd day of each week. The rats received a 0.2% solution of thiourea in the drinking water following the initial treatment. Five out of six

rats surviving 12-16 months developed tumors involving the area between the ear duct and the orbit, compared with no tumor development in the controls. Three of the tumors were diagnosed as squamous cell carcinomas, one a mixed sarcoma and squamous cell carcinoma, and one a mixed-cell sarcoma.

Of 42 newborn ICR Swiss mice administered a single subcutaneous injection of 2,500 mg/kg thiourea, no increase in the number of lung adenomas was observed compared with controls in mice killed 6 months after injection [248].

In another study [249], 0.2% thiourea was administered in the drinking water of 19 male albino rats for up to 26 months. One rat developed a myxomatous tumor of the nose and another developed epidermoid carcinomas in the area of the ear duct and the orbit. No tumors occurred in 12 control rats observed for 104 weeks. A significant increase in tumors was rot demonstrated.

While design and analysis are inadequate for some of these studies and results are contradictory, some carcinogenicity may be indicated and further test data are necessary.

(xxv) Trichlorfon

In a study by Gibel et al [224], trichlorfon, a chlorinated OP pesticide, was tested orally and intramuscularly in rats and topically (dose not given) in mice. The rats were given total doses of 15 mg/kg by mouth and injections of 15 mg/kg. These groups had 7 malignant tumors in 28 animals treated orally (including 1 lung carcinoma, 1 malignant reticulosis, 2 spleen sarcomas, 1 liver carcinoma, 1

maxillary carcinoma, and 1 forestomach cancer in situ) and 4 malignant tumors in 27 injected animals (including 1 malignant reticulosis, 1 liver sarcoma, and 2 spleen sarcomas). No control rats (36 oral, 35 im) developed cancer, but no probability statistics were presented. Five of 14 mice developed myeloid leukosis. No control mice were reported and no probability statistics were presented [224]. NCI has tentatively selected trichlorfon for bioassay testing.

(xxvi) 2,4,6-Trichlorophenol

Bionetics Research Lab [4] tested 2,4,6-trichlorophenol in B6C3F1 and B6AKF1 male and female mice for NCI. Eighteen mice of each sex and strain were administered the compound at 100 mg/kg by oral intubation on days 7-28, after which it was mixed with the ground feed at 260 ppm for the duration of the 83-week experiment. The total number of tumors was significant at a level of P=0.01. Reticulum cell sarcomas and hepatomas were determined to be significant (P=0.05 among the tumor types found. In B6C3F1 mice, 9/18 males developed 10 tumors, 4 of which were reticulum cell sarcomas and 3 of which were hepatomas. In the control group, 23 tumors (including 5 sarcomas and 8 hepatomas) were found in 22/79 male mice. Seven out of 18 female B6C3F1 mice developed 8 tumors in all; 2 were sarcomas and 2 were hepatomas. In the control group, 4 of 8 tumors found in 87 females were sarcomas and no hepatomas were reported. Total tumors in 3/17 B6AKF1 male mice numbered 4, and 1 was a hepatoma. Values for controls were 1 sarcoma and 5 hepatomas out of 17 total tumors counted in 16/90 male mice. Of

the 2 tumors found in 17 female B6AKF1 mice, 1 was a sarcoma and 1 was a hepatoma. In the control group, 3 sarcomas and 1 hepatoma were found in a total of 9 tumors in 7/82 female mice. Other tumor types not included in the totals were insignificant in occurrence [4].

(C) Pesticides for Which Test Results Were Not Positive

The administration of picloram, dichlorvos, methyl chloroform, methoxychlor, and malathion to rats and mice showed no clear evidence of association with tumor incidence in NCI bioassay tests. Male and female mice and male rats fed picloram did not develop tumors significantly associated with the pesticide. Although female rats did develop hepatic nodules, they were benign [250]. A few tumors developed in mice fed dichlorvos, but there was not sufficient evidence to indicate that the tumors resulted from dichlorvos treatment [251]. Results for methyl chloroform were considered negative due to the high mortality and the similarity of tumors in control and in treated animals [252]. Tumor incidences for mice and rats administered malathion were not significantly different from incidence in controls. No tumors were judged to be related to malathion [253]. No significant tumor rates occurred for mice or rats of either sex in a bioassay of methoxychlor [254]. Fifty-five additional pesticides tested by Bionetics Research Labs for NCI [4] did have significant effects. These are listed in Table XIV-11.

It should be noted that relatively few of the

approximately 1,500 registered pesticides have been adequately tested for carcinogenicity, and very little is known about the carcinogenic risk of many of these substances. Accordingly, there is an obvious need for testing many registered pesticides for carcinogenic potential.

(2) Mutagenesis

In recent years, there has been a heightened interest in mutagenesis and related research. This interest has occurred for principally two reasons: (1) to assess the possibility that chemical substances may be producing genetic mutations which might alter the hereditary characteristics of humans [255], and (2) to use mutagenic assays as rapid and inexpensive surrogate methods to detect and to evaluate chemicals for potential carcinogenic effects [256]. The rationale for the latter lies in the prevailing theory that cancer is the result of a somatic mutation. The result of this interest has been the development of a large variety of experimental tests for mutagenesis utilizing bacteria, Drosophila, and various mammalian species [257-261].

The ubiquity of pesticides in the general and occupational environment has increased their importance as candidates for mutagen testing; however, the testing systems are varied, complex, and not easily correlated to human response. DDT has been most extensively studied in this regard [255] and the tests have yielded a mixture of positive and negative findings. DDT and its metabolite, DDE, have been consistently negative in bacterial assays. However, DDT and DDE have caused chromosomal

breaks and gaps in a number of in vivo and in vitro experiments. The occurrence of chromosomal damage in these experiments may correlate with the positive findings of similar chromosomal damage in occupationally exposed workers [255]. In the study by Rabello et al [255], 30 workers with high plasma DDT levels (average 0.993 mg/ml) were compared with 20 controls (average 0.275 mg/ml), and chromatid aberrations were found in 12% of the examined cells from the exposed group and in 8.8% of the examined cells of controls ($P<0.05$). Aldrin and dieldrin also caused chromosomal damage in mammalian cells in vivo and in vitro, yet they consistently produced negative results in bacterial reversion assays. Other pesticides which produce mutagenic effects in various organisms are shown in Table XIV-10. The ability of these pesticides to induce mutations in a wide variety of test systems is suggestive of their potential to induce mutations in human populations, but there is no evidence available to enable an adequate assessment of the quantitative aspects of relative risks for human populations. While the exact mechanisms and mutagenic potentialities cannot be stated with certainty, alkylating pesticides are most suspect of altering human genetic material. For example, ethylene dibromide is a bifunctional alkylating agent, and the most likely mode of mutagenic activity is the covalent bonding of ethylene dibromide to DNA. It must be realized, however, that there is no evidence to indicate that pesticides or any other organic compounds actually have caused mutagenesis in humans. For this reason, NIOSH does not recommend at this time that any pesticide be

controlled because of mutagenicity demonstrated in a test system.

While mutagenicity studies may not demonstrate toxicologic effects as dramatically as acute toxicity and carcinogenicity studies, they are nevertheless important. These studies show that genetic material is affected, and it is only prudent to avoid or greatly minimize exposure to material capable of such effects.

(3) Teratogenesis

The effects from the drug thalidomide have clearly shown that there is a potential for chemical substances to produce profound teratogenic abnormalities in humans. Since that finding, many tests have been conducted to determine the teratogenicity of chemicals in laboratory animals such as mice, rats, and hamsters. However, many of these tests have results from which it is difficult to draw conclusions regarding the teratogenicity of the compound due to the small sample size and to the lack of reporting numerical values or experimental methods critical to the findings. Pesticides, as a group, have been no exception. A summary of pesticides reported in the literature and labeled by the authors as being teratogenic, having teratogenic effects, or producing terata, appear in Table XIV-12.

Of the 15 pesticides listed , 8 of them (thiram, aldrin, dieldrin, endrin, folpet, captan, captafol, and 2,4,5-T) have sufficient data to indicate that these might pose a potential problem in humans.

Based on available evidence, four pesticides (parathion, dichlorvos, diazinon, and phosmet) tested in a small number of

animals, can be considered to be possibly teratogenic and should be handled with caution by women of childbearing age.

For the remaining three pesticides tested (diquat, paraquat, and trichlorfon), there is insufficient evidence to label these compounds as teratogens or possible teratogens.

The pesticides listed in Table XIV-12 can be grouped into OP, CC, dipyridyl, dithiocarbamate, phthalimide derivatives, and 2,4,5-T.

(A) Organophosphorus Compounds

The teratogenic potential of the OP compounds parathion, dichlorvos, and diazinon, among others, were studied using the Sherman rat fetus [262]. The highest nonfatal dose for each compound was: parathion, 3.5 mg/kg; dichlorvos, 15 mg/kg; diazinon, 100 and 200 mg/kg. Each dose was injected intraperitoneally for each compound on the 11th day after insemination. On the 20th day of gestation, the fetuses were removed for examination. Toxic levels of parathion and diazinon caused a high incidence of resorptions and reduced fetal weight. Parathion, dichlorvos, and diazinon produced various malformations: 1/28 fetuses exposed to 3.5 mg/kg parathion were edematous, 3/41 exposed to 15 mg/kg dichlorvos were omphalocelic (intestine herniated through umbilicus), 1/6 of those exposed to 200 mg/kg diazinon were hydrocephalic (cranial vault enlarged), 1/6 were missing the first distal phalanx, 1/6 were ectromelic (marked shortening or absence of long bones), and 6/30 exposed to 100 mg/kg diazinon had a dilated renal pelvis. The authors concluded that these three compounds were slightly teratogenic

[262]. After reviewing additional information on parathion, NIOSH concluded that parathion is not an active teratogen [89].

Two additional OP pesticides, trichlorfon and phosmet, have been labeled by Martson and Voronina [263] as having embryotoxic and teratogenic effects. These compounds were administered orally to groups of pregnant Wistar rats on days 9 or 13 of gestation, and daily or every other day throughout gestation. An 80 mg/kg dose of trichlorfon on day 9 resulted in an insignificant increase in embryo deaths. However, the same dose when given on day 13 resulted in a decrease in the number of developing fetuses, although corpora lutea production matched that of the controls. Post-implantation mortality in trichlorfon-treated animals increased significantly, and examination of dead fetuses revealed general edema and abnormalities such as exencephaly and "nonclosing eyelid" symptom. Trichlorfon administered at a dose of 8 mg/kg/day throughout gestation failed to show any significant deviation in embryogenesis. Analysis of embryoskeletal systems revealed only a few cases of wavy ribs. Trichlorfon administered in a dose of 1.5 mg/kg every other day throughout pregnancy resulted in a statistically verifiable reduction in the number of live fetuses. Hydrocephaly and subcutaneous hemorrhages were also seen. At a dose of 0.06 mg/kg given on alternate days, trichlorfon produced no adverse effects.

A 30 mg/kg dose of phosmet on day 9 resulted in malformations such as hypognathia and dislocation of extremities, but an insignificant increase in post-implantation mortality was

seen [263]. A 30 mg/kg dose of phosmet when administered on day 13 had no effect on embryo mortality before or after implantation. Examination of these embryos did reveal hydrocephaly in 33 of the 55 embryos studied.

(B) Organochlorine Compounds

The teratogenic potential of aldrin, dieldrin, and endrin was studied in 221 pregnant Syrian golden hamsters and in 50 pregnant CD1 mice [264]. These pesticides were given by oral intubation on days 7, 8, or 9 of gestation to hamsters and on the 9th day of gestation to mice, in doses equivalent to half the oral LD50 of each substance for both species. The abnormalities produced in both species included soft-tissue malformations, while the most frequent were cleft lip, webbed foot, and open eye. The latter two often occurred in combination with low fetal weight, indicating simply a suggestion of growth retardation. In hamsters, 62% of the 216 abnormal fetuses had only one abnormality, 23% had two abnormalities, and 15% had three or more. Also in this species, fused ribs occurred as a single defect, whereas 40-50% of cleft lip, cleft palate, open eye, and 87% of webbed feet occurred in combination with one or more other defects. The incidence of open eye, webbed foot, and cleft palate was uniform for all three pesticides in hamsters; however, in mice, different abnormalities were associated with each pesticide. Open eye and webbed foot were more frequent for aldrin in mice; dieldrin was associated with cleft palate and webbed foot. In both species, cleft palate, cleft lip, and fused ribs occurred significantly as a single

malformation, an indication of the teratogenicity of these pesticides [264].

(C) Dithiocarbamate Compounds

Thiram, dissolved in dimethylsulfoxide (DMSO) or carboxymethylcellulose (CMC) and administered to hamsters, was teratogenic in the hamster [265]. When DMSO was used, incidence of terata varied from 0/22 fetuses at 125 mg/kg to 11/48 (23%) at 31 mg/kg and to 6/6 (100%) at 250 mg/kg; controls had 27/149 (18%). In CMC, incidence progressed from 2 terata/68 fetuses (3%) at 125 mg/kg to 10/49 (20%) at 250 mg/kg to 5/15 (33%) at 300 mg/kg; controls had 3/714 (0.4%) [265]. Defects found with both solvents included head and limb abnormalities, fused ribs, maxillary or mandibular shortening, and umbilical hernia.

(D) Dipyridyl Compounds

Khera and Whitta [266] summarized the effects of paraquat and diquat in rats and found that a single 7 mg/kg injection of diquat on days 6-15 of gestation produced retarded growth of the sternum and auditory ossicles. Paraquat at 6.5 mg/kg on day 6 produced costal cartilage malformations. The number of animals tested and the incidence of malformations were not reported. Higher doses of both compounds caused increased abortion rates and, for diquat, more pronounced embryonic defects.

(E) Phthalimide Derivatives

Robens [267] found the following compounds to be teratogenic in hamsters: captan (300 mg/kg), folpet (500 mg/kg), and captafol (200 mg/kg); they were studied because of

their relationship to thalidomide. The compounds were administered orally in CMC to hamsters at a constant volume of 1 ml/100 g body weight. Administration was between days 6 and 10 of gestation with fetuses examined on day 15. Controls had 4 terata/1,081 fetuses (0.4%). At 300 mg/kg, captan caused 9/111 (8.1%), at 500 mg/kg, folpet had 3/91 (3.3%), and at 200 mg/kg, Captafol had 4/145 (2.8%). No single pesticide had a distinctive abnormality associated with it. Teratogenic defects included head abnormalities and exencephaly, short or curved tails, fused ribs, limb anomalies, and vertebral defects. The author was not able to relate these hamster data to humans [267].

(F) 2,4,5-T

Several studies have been conducted with the compound 2,4,5-T in mice to assess both the teratogenic/embryotoxic effect and the enhancement of these effects by the impurity, dioxin [268-270].

In a study by Neubert and Dillmann [268], the purest sample of 2,4,5-T available (containing less than 0.02 mg/kg dioxin) induced embryotoxic effects in NMRI mice when given orally on days 6 through 15 of gestation. The frequency of cleft palate exceeded that in controls when doses higher than 20 mg/kg (about 1/6 the LD50) were administered. While reductions in fetal weight were found with 10-15 mg/kg, there was no definite increase in embryo lethality over that seen in controls. Cleft palates were produced with a single oral dose of 300 mg/kg of 2,4,5-T; the maximal teratogenic effect was obtained when the compound was administered on day 12 or 13 of gestation. When

doses of 150-300 mg/kg were given on days 6-15 of pregnancy, 5% of the fetuses had cleft palates, as compared with about 0.7% of control animals (P=0.01). With doses of 20-30 mg/kg, no significant increase was observed [268].

In a second study, in two strains of mice (C57BL/6 and AKR), 2,4,5-T containing 30 mg dioxin/kg was teratogenic and feticidal when given orally at a dose of 113 mg/kg/day in honey or subcutaneously in dimethyl sulfoxide on days 6-14 or 9-17 of gestation. Cleft palates and cystic kidneys were seen in C57BI/6 mice, while only cleft palates occurred in AKR mice. No teratogenic effect was observed in C57BL/6 mice given lower doses (21.5 mg/kg), but cystic kidneys occurred with a dose of 46.4 mg/kg. A dose-response relationship was suggested for the feticidal and teratogenic properties of 2,4,5-T administered by either oral or subcutaneous routes [269].

In a third study, commercial samples of 2,4,5-T (containing 0.1, 0.5, 2.9, or 4.5 mg/kg of dioxin) were feticidal and teratogenic in Syrian golden hamsters when administered orally on days 6-10 of pregnancy at levels of 20, 40, 80, or 100 mg/kg; the incidence of effects increased with both the 2,4,5-T dosage and the content of dioxin. No malformations were produced by doses of less than 100 mg/kg 2,4,5-T containing no detectable dioxin. Bulging eyes (absence of eyelid) accounted for the majority of the effects caused by 2,4,5-T containing dioxin. Dioxin contamination increased the incidence of hemorrhages in liveborn hamsters and also produced marked edema [270].

(4) Reproductive Effects

The term "reproductive effects" refers to the inhibition of reproduction as distinct from teratogenesis which refers generally to the induction of malformations in the fetus. Some compounds such as DDT and Kepone, however, may exhibit both reproductive and teratogenic effects. Reproductive effects are believed to be mediated through some hormonal action of the pesticide or some effect on the endocrine system. This section discusses some pesticides, including Kepone, mirex, aldrin, DDT, dieldrin, 2,4,5-T, carbaryl, heptachlor, and crufomate, that have caused adverse reproductive effects in animals. The results are summarized in Table XIV-13.

Kepone has produced adverse reproductive effects in mice. Good et al [271] fed Kepone to mice in three experiments.

In the first experiment, Kepone at levels of 10, 17.5, 25, 30, and 37.5 ppm was fed to 66 pairs of mixed laboratory mice (7-16 pairs per treatment group including controls) from 1 month before mating until 5 months after. The average number of litters per pair decreased from 1.67 (0 ppm) to 0.2 (37.5 ppm) except for the 10 ppm group, which had 2.0. The average number of young per litter decreased from 7.93 (0 ppm) to 3.0 (30 ppm) and to 5.0 (37.5 ppm).

In the second experiment, 5 ppm Kepone was fed to 36 pairs of BALB/c mice from 1 month before mating until 4 months after. Twenty-four other pairs served as untreated controls. Treated pairs had 15.3% fewer first litters (95.8% in controls vs 80.55% in treated animals) and 28.2% fewer second litters (78.2% vs 50.0%, P=0.05).

In the third experiment, 5 ppm Kepone was fed to 20 pairs of the progeny of the treated animals in experiment two. No Kepone was fed to 23 other pairs of the progeny of the treated animals in experiment two, and no Kepone was fed to 21 pairs of the progeny of the untreated controls in experiment two. For treated animals in experiment three, Kepone was fed from 1 month before mating until 3 months after. Reproduction was decreased in treated animals and in the untreated test progeny when compared with untreated control progeny. The percent of pairs bearing first litters was 25.4%, 30.4%, and 71.4% (P=0.05), respectively. The percent of pairs bearing second litters was 15.0%, 8.7%, and 28.6%, respectively. The numbers of pups per litter were 4.40, 4.29, and 5.60, respectively, for the first litter and 5.34, 6.0, and 6.5 for the second litter. The author concluded that reproductive physiology would probably be affected by doses considerably lower than those tested in this study [271].

In another study [272], reproduction, as indicated by total offspring produced by groups of 8 pairs of mice, decreased by 23.9-87% compared with controls in BALB/cJaxGnMc mice fed 10-37.5 ppm Kepone. Average number of animals per litter, number of litters produced, and survival to weaning also decreased as Kepone concentration increased. Matings between Kepone-fed females and control males as well as between control females and Kepone-fed males at 40 ppm indicated that Kepone had an effect on the reproductive systems of both sexes, although mostly on the female [272].

Two experiments were conducted to determine the effect of mirex-induced ovulation in rats [273]. Immature Long-Evan strain rats were injected with 0.2-50 mg of mirex. After administration of pregnant mare serum (PMS), the number of ova was reduced 40-80% at all treatment levels, except at 0.2 mg, with a progressively greater effect as the insecticide dosage was increased ($P<0.05$ at 0.04 mg mirex and $P<0.01$ at 0.8-50 mg mirex). In a second study undertaken to determine whether mirex was affecting the release of luteinizing hormone (LH) or directly inhibiting follicular rupture, injections of 6, 25, and 50 mg of mirex administered 48 hours after PMS were followed by the administration of Human Chorionic Gonadotrophin (HCG). HCG overcame the inhibitory effect of the insecticide, suggesting the ovary was not the primary site of action for mirex. Injections of mirex preceding the PMS-induced release of LH inhibited ovulation, but injections following the release of LH did not effect ovulation. The data suggest mirex affects neural mechanisms which control the release of LH to inhibit PMS-induced ovulation [273].

During 1971, Deichmann and MacDonald [274] and Deichmann and associates [275], treated male and female beagle dogs orally by capsule for 14 months with either 0.15 mg/kg aldrin, 0.3 mg/kg aldrin, 12 mg/kg p,p'-DDT, or with a mixture of 0.15 mg/kg aldrin plus 6 mg/kg p,p'-DDT. Breeding occurred 0.5-9 months after dosing. Reproduction was severely affected in all four treatment groups. Out of 15 treated females, 6 had delayed estrus (with 1 or 2 of these 6 dogs in each treatment group). Two females given

DDT did not conceive after several matings. Of 11 males, 2 given 0.3 mg/kg aldrin and 1 given 12 mg/kg DDT could not mate during the study. One male given 0.3 mg/kg aldrin and one given the aldrin/DDT mixture could not mate until late in the study. All treated females had decreased mammary development and milk production associated with first litters. Regardless of treatment, half of all deliveries had stillbirths (usually one). Survival of 8 first litters until weaning was only 32% (13/32) compared with 84% of the pups of controls. All 18 control females conceived and 16 nonbreech deliveries produced 93 pups. In summary, the adverse reproductive effects from these pesticides included delayed estrus, diminished libido, presence of stillbirths, reduction in mammary development and milk production, and high offspring mortality [274,275].

Thomas [276] tested a number of pesticides for male reproductive effects in mice. Technical DDT orally administered by intubation at doses of 12.5-50 mg/kg caused a significant reduction ($P \leq 0.05$) in the prostate gland's ability to assimilate radioisotope-labeled testosterone. Labeled DDT was found to concentrate in the prostate and testes within 1-2 hours after oral administration, and amounts were still present in epididymal fat as long as 12 days later. Ten daily oral administrations of DDT at 25 or 50 mg/kg resulted in significant reductions ($P<0.05$) in testosterone accumulation in the anterior prostate. Dieldrin administered to mice similarly at 1.25-5 mg/kg for 5 days significantly reduced ($P<0.05$) uptake of labeled testosterone by the anterior prostate; 2,4,5-T likewise reduced testosterone

assimilation by the prostate at 6.25-25 mg/kg ($P \leq 0.05$) [276].

Negative results were also obtained when Thomas [276] tested parathion at 1.3-5.3 mg/kg and carbaryl at 8.5-34 mg/kg in the same manner. Neither of these inhibited testosterone uptake by the anterior prostate. Labeled carbaryl was administered once orally and amounts were found in the prostate, seminal vesicles, testes, epididymal fat, and seminal plasma. The fungicide thiophanate did not affect testosterone absorption either, although the prostate and adrenal glands showed significant increases in weight [276]. Based on the reduction of testosterone uptake by the anterior prostate, the author suggested that the OC pesticides were the principal class of pesticides that exert significant changes upon male reproductive systems.

In contrast to the previous study, Shtenberg and Rybakova [277] found that daily doses of 7, 14, or 70 mg/kg carbaryl fed to rats for up to 12 months did affect the reproductive system. After 6 months, estrous cycles were unusually prolonged ($P \leq 0.05$) by 14 mg/kg/day and likewise at 3 months by 70 mg/kg/day ($P \leq 0.002$). Sperm motility reduction, disturbed spermatogenesis, and an increase in the number of corpora lutea and atretic follicles of the ovaries were also observed [277].

Heptachlor was studied in rats by Mestitzova [278] for reproductive effects. At 6 mg/kg in the diet, litter sizes in several generations decreased and the offspring death rate increased, especially from 24 to 48 hours after birth [278].

Crufomate caused a decreased number of litters among rats

dipped once in a 10 g/liter xylene-water-emulsifier solution of the test material. The ratio of the number of litters to the number of bred females was calculated for each day of application. The only days of dipping causing significant occurrence were 2 days before and 10 days after mating [279]. Other reproduction indicators such as number of pups/litter were not significant.

These demonstrated reproductive effects in animals have taken on new importance in light of recent incidents of reduced human fertility after exposure to Kepone and DBCP as discussed in the previous section. In light of inconsistencies and insufficient evidence in studies reported above, guidelines on testing for reproductive effects are needed in order that results are consistent, repeatable, and interpretable. It is only prudent that employers and other responsible parties be aware of the possible reproductive effects cited above and take necessary precautions.

(5) Neurotoxic Effects

While acute neural intoxication by OP and carbamate pesticides through inhibition of AChE is fairly well understood, much less is known about the mechanism of the neurotoxic effects by other types of pesticides. Experimental evidence indicates that DDT and perhaps other chlorinated hydrocarbon pesticides produce neurologic effects by the inhibition of Na^+, K^+, and Mg^{2+} ATPase activity, which controls the migration of these ions at nerve endings [55]. Following is a discussion of neurological and behavioral effects observed in animals administered OC and CP

pesticides.

(A) Organochlorine Pesticides

Symptoms of acute poisoning by DDT result from effects of DDT on the CNS. DDT induced symptoms, which are generally similar in different species, begin with abnormal susceptibility to alarm stimuli, motor unrest, and increased frequency of spontaneous movements. These symptoms are followed by tremors which become constant, and as severity increases, attacks of epileptiform tonoclonic convulsions occur. DDT poisoning may ultimately result in death from ventricular fibrillation. These symptoms may be caused by a single large dose of DDT as well as by repeated exposures to the pesticide [73].

NIOSH reviewed literature on the OC pesticides DDT and aldrin/dieldrin. Several experiments discussed therein indicated that neurotoxic effects were produced by these pesticides [2,31]. Khairy [280] observed a progressive deterioration of muscular efficiency related to the amount of dieldrin administered to rats.

London and Pallade [281] found that after chronic dietary administration of aldrin to rats, at the rate of 13 mg/kg/day, 6 days/week, for 6 months and then at 4.5 mg/kg/day for 7 months, a shock-withdrawal reaction required a longer-duration electric shock to elicit the withdrawal response in treated rats than in controls. Acute dosing with a higher level of aldrin (97 mg/kg) had the reverse effect of increased excitability.

Regardless of the foregoing, the mechanism of

aldrin/dieldrin activity is not completely understood, although a metabolite may be the actual active agent [2]. The general symptoms attributed to aldrin/dieldrin poisoning include CNS stimulation, convulsions, headache, nausea, vomiting, and dizziness. Unlike DDT, convulsions may occur without previous symptoms [55].

Huber [272], as reported in 1965, fed Kepone to BALB/c strain mice and found 80 ppm or higher to be lethal to all animals in no more than 32 days. No deaths occurred when Kepone was fed at 40 ppm over 12 months. Within 4 weeks, all mice fed Kepone at 30 ppm or higher developed a constant tremor syndrome which terminated no later than 4 weeks after withdrawal of Kepone from the diet. These neurologic signs are probably correlated with the neurologic effects observed in workers poisoned by occupational exposure to Kepone [282]. Such effects included weight loss, tremor, unusual ocular motility, and arthralgia. Kepone appears to produce neurologic effects involving the central and peripheral nervous systems.

While differences exist among the symptoms caused by the OC pesticides, they do have a tendency to affect the CNS. Increased sensitivity often results and causes tremors or convulsions. These effects can be caused by high, acute dosage or by low, chronic administration.

(B) Organophosphorus Pesticides

Delayed neurotoxic effects in humans have been reported earlier for the OP pesticides leptophos and mipafox [40,87]. Axonal damage and secondary demyelination are suspected

to be the causes of such effects and result in weakness of the muscles innervated by the damaged nerve fibers. Regeneration of the nerve occurs slowly and not always completely [55].

Damage and demyelination of peripheral nerves and, in some cases, tracts in the spinal cord have been demonstrated unequivocably in experimental animals. Leptophos and mipafox, two compounds which have demonstrated similar neurotoxicity in humans, produce like effects in chickens. Chickens are the species most sensitive to these toxic effects and have been used extensively to test OP pesticides.

The neurotoxicity of OP compounds has been reviewed [283, 284]. In 1975, Johnson [283] reviewed the neurotoxicity of 226 compounds; however, most of these were not registered pesticides. Of those compounds reviewed by Johnson, haloxon [285], EPN [286,287], S,S,S-tributylphosphorotrithioate [288], S,S,S-tributylphosphorotrithioite [288], and carbophenothion [99,282] are currently registered pesticides which produced persistent ataxia when administered to chickens (see Table XIV-14). Although ataxia produced by EPN was not delayed for the usual 8-14 days [283], as is characteristic with most neurotoxic OP compounds, the ataxia was persistent and lasted more than 308 days. In addition, Gaines [99] reported persistent ataxia in chickens, lasting more than 330 days, after subcutaneous administration of EPN. Ataxia produced by S,S,S-tributylphosphorothioite, S,S,S-tributylphosphorotrithioate, and haloxon was both persistent and delayed.

In addition to EPN, Gaines [99] also found delayed ataxia

in chickens when dosed with S,S,S-tributylphosphorotrithioite, S,S,S- tributylphosphorotrithioate, and chlorpyrifos. Although delay of ataxia was 14 days for S,S,S-tributylphosphorotrithioite and S,S,S-tributylphosphorotrithioate, it was only 3 days for chlorpyrifos and was not persistent. Possible delayed ataxia caused by chlorpyrifos has not been reported elsewhere [283,284] and, consequently, further work is needed to clarify the possible neurotoxicity of this compound.

MK Johnson (written communication, May 1978) reported that carbophenothion caused delayed ataxia at 2 subcutaneous doses of 500 mg/kg. Ataxia produced by carbophenothion in Gaines' work [99] was not delayed, but was persistent, lasting more than 53 days (pericd cf observation,.

Casida et al [289] tested O-(2,4-dichlorophenyl) O-methyl(1-methylethyl)phosphoramidothioate (DMPA) by injection in hens and found that either 50 mg/kg for 10-14 days or 100 mg/kg for 7 days produced ataxia signs in 21 days. Partial recovery from the delayed neurotoxicity occurred slowly after a period of three months.

It should be noted that with few exceptions, fluoride esters of OP acids cause delayed ataxia in chickens. DFP, mipafox, dimefox, and butafox [283] are examples. Although these compounds are not registered for sale in the US, experimental or developmental use or importation of the compounds in this class could lead to undesired effects in humans. As reported earlier, two cases of delayed neurotoxicity caused by mipafox occurred in laboratory workers who were experimenting with insecticides.

(C) Behavioral Effects

Concern that pesticides may have subtle effects on human behavior, at doses lower than those which produce grossly observable neurologic effects, directed attention towards behavioral effect experiments. A number of pesticides have been tested for behavioral effects in animals. Several will be discussed here, including dichlorvos, parathion, DDT, dieldrin, and crufomate.

Behavioral effects have been observed in rat experiments with a series of chlorovinyl OP esters. Brimblecombe et al [290] found that preening activity, rearing, and defecation frequency in an open field were affected by phosphate esters at dose levels which did not cause detectable inhibition of AChE activity. The dose levels at which these effects were observed were substantially below the pesticides' LD50 values. One of the compounds tested was dichlorvos (DDVP). The minimal effective single dose of DDVP required to produce the behavioral changes observed was approximately 0.2 mg/kg. The ratio of the LD50 of DDVP to the effective behavioral dose was 175. Other compounds tested were 2-chloroethyl-2,2-dichlorovinylethylphosphonate, 2-fluoroethyl-2,2-dichlorovinyl methylphosphonate, and 5 similar synthesized esters. The ratio of LD50 to effective behavioral dose for these compounds ranged from <3 to >100.

In another study, Reiter et al [291] studied parathion's effect on learning in mice. Subchronic oral doses consisting of 1-4 mg/kg parathion on 6 successive days prior to a learning trial did not affect learning, despite observed depression of

blood AChE activity. The authors suggested that compensatory mechanisms were operating which allowed the mice to learn even after repeated parathion administration. However, in a separate experiment a single acute dose of 6 mg/kg parathion after an 18-hour fasting period (approximately one-third of the LD50) abolished learning capabilities in the 90% surviving mice.

Despite the apparent effects of acute and chronic exposure to OC insecticides upon the CNS, until recently, little attention had been paid to the influence of these compounds on behavioral systems. Scudder and Richardson [292] found that DDT in very low doses (0.1 or 1.0 mg/l) in drinking water of pregnant mice and their offspring resulted in a significant decrease in the aggressivity of isolated young males. Sobotka [293] reported alterations in several behavioral and neurophysiologic parameters in mice after single low doses of DDT. Exploratory activity in an open field was significantly enhanced 24 hours after a single oral dose of 25 mg/kg of DDT. At the same time, the ability of animals to habituate to the open-field situation was reduced. In a passive avoidance test, DDT in doses lower than 25 mg/kg alleviated stress-induced motor depression. Changes in the maximum electroshock seizure patterns reflected an increase in brain excitability. It has been suggested that DDT may facilitate the central excitatory process, at least partially, by a disinhibitory mechanism.

Peterle and Peterle [294] studied the effects of feeding 7 ppm technical DDT on the aggressive behavior of male mice. Mice given DDT "lost" more bouts (as determined by posturing and

avoidance behavior) and made fewer biting attacks than controls. The DDT-fed mice were significantly less aggressive than control mice and were more likely to submit in territorial fights.

Smith et al [295] exposed 7 squirrel monkeys to dieldrin at two oral doses of 0.10 and 0.01 mg/kg/day for 55 days. Two zero-dose controls were included. All 9 monkeys were taught a visual nonspatial successive discrimination task. Ability to learn this task was severely retarded in the high-dose group ($P<0.003$) but not in the low-dose group, when compared with controls.

At the end of 55 days, the higher dose group was shifted to the low dose, the low-dose group was shifted to the high-dose, and the controls continued at zero exposure. During the following 54 days, all group performances remained at approximately the levels achieved at the end of the preshift period. The authors concluded that the high dose had disrupted learning acquisition, and speculated that this effect was due to disruption of the activity of the hippocampus, which is necessary for initial learning but not for retention of a learned task. This appears to demonstrate state-dependent learning.

Results of the animal behavioral studies cited above are in some cases inconsistent and are often too scant to serve as a basis for recommended control procedures. However, they do point out the possible subtle changes that may take place in humans exposed to various pesticides.

In summary, many factors, including individual susceptibility due to variations in genetic makeup of

individuals, age, sex, synergism caused by multiple exposures, fractionization of doses, and extrinsic factors such as temperature, affect the toxicity of pesticides. Attention should be given to the chronic irreversible effects demonstrated for certain pesticides. Carcinogenic and neurotoxic effects of certain pesticides have been demonstrated in man and animals, and the literature reveals a substantial number of reports on teratogenic and mutagenic effects in laboratory animals and in in vitro test systems. A continuing controversy is the use of animal toxicologic data to predict effects in man, but it is well established that many toxic effects observed in humans were observed first in experimental animals. Accordingly, the use of animal data as a surrogate for human data is warranted in order to anticipate potential human toxic effects and to develop the best and safest protective mechanisms in the manufacture and formulation of pesticides.