Int. J. Environ. Res. Public Health **2005**, *2(3)*, 430-441

International Journal of
Environmental Research and Public Health
ISSN 1660-4601
www.ijerph.org

Environmental Toxicology and Health Effects Associated with Methyl Parathion Exposure – *A Scientific Review*

Falicia L. Edwards[1] and Paul B. Tchounwou[1*]

[1]Environmental Toxicology Research Laboratory, NIH-RCMI Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University. Jackson, Mississippi 39217, USA
*Correspondence to Dr. Paul B. Tchounwou: email: paul.b.tchounwou@jsums.edu

Received: 03 July 2005 / Accepted: 04 December 2005 / Published: 30 December 2005

Abstract: Methyl parathion - MP ($C_8H_{10}NO_5PS$) is a restricted-use pesticide that has been widely used as an agricultural insecticide. It belongs to the class of organophosphate chemicals characterized by their ability to inhibit acetylcholinesterase activity. The main route of human exposure is inhalation, but dermal contact and inadvertent ingestion can also be substantial. Populations that are susceptible to MP exposure primarily are applicators, manufacturers and individuals living near application and/or disposal sites. Exposure has also been reported as a result of illegal indoor application. MP related health effects include headaches, nausea, night-waking, diarrhea, difficulty breathing, excessive sweating and salivation, incoordination, and mental confusion. Other symptoms including behavior problems, motor skill problems and impairment of memory recall have also been reported. The primary targets of toxicity are the hematopoietic system (serum cholinesterase inhibition), the cardiovascular system (cardiovascular lesions, abnormalities in heart rate and increase in heart-to-body ratio), the reproductive system (placental morphology, fibrosis and hemorrhage, and inhibition of DNA synthesis in seminiferous tubules), and the nervous system (headache, muscle weakness, insomnia, dizziness, and impaired memory). MP is believed to not have any carcinogenic effects. In an attempt to update its toxicologic profile, we hereby provide a critical review of MP-related environmental and toxicologic effects, with a special emphasis on their potential implications for public health.

Keywords: Methyl parathion, toxicokinetics, systemic toxicity, genotoxicity, neurotoxicity, carcinogenicity, health effects

Introduction

Organophosphates (OP) are a group of compounds that have historically been used as pesticides as well as chemical warfare agents. These compounds are potent irreversible acetylcholinesterase inhibitors that have a profound effect on the nervous system of exposed organisms. OP are phosphorus-containing insecticides whose insecticidal qualities were first observed in Germany during World War II in the study of the extremely toxic OP nerve gases *sarin*, *soman,* and *tabun*. This group of chemicals includes insecticides such as malathion, diazinon, chlorpyrifos, azamethiphos, dichlorvos, parathion and methyl parathion. These chemicals are characterized by their ability to inhibit acetylcholinesterase activity [1-4]. They are lipid-soluble and are capable of penetrating the skin, the placenta, and into the fetus [5-14].

Organophosphates, introduced in the 1930s, are manufactured chemical substances that are produced by the reaction of alcohols and phosphoric acid [15]. This class of chemicals is divided into several forms; however, the two most common forms are the phosphates and phosphorothionates. Methyl parathion (*O, O*–dimethyl *O*-4-nitrophenyl phosphorothioate) is a class I insecticide [15] and has been classified as restricted use pesticide by the U.S. Environmental Protection Agency due to its high level of toxicity; therefore, only certified applicators should purchase and use this product [16].

Methyl parathion, used as a dust, emulsion concentrate, granular, ULV liquid, and/or wet-table powder, is applied to alfalfa, barley, corn, cotton, sorghum, soybeans, sunflowers and wheat. MP is used to control boll weevils and many biting insects of agricultural crops, primarily cotton. Its chemical structure is diagramed in Figure 1. It appears as a white crystalline substance (pure) or as a technical grade liquid chemical with a light to dark tan color, and contains 80% active ingredient, 16.7% xylene, and 3.3% inert ingredients.

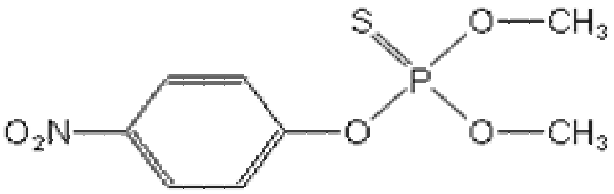

Figure 1: Structure of methyl parathion

The physiochemical characteristics of methyl parathion are presented in Table 1 [16-18].

Table 1: Physiochemical characteristics of methyl parathion

Properties	*MP Characteristics*	*Reference*
Cas Reg. No.	298-00-0	16
Synonyms	Dimethyl 4-nitrophenyl phosphorothionate	16
Molecular weight	263.23	16
Color	White	16
Physical state	Solid	16
Melting point	37-38^{o}C	16
Boiling point	143^{o}C	17
Density/specific gravity	1.36	17
Odor	Rotten eggs or garlic	16
Solubility		
Water (mg/L)	50 mg/L	16
Organic solvent	Soluble in ethanol, chloroform	
Vapor pressure (mmhg)	9.7x10^{-6} @ 20^{o}C	16
Henry's law constant	1.0x10^{-7} @ 25^{o}C	18

Methyl parathion, known as the "cotton poison" [19], is applied in fields containing crops such as cotton, corn, apples, soybeans, rice, wheat, peaches, alfalfa, sunflower, and sweet corn [20]. While methyl parathion was initially registered in 1954 in the United States [21], its use was restricted in 1978 as a result of detrimental effects to humans [22]. EPA has now classified methyl parathion as a restricted-use pesticide. Although this organophosphate is restricted specifically to outdoor use and application by a licensed applicator, there have been several incidences of illegal applications of methyl parathion across the United States as well as suicidal ingestions. These illegal applications have occurred in the homes of families living in Mississippi, Ohio and Louisiana, Alabama, Arkansas, Illinois, Michigan, Tennessee, and Texas [19]. In 1994 it was discovered that illegal applications had taken place in homes in Lorain County, OH; in April 1995, contamination of homes and businesses was discovered in Detroit, and in November 1996, it was discovered that illegal applications of methyl parathion had taken place in homes over a 10 year period [23].

There are about 20,000 cases of organophosphate intoxication reported yearly and of these reported cases 2.3 – 2.6 per 10 million of the cases represent suicidal ingestion. In addition to these figures, it is believed that a true number of exposures are unlikely to be attained due to the number of cases that go unreported with respect to exposure of agricultural workers [15]. There is no specific group of individuals susceptible to methyl parathion exposure. Race is not a specific factor that relates to exposure; however, it has been pointed out pointed out that African-Americans have a reported 3-fold greater incidence of being exposed because African-Americans are associated with at-risk populations [15]. It has been further indicated that exposure would mostly be in the male population because most of this exposure involves agricultural workers [15].

People may be exposed to methyl parathion in a number of other ways as well. Applicators and manufacturers of this product may be exposed occupationally, although, there are some individuals that are exposed via food and water. Illegal application of methyl parathion increases the risk of exposure to the chemical for those who do not have occupations that involve MP handling. Individuals residing near hazardous waste disposal sites may be subject to higher levels of methyl parathion exposure [24]. The EPA has identified methyl parathion in at least 16 of 1585 hazardous waste sites that are considered for inclusion on EPA National Priority List (NPL) [25]. Once exposed, a number of biomarkers have been proven to be effective in determining the extent of organophosphate exposure. Blood and urine are the primary specimens used for biomarkers assessment; however, postpartum meconium has been studied to determine exposure, and in addition, studies are in progress to determine the effectiveness of using saliva and amniotic fluid for biomarkers evaluation [26-28].

To determine the level of prenatal exposure, six metabolites of organophosphates were measured [29]. Four of the studied metabolites – dimethylphosphate (DMP) and dimethylthiophosphate (DMTP), and diethylphosphate (DEP) and diethylthiophosphate (DETP) – were a result of possible methyl parathion and parathion exposure, respectively. This study showed that 1 out of the 20 samples taken showed that the methyl parathion metabolite, DMP, was present at a level of 16.0 μg/g; however, the metabolite DMTP was not detected. It was concluded that measurements of organophosphate metabolites in meconium may prove as a significant biomarker of prenatal exposure [29].

A case study involving organophosphates, chlorpyrifos and methyl parathion, was conducted in Thailand to determine the exposure levels of traditional farmers versus integrated pest management (IPM) farmers [30]. This study showed that traditional farmers were exposed to higher levels of pesticides than IPM farmers. Ambient air breathed by farmers was measured from thirty-three samples collected. The results showed that an average concentration exposure for traditional farmers was 0.19 mg/m3 compared to 0.037 mg/m3 for IPM [30].

If applied as required, the general public should not be exposed to large amounts of MP. The only persons at risk should be limited to manufacturers, applicators and possibly individuals living in close proximity to areas where these chemicals are applied. However, some incidents have occurred where exposure occurred as a result of touching contaminated plants, breathing mist formed from the sprayed chemical, drinking contaminated water, or eating recently sprayed fruits and vegetables.

MP exposure has been linked to substantial adverse health effects on several organ systems including the heamatopoietic system (cholinesterase inhibition), the cardiovascular system (cardiovascular lesions, abnormalities in heart rate and increase in heart-to-body ratio), the reproductive system (placental morphology, fibrosis and hemorrhage, and inhibition of DNA synthesis in seminiferous tubules), and the nervous system (headache, muscle weakness, insomnia, dizziness, and impaired memory). MP is believed to not have any carcinogenic effects. In an attempt to update its toxicologic profile, we hereby provide a critical review of MP toxicology with special emphases on its environmental fate and transport, toxicokinetics (absorption, distribution, mode of action, metabolism and excretion), systemic health effects (genotoxicity, neurotoxicity, reproductive and developmental toxicity), carcinogenicity, and regulatory guidelines.

Environmental Fate and Transport

Organophosphates are transported through the environment in various ways. When applied as an insecticide, methyl parathion breaks down rapidly primarily by photolysis and biodegradation from microorganisms in sediment and water [31, 32]. MP can be transported by rain, fog, and wind to surrounding areas [31].

To determine the degradation of organophosphates, selected chemicals including MP were exposed to various water and soil conditions under environmental conditions [31]. This research reported that the degradation of organophosphates is influenced by hydrolysis, mainly at pH >7, microbial degradation and photolysis. Degradation half lives and photolysis constants of methyl parathion are presented in Table 2.

Table 2: Half life (days) and photolysis constants of methyl parathion in selected water and soil [31]

Media	*Methyl Parathion*	
	HL (days)	*PC*
Water		
Lake water	25.6	2.71
River water	24.6	2.82
Marine water	27.3	2.54
Ground water	27.4	2.52
Distilled water	35.4	1.96
Soil		
Sandy clay loam	11.2	6.18
Clay loam	5.6	12.39
Sandy loam	9.1	7.58

Because methyl parathion hydrolyzes quickly, and due to the nature of its physical and chemical properties, it does not transfer through the food-chain [32]. If applied properly in agricultural settings where it is exposed to water and ultraviolet light, methyl parathion has an environmental half-life of days or weeks; however, when applied indoors, the half-life increases considerably [33]. Further studies completed to determine the effect of temperature and relative humidity on methyl parathion showed that the half-lives for methyl parathion were from 48 to 57 days at 0^{o}C, from 9.2 to 10.5 days for 20^{o}C, and from 1.3 to 1.5 days at 40^{o}C thereby concluding that the decomposition of methyl parathion was mainly affected by environmental factors as opposed to biological factors [34].

Toxicokinetics

Absorption

Absorption of methyl parathion occurs through the gastrointestinal tract, the respiratory tract and the skin [22, 35]. Previous studies have indicated that dermal absorption is the primary route of methyl parathion exposure, and this exposure is a result of occupational exposure. Exposure to methyl parathion has been known to occur via inhalation and contact with contaminated material, as has been the case where illegal applications have occurred. This exposure can be measured primarily in the blood and urine; however, postpartum meconium, saliva and amniotic fluid have been used to determine exposure [26].

Distribution

After exposure to methyl parathion, a large concentration of the chemical was found in the adipose tissue, while there were larger concentrations found in the liver and the kidney one hour after exposure [5]. Studies showed that within 12 hours of a single dermal dose of 10 mg/kg of methyl parathion, tissues with the highest concentrations of methyl parathion were the adipose tissue (67,532 ng/g), the kidney (1571 ng/g), spleen (1004 ng/g), heart (729 ng/g), liver (706 ng/g), brain (546 ng/g), placenta (389 ng/g), and fetus (256 ng/g) [6, 36]. These studies also pointed out that methyl parathion's primary mechanism of toxicity is via storage in the adipose tissue; from there, its slow release into circulation and subsequently into the nervous system makes it possible to result in toxicity.

Mode of Action

The primary detrimental action associated with exposure to organophosphates involves effects associated with the nervous system and consequential effects. Acetylcholine, a neurohumural transmitter, is present in the peripheral autonomic nervous system, in the somatic motor nervous system, and in some portions of the central nervous system. Following release of acetylcholine at a nerve synapse or at a neuromuscular junction, the transmitter is rapidly hydrolyzed by an acetylcholinesterase. Muscles, glands, and nerve fibers

called neurons are stimulated or inhibited by the constant firing of signals across the "electrical twitching" called synapses [37]. This is an ongoing reaction that happens quickly with acetylcholine causing the stimulation and acetylcholinesterase ending the signal. Proper functioning of the nervous system requires an enzyme called cholinesterase (ChE), which facilitates the transmission of nerve impulses. When cholinesterase-inhibiting pesticides enter the system, this function is negatively affected. Cholinesterase inhibiting pesticides disable this enzyme, resulting in symptoms of neurotoxicity [38].

Figure 2 depicts normal functioning as the conduction of a nerve impulse by acetylcholine across the junction between the nerve and the muscle stimulating the muscle to move. Normally after the appropriate response is accomplished, cholinesterase is released which breaks down the acetylcholine thereby ending the stimulation of the muscle [38]. If acetylcholinesterase is unable to breakdown or remove acetylcholine (Figure 3), the muscle will continue to move uncontrollably [38].

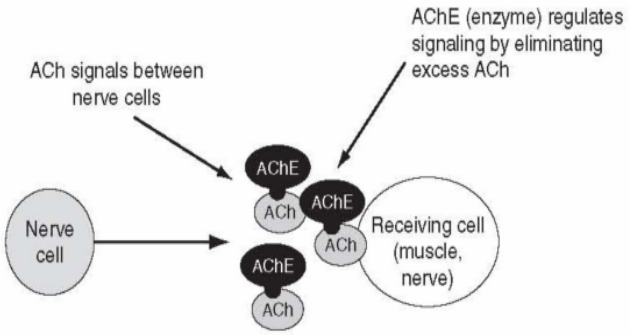

Figure 2: Normal nerve signals [38]

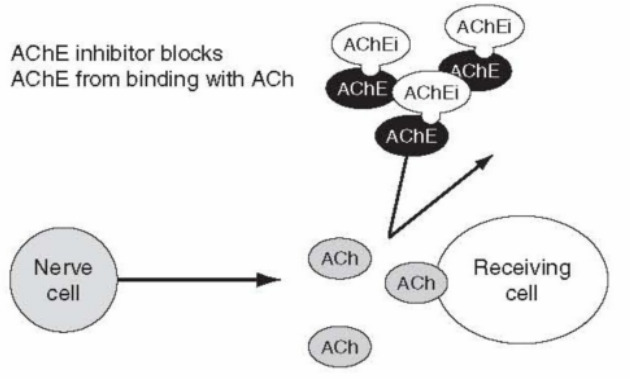

Figure 3: Effect of AChE inhibitors (Organophosphates) [38]

The mechanism of toxicity of organophosphates (OP) involves the process of phosphorylating the serine hydroxyl group at the site of acetylcholine and binding irreversibly thereby deactivating the esterase and resulting in accumulation of acetylcholine at the endplate [15, 38]. As a result of the accumulation of acetylcholine at the neuromuscular junction, persistent depolarization of the skeletal muscle occurs and this results in weakness and fasciculations, and disruption of neural transmission in the central nervous system, [39].

Acetylcholinesterase is also contained in the erythrocytes, and is identical to that which is found in the nervous system; however, the function there is to control, to a certain extent, permeability of the cell membrane. As a result, exposure to methyl parathion will result in inhibition of acetylcholinesterase in the erythrocytes [24].

Methyl parathion's primary mechanism of toxicity is the inhibition of acetylcholinesterase activity in the nervous system and at the motor end-plate. Methyl paraoxon, the active metabolite of methyl parathion, inactivates acetylcholinesterase by phosphorylating the active site of the enzyme. This action inhibits the hydrolysis of acetylcholine and the neurotransmitter accumulates at the site of action thereby producing over-stimulation of cholinergic end organs [24].

Metabolism

Once organophosphates (OP) enter the body, they are metabolized and are distributed into various areas of the body where they can cause damage. They are enzymatically converted to their oxon forms that react with available cholinesterase to cause subsequent toxicity [26]. Accordingly, research has reported that methyl parathion must be metabolized in order to bind to acetylcholinesterase and prevent the hydrolysis of acetylcholine [22].

A recent review of health effects resulting from methyl parathion exposure outlines the metabolic pathways of methyl parathion (Figure 4). MP undergoes metabolic biotransformation via two phases including the hepatic (phase I) and extrahepatic (phase II) phases [6]. Phase I reactions include methyl parathion desulfuation by cytochrome P450 [6, 22].

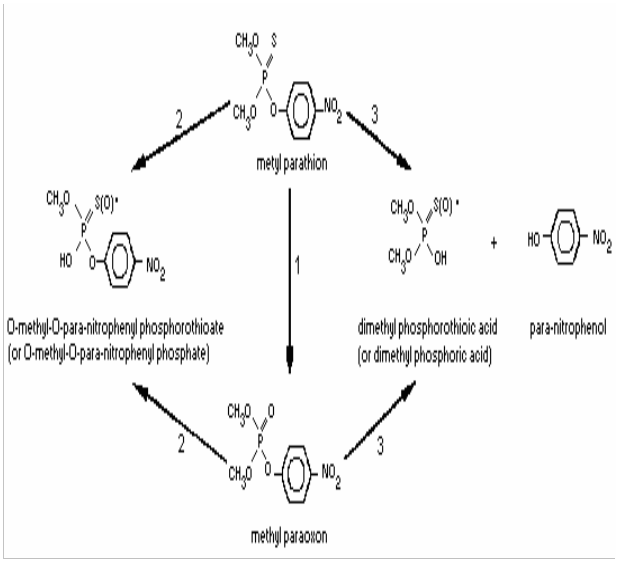

Figure 4: Metabolic pathways of methyl parathion [22]

When methyl parathion enters the body, it is either detoxified or activated. It is oxidized to form methyl paraoxon, which is extremely more potent [40-42]. After oxidative desulfuration, MP is hydrolyzed to p-nitrophenol and other metabolites [22, 43]. In other words, the P = S derivative is converted to the P = O form, and it (P = O) is the direct inhibitor of acetylcholinesterase.

Excretion

An exposure assessment of individuals who were exposed to methyl parathion indicates that MP is well-absorbed following inhalation or dermal contact and is excreted through the urine. When MP is metabolized, metabolism results in the urinary excretion of dimethylthiophosphate (DMTP), dimethylphosphate (DMP) and p-nitrophenol (PNP). P-nitrophenol is also a metabolite of parathion [33]. Assessing the amount of p-nitrophenol (a metabolite of parathion and methyl parathion) eliminated in humans, is an approach that was used to identify individuals exposed to methyl parathion. This was found in a study completed using humans exposed to MP as a result of illegal application [33, 44].

Methyl parathion enters the body and moves into the bloodstream as well as the brain, the liver and other organs. The liver changes some of the methyl parathion into a more harmful chemical methyl paraoxon. Within hours or days, both chemicals subsequently leave the body through urination [24].

Systemic Health Effects / Epidemiologic Studies

A number of epidemiological studies have been done to assess the effects of human exposure to organophosphates. An exposure assessment completed by the California Department of Pesticide Regulation in 1997 indicated that between 1986 and 1995 a total of 18 illnesses, systemic in nature, were reported as a result of exposure to methyl parathion either alone or in a combination of chemicals. These cases were associated with aerial application [45]. Breast milk was extracted from 12 mothers between 19 and 45 years of age from the lower socioeconomic class [46]. While 30% of the pesticides applied were representative of the organophosphate pesticide class, methyl parathion was only present in negligible quantities. However, this finding showed that infant exposure to methyl parathion could be accomplished through breast milk.

General Health Effects

A two-year study was conducted by the Centers for Disease Control and Prevention in conjunction with county and city health officials about exposure and health effects among residents. Indoor applications over a 5-7 year span had been made by a pesticide applicator. Individual questionnaires indicated that two weeks following application, household members reported headaches (30%), nausea (29%), night-waking (sleeplessness) (28%), diarrhea (26%), restlessness (23%), difficulty breathing (21%), dizziness (21%), abdominal cramps (20%), excessive sweating (13%), in-coordination (11%), excessive salivation (9%), and mental confusion (7%) [47]. These are all classical symptoms of organophosphate poisoning [47, 48]. Symptoms were further categorized to include the following: effects of the central nervous system (CNS): anxiety, seizures, skeletal nerve-muscle junctions, autonomic ganglia – twitching, tachycardia, muscle weakness which are all nicotinic effects; peripheral cholinergic neuroeffector junctions effects including sweating, salivation, diarrhea, tearing – the muscarinic effects; miosis or pinpoint pupils, and mydriasis secondary to epinephrine release from adrenals as a result of nicotinic receptor stimulation has been observed in about 15% of individuals exposed to organophosphates [48].

Neurotoxic Effects

Batteries of pediatric environmental neurobehavioral tests were completed to evaluate cognitive, motor, and sensory effects essential to neurobehavioral assessment in children 4 years or older [19]. These tests were administered to children exposed to methyl parathion in Mississippi and Ohio. The results indicated that there were no effects observed with reference to general intelligence, integration of visual and motor skills and multi-step processing. Conversely, there was a problem observed in memory recall after a period of delay, attention, and some parents whose children who were exposed complained that their children had exhibited behavioral and motor skill problems as opposed to parents whose children were not exposed. Behavioral problems included general mischief, acting on impulse, anger management, being distressed or withdrawn, and having problems relating to other children.

The Mississippi State Department of Health (MSDH), Agency for Toxic Substances and Disease Registry (ATSDR) and the U.S. Environmental Protection Agency (EPA) reported that the largest human-induced incidence in U.S. history had taken place in Jackson County, Mississippi, in November 1996 [23]. Over a 10-year period, 1,800 homes and businesses including day care centers, churches, and restaurants had been contaminated with methyl parathion. Homes were usually sprayed twice a year for $35 per application. Research was conducted to determine if individuals who were more exposed to methyl parathion exhibited greater levels of depression. In determining this, students seeking a master's degree in a social work program, over a 2½ month period, conducted in-home or telephone interviews of 115 heads of household. These interviews revealed that respondents with a higher number of depressive symptoms had been exposed to methyl parathion longer. The study further categorized the respondents by gender, race, and income. It was observed that women, African Americans and households with smaller incomes had more depressive symptoms than males, whites and household with larger incomes, respectively. In addition, it was noted that African Americans had been exposed to methyl parathion for a significantly longer period of time than whites, i.e., their homes had been sprayed four years longer than that of whites [23].

Extensive studies have shown organophosphates to be neurotoxic chemicals. As previously mentioned [22], methyl parathion has the ability to bind to acetylcholinesterase and to prevent the hydrolysis of acetylcholine. Neurotoxicity as it is related to high-level exposure, low-level exposure, and neurodegenerative diseases has been studied [49]. A compiled review noted that there is an association of pesticide exposure and Parkinson's disease with a 1.5 – 7 –fold increase in risk; however, most studies of pesticide exposure and

Parkinson's disease have been unable to implicate specific pesticides [49]. Although a specific chemical exposure has not been associated with the development of amyotrophic lateral sclerosis (ALS) and Alzheimer's disease, both diseases have been noted to possibly be attributed to exposure to organophosphates [49, 50].

Hematologic Effects

A group of 1000 individuals that were susceptible to organophosphate was studied with respect to their serum cholinesterase activity; in addition, fifty individuals were selected, studied, and used for controls [51]. Blood samples were collected and serum cholinesterase level was determined for both groups. The results indicated that the average level of serum cholinesterase (SChE) in workers was lower than that of controls. Factory workers had lower SChE levels than agricultural workers. Researchers attributed this finding to the fact that factory workers were exposed almost on a daily basis, while agricultural workers were only intermittently exposed [51].

Respiratory, Cardiovascular, Hepatic and Renal Effects

Methyl parathion has been found to affect other organs of the body as well. In studies of inhaled methyl parathion intoxication, the following effects have been reported: pulmonary edema, cardiovascular lesions, acute nephrosis of the kidney, and liver lesions [24, 52]. Pulmonary edema was found in a man who died 2 hours after intoxication and in others who died as long as 9 days after exposure [52]. With respect to cardiovascular lesions, the study [52] showed that patients who survived at least 20-24 hours had degeneration of the heart muscle with segmentation, fragmentation, and splitting of myofibers. Patients surviving 28 hours to 9 days after intoxication had widespread swelling of vascular endothelium even after receiving intensive therapy. Congestion of the esophageal mucosa and petechial hemorrhages has been associated with a lethal dosage of methyl parathion taken for suicide [24, 53].

Genotoxic Effects

Human peripheral lymphocytes were exposed to methyl parathion and tested for genotoxicity using the single cell gel electrophoresis (comet) assay [54]. The cells were incubated with 10, 50, 100 and 200 µg/mL concentration of methyl parathion for 0.5 hour at 37^{o}C and their amount of DNA damage compared to that of untreated peripheral lymphocyte cells from the same donor. Results showed that methyl parathion at 100 and 200 µg/mL significantly increased DNA damage in human lymphocytes.

Five patients who ingested methyl parathion in an apparent suicide attempt were studied to determine chromosomal effects associated with methyl parathion exposure. There was no significant difference observed between the data obtained from control and the experimental groups; in aneuploidy, chromatid aberrations, and chromosome aberrations observations [55]. However, a previous study completed on the lymphocytes from 31 patients, 5 of whom were exposed to methyl parathion, indicated a significant increase in the frequency of stable chromosomal aberrations in patients acutely exposed to methyl parathion [56].

Assessments aimed at determining the frequency of sperm aneuploidy (X, Y, and 18) and their relationships with urinary organophosphate metabolites in agricultural workers were analyzed [57]. These analyses involved the following metabolites: dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), and dimethylthiophosphate (DETP). Two of these metabolites, DMP and DMTP are end-products of methyl parathion. Overall, there was no significant difference in the prevalence of the three most frequent aneuploidies when comparing before and during spraying season; however, the frequency of total aneuploidies was slightly higher during the spraying season. Moreover, there were significant associations between DEP, a metabolite of parathion, and sex null, and smaller, yet still significant associations, with relation to DMTP metabolites. It was further explained that sperm sex aneuploidies may be responsible for some of the most common genetic syndromes such as Turner and Klinefelter [57]. Further, it has been noted that recent evidence shows that Turner syndrome has an estimated frequency of 1-2% among all clinically recognized pregnancies and that 70-80% of these patients retain the maternal X chromosome because the paternal X is missing. In agreement, another report indicated that increased frequency of total sperm aneuploidies was observed in Chinese workers manufacturing methyl parathion and parathion as compared to their controls [58].

Systemic Health Effects / Experimental Studies

General effects

Single and repeated doses of methyl parathion were administered to female Sprague-Dawley rats to assess acute toxicity [59]. Rats were treated with 6.25, 12.5 or 50 mg/kg for single doses and 0.1 or 1 mg/kg/day of methyl parathion for repeated doses. This study showed that a single dose of 50 mg/kg of methyl parathion resulted in severe signs of acute toxicity within 24 hours of dosing and animals died within 72 hours. Animals treated with 6.25 or 12.5 mg/kg of methyl parathion showed little or no signs of toxicity. Research associated with repeated doses of methyl parathion (i.e., 0.1 and 1.0 mg/kg) also showed little or no signs of acute toxicity. A single topical application of 10 mg/kg did not show significant differences in the weight, size, shape, or color of various tissues between the control and experimental animals [6].

Cardiovascular Effects

Methyl parathion exposure has also resulted in cardiovascular effects on organisms as reported in various studies. Abnormalities in heart rate and electrocardiograms were observed [24, 60]. Also found was an increase in heart-to-body ratio in female rats

exposed to 2.5 mg/kg/day in the diet for two years; however, this same effect was not observed when exposed to 0.025 or 0.25 mg/kg/day [24, 61].

Genotoxic Effects

Mutagenic effects of methyl parathion have been studied to determine the chemical's ability to cause a change in the DNA sequence of a gene. A study involving three organophosphates was completed and it was determined that methyl parathion, when administered to Wistar rats for a 6-week period of five treatment days per week at doses of 1/100, 1/75 and 1/50 of the LD_{50}, displayed no significance in mutagenicity [62].

The cytogenetic and cytotoxic effects of organophosphorus and organochlorine compounds following a single dose administration were studied [63]. It was found that the frequency of chromosomal aberrations and micronuclei in bone marrow cells and an assay of the liver expressed the genotoxic capabilities of these chemicals. Further, it was found that methyl parathion, as a result a single-exposure, was the most hazardous organophosphate tested showing definite pathology in the livers of treated rats. It was also concluded from genotoxicity studies of organophosphorus pesticides that methyl parathion had some genotoxic effects [64].

Neurotoxic Effects

A group of male Sprague-Dawley rats were treated with 3 mg/kg/day of methyl parathion (MP) for one or three weeks to observe the neurochemical and behavioral effects after repeated administration [65]. There was no evidence that repeated MP exposures caused significant decreases in body weight gain except on day 7, with a slight delay in growth observed on day 10. Weight gain after day 10 increased at a steady rate. Tremor, irritating, purposeless chewing and lacrimation were only slightly observed after repeated MP exposure at 3 mg/kg/day [65].

The behavioral effects associated with single and repeated doses of methyl parathion were studied using female Sprague-Dawley rats [60]. Rats treated with a single dose of 50 mg/kg of methyl parathion showed a total loss of spontaneous locomotor activity and a 90% reduction in neuromuscular coordination. Rats treated with a 12.5 mg/kg dose of methyl parathion showed a significant reduction in spontaneous locomotor activity and neuromuscular coordination 2 days after dosing; however recovery was obtained 7 days afterwards. Repeated administration of methyl parathion (1 mg/kg/day) decreased spontaneous locomotor activity and impaired memory.

In a previously mentioned study involving the exposure of adult male rats to 3 mg/kg/day of methyl parathion for 1 or 3 weeks, animals were sacrificed and their brains were removed to determine the level of acetylcholinesterase activity and binding of radioligans, [3H]QNB (nonselective), [3H]pirenzepine (M1 selective), and [3H]AF-DX384 (M2 selective) to muscarinic receptors. AChE activity measured in the brain indicated a 54 – 74% decrease in activity following weeks 1 and 3 of treatment. This experiment also found that acetylcholinesterase activity in the blood decreased after successive injections until minimal activity was reached and maintained throughout the treatment. Minimal activity was considered to be 30% of control AChE [65].

Another study involving [3H]QNB was completed to determine the *in vivo* and *in vitro* effects of methyl parathion on cholinergic neurotransmitter systems in the brain after exposure to 0, 0.1, and 1.0 mg/kg of methyl parathion [66]. This study revealed that exposure to 0.1 mg/kg of methyl parathion produced inhibition of acetylcholinesterase in the caudate-putamen and thalamic nuclei; however, 1.0 mg/kg resulted in inhibition of acetylcholinesterase in most brain regions, but these same doses had no effect on [3H]QNB binding to muscarinic receptors in the brain regions examined. Furthermore, the *in vitro* study resulted in preferential inhibitory effects of acetylcholinesterase and [3H]QNB binding in specific brain regions.

Methyl parathion was administered to adult female rats via intravenous, oral and dermal routes, and it was found that dermal administration of methyl parathion resulted in a dose-dependent inhibition of acetylcholinesterase that developed slowly and was prolonged; however, intravenous and oral administration of methyl parathion resulted in rapid decreases in cholinesterase activity which was later fully recovered within 30-48 hours [67]. This supports the claim that dermal exposure to methyl parathion is more effective than other routes of exposure.

Neurochemical effects associated with repeated methyl parathion (MP) exposure in neonatal and adult rats were studied to determine if there were any age related effects for methyl parathion. MP was administered to neonatal (7 days of age) and adult rats (90 days of age) daily for 14 days and neurochemical endpoints, cholinesterase inhibition, total muscarinic receptor ([(3H]quinuclidinyl benzilate, QNB) and muscarinic M2 subtype-preferential ([(3)H]AF-DX 384) binding in frontal cortex and striatum were measured at timepoints during (1 day after the 7^{th} and 14^{th} dose) and after (8 days after the 14^{th} dose) exposures [68]. This study found that ChE activity and muscarinic receptor binding were more reduced in neonatal brain regions as opposed to activity levels measured in adult brain regions following repeated exposure. In addition, the relationship between the degree of ChE inhibition and the reduction in cortical muscarinic receptor binding appeared different between the age groups with more extensive reduction noted in neonates as opposed to adults at a given level of ChE inhibition [68].

Reproductive and Developmental Effects

Several studies have demonstrated the detrimental effects of methyl parathion exposure to female and male reproductive organs. Studies of the placenta of rats treated pre-natally with methyl parathion showed detrimental results [69]. Rats were treated with 0.0, 1.0, 1.5 or 2.0 mg/kg of methyl parathion. In the presence of methyl parathion, trophoblast giant cells showed either significant degeneration or normal morphology with

many phagosome vacuoles containing cell debris that is believed to be dead cells from the maternal-placental interface. Other results associated with exposure to methyl parathion included fibrosis and hemorrhage in the decidua, decidual cells presenting with pyknotic nuclei and acidophilic cytoplasm and vascular congestion in the labyrinth [69].

The effects of methyl parathion on the period of when a female is willing to accept mating (estrous cycle) and reproductive performance in albino rats were studied to determine if exposure had any detrimental effects [70]. The animals were exposed intraperitoneally with doses of methyl parathion ranging from 1.5 to 3 mg/kg of body weight for 15 days. This study showed that while there were no significant changes in reproductive indices like pregnancy, parturition, live birth and viability with the exception of the viability index in the highest dose, there were significant changes in the duration of estrous cycle, duration of the proestrus and diestrus in the groups treated with 2.5 and 3 mg/kg [70].

Carcinogenic Effects

Although reviewed research [24] has indicated that there is no evidence that methyl parathion (MP) causes cancer to individuals regularly exposed to the chemical, other studies have shown that carcinogenic endpoints have been observed in rats. MP was administered to F344 rats and B6C3F1 mice in feed for the purpose of determining whether or not it has any carcinogenic effects. Groups were initially given 62.5 or 125 mg/kg of MP. Doses were decreased thereafter as a result of a decrease in mean body weight. As compared to the control groups, there was not a significant increase in the occurrence of tumors in either rats or mice. It was concluded that methyl parathion was not carcinogenic for either F344 rats or B6C3F1 mice [71]. Although it has been concluded that methyl parathion is not carcinogenic to animals or humans, a compilation of known adverse effects of agrochemicals was completed to assess the links between reproduction and health effects of these chemicals [72]. It was reported that chronic low-level exposure to parathion and methyl parathion from early infancy may lead to cancer formation later in life [1].

Regulatory Guidelines

United States federal agencies that develop regulations for toxic substances are the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA) and the Occupational Safety and Health Administration (OSHA). These agencies have implemented regulatory guidelines to protect the public health from the detrimental damage of chemical compounds. As required by the Federal Insecticide, Fungicide, and Rodenticide Act of (FIFRA), every pesticide product manufactured in the United Stated must be classified as either "general use" or "restricted use". According to FIFRA, a general use pesticide "when applied in accordance with its directions for use, warning and cautions will not generally cause unreasonable adverse effects on the environment." On the other hand, a restricted use pesticide "may generally cause, without additional regulator restrictions, unreasonable adverse effects on the environment, including injury to the applicator." Since 1978, methyl parathion has been considered as a "restricted use" pesticide by the U.S. EPA because of its toxic effects on exposed organisms [24, 25].

In August 1999, EPA accepted voluntary termination of methyl parathion application on certain items for dietary uses as well as non-food use items. Application of methyl parathion was prohibited for the next growing season (2000) on the following items: all fruits, carrots, succulent beans, succulent peas, tomatoes, artichokes, broccoli, brussel sprouts, cauliflower, celery, collards, kale, kohlrabi, lettuce, mustard greens, rutabagas, spinach, turnips, ornamental plants, grasses grown for seed, mosquito use, and nursery stock. EPA allows a maximum of 0.1 – 1 ppm of methyl parathion in or on crops that are still approved for methyl parathion usage [24]. EPA's water limits include the following: 0.3 mg/L for 1 or 10 days of exposure for children, 0.03 mg/L for longer term exposure for children and 0.002 mg/L for lifetime exposure for adults [24]. In addition, reentry into areas where the methyl parathion has been applied has been increased from 2 days to 4-5 days [24].

As previously mentioned, most incidents of methyl parathion exposure occur as a result of occupational exposure. The Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) set the permissible exposure limits (PELs), which are legal parameters, and the threshold limit values, which are recommended parameters based on scientific research [73-76]. Initially, OSHA had not set a PEL for methyl parathion; however, the ACGIH did set a TLV (threshold limit value) for the chemical. The average TLV for methyl parathion is 0.2 mg/m^3 over an 8-hour workday [73]. However, two years later, OSHA had proposed a final PEL based on an analogy of methyl parathion to a related substance, parathion, of 0.2 mg/m^3 TWA, skin [74]. It has been recommended that a person in the workplace should not be exposed to more than 0.2 mg/m^3 of methyl parathion for a 10-hour workday or 40-hour workweek [75]. A healthy worker can be exposed to this level of methyl parathion exposure without unreasonable risk of injury or disease.

In addition to aerial exposure, organisms are also exposed to methyl parathion via food and water. The World Health Organization has set an acceptable daily intake of 0.003 mg/kg of body weight established on the basis of a no observed adverse effect level (NOAEL) of 5 mg/kg obtained from combined studies conducted in humans [76].

Conclusions

A comprehensive review of the published literature indicates that methyl parathion is not persistent, bio-accumulative, or transferable through the food-chain. Exposure predominately occurs via occupational exposure or via food intake. Its primary route of exposure is via inhalation or skin contact. Most individuals should not be susceptible to methyl parathion

exposure due to its classification as a "restricted use" pesticide by EPA. This restriction not only eliminates the possibility of spraying in areas that may be extremely harmful to individuals (i.e., inside homes, offices, restaurants, etc); it also eliminates exposure from food chain contamination. Only those who are in direct contact (individuals involved in production of the chemicals, applicators, etc.) as well as those who live near areas where methyl parathion is applied and possibly disposed of, are at high risk.

Methyl parathion is an organophosphate that is capable of causing substantial health effects to exposed individuals. It enters the body and moves into the blood stream, brain and other organs where it elicits toxic effects, disturbances, chromosomal aberrations, and cardiovascular abnormalities. There is no evidence that MP causes birth defects in humans nor does it affect the ability of humans to produce children. There is also no proof that MP causes cancer in persons such as farmers and pesticide applicators who may be commonly exposed. However, laboratory animals chronically exposed to methyl parathion elicit cancer formation later in life.

There is evidence that methyl parathion causes neuropsychiatric disorders in humans after chronic exposure as well as hematological and ocular alterations. Acute exposure on the other hand, has been found to cause reduced cholinesterase levels in the brain, erythrocytes, and plasma. Clinical symptoms of acute exposure include tremors, convulsions, and cardiac arrhythmia. MP exposure has been also found to result in pulmonary edema, cardiovascular lesions, acute nephrosis of the kidney, and liver lesions.

Acknowledgements: This research was financially supported in part by the U.S. Department of Defense Cooperative Agreement (Grant No. W912HZ-04-2-2002), and in part by the National Institutes of Health - RCMI Program (Grant No. 1G12RR13459) at Jackson State University (JSU). We thank Dr. Abdul K. Mohamed, Dr. Richard Price, and Mr. Shelton Swanier for their support in this research.

References

1. Tourmaa, T E.: Adverse effects of agrochemicals on reproduction and health: A brief review from the literature. *Journal of Nutritional & Environmental Medicine* **1995**; *5(4)*:353-366.
2. United States Environmental Protection Agency. Methyl parathion risk management decision. *Updated 2 August* **1999**. Available [http://www.epa.gov].
3. Eskenazi, B.; Bradman, A.; Castorina, R.: Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environmental Health Perspectives,* **1999***, 107(3)*:409-419.
4. Cabello, G.; Valenzuela, M.; Vilaza, A.; Duran, V.; Rudolph, I.; Hrepic, N.; Calaf, G.: A rat mammary tumor model induced by the organophosphorus pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. *Environmental Health Perspectives,* **2001,** *109(5)*:471-479.
5. Kramer, R. E.; Wellman, S. E.; Rockhold, R. W.; Baker, R. C.: Pharmacokinetics of methyl parathion: A comparison following single intravenous, oral or dermal administration. *Journal of Biomedical Science*, **2002**; *9:*311-320.
6. Abu-Qare, A. W.; Abdel-Rahman, A. A.; Kishk, A. M.; Abou-Donia, M. B.: Placental transfer and pharmacokinetics of a single dermal dose of [^{14}C] methyl parathion in rats. *Toxicological Sciences* **2000***; 53*:5-12.
7. Baynes, R. E.; Bowen, J. M.: Toxicokinetics of methyl parathion in lactating goats. *Journal of Agricultural Food Chemistry,* **1995***; 43*: 1598-1604.
8. Carver, M. P.; Riviere, J. E.: Percutaneous absorption and excretion of xenobiotics after topical and intravenous administration to pigs. *Fundamental and Applied Toxicology,* **1989***; 13*: 714-722.
9. Draper, W. M.; Street, J. C.: Drift from a commercial aerial application of methyl parathion and ethyl parathion: An estimation of potential human exposure. Bull. *Environmental Contamination Toxicology,* **1981***; 26*: 530-536.
10. Nolan, R. J.; Rick, D. L.; Freshour, N. L.; Saunders J. H.: Chrorpyrifos: Pharmacokinetics in human volunteers. *Toxicology and Applied Pharmacology* **1984***; 73*: 8-15.
11. Wester, R. C.; Sedik, L.; Melendres, J.; Logan, F.; Haiback, H. I.; Russell, I.: Percutaneous absorption of diazinon in humans. *Food Chemistry Toxicology* **1993***; 31*: 469-572.
12. Salama, A. K.; Bakry, N. M.; Aly, H. A.; Abou-Donia, M. B.: Placental acid with transfer, disposition, and elimination of a single oral dose of [^{14}C-acetyl]acephate in Sprague-Dawley rats. **1992a**; *1:* 265-274.
13. Salama, A. K.; Bakry, N. M.; Aly, H. A.; Abou-Donia, M. B.: Placental and milk transfer, disposition, and metabolism of a single oral dose of [$^{14}CH_3S$] methamidophos in Sprague-Dawley rats. *Journal of Occupational Medical Toxicology,* **1992***; 1:* 275-291.
14. Villeneuve, D. C.; Willes, R. F.; Lacroix, J. B.; Phillips, W. E.: Placental transfer of ^{14}C-parathion administered intravenously to sheep. *Toxicology and Applied Pharmacology,* **1972***; 21*: 542-548.
15. Dyro, F. M.: Organophosphates. *eMedicine*. Updated 13 March **2003**. Available [http://www.emedicine.com/neuro/topic286.htm].
16. U.S. Environmental Protection Agency. Chemical Emergency Preparedness and Prevention: *EPA Chemical Profile*. Revised 30 November **1987**. Available:http://yosemite.epa.gov/oswer/ceppoehs.nsf/EHS_Profile?openform.
17. World Health Organization (WHO). Methyl parathion: Health and safety guide, 75, **1992**. Geneva, World Health Organization. *NLM Classification: 240.*
18. Hazardous Substances Data Bank (HSDB). Bethesda, Maryland, USA: *National Library of Medicine*, **2004**.
19. Ruckart, P. Z.; Kakolewski, K.; Bove, F. J.; Kaye, W. E.: Long-term neurobehavioral health effects of

methyl parathion exposure in children in Mississippi and Ohio. *Environmental Health Perspectives,* **2004***; 112(1)*:46-51.

20. United State Geological Survey. 1997 Pesticide use maps: Methyl parathion-insecticides estimated annual agricultural use. *Updated 13 August* **2003**. Available [http://ca.water.usgs.gov/cgi-bin/pnsp/pesticide_use_maps_1997.pl?map=W6042].
21. United States Environmental Protection Agency. Interim registration eligibility decision (IRED) Facts: *Methyl parathion. Washington, DC, USA*. **EPA-738-F-03-005**.
22. Garcia, S. J.; Abu-Qare, A. W.; Meeker-O'Connel, W. A.; Borton, A. J.; Abou-Donia, MB. Methyl parathion: A review of health effects. *Journal of Toxicology and Environmental Health,* **2003b**; *6*:285-210.
23. Rehner, T, A.; Kolbo, J. R.; Trump, R.; Smith, C.; Reid, D.: Depression among victims of south Mississippi's methyl parathion disaster. *Health & Social Work,* February **2000***; 25(1):* 33-40.
24. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for methyl parathion. *Atlanta, Georgia, USA,* September **2001**.
25. Agency for Toxic Substance and Disease Registry (ATSDR). Toxicological profile information sheet. *Atlanta, GA, USA*.8 December **2004**. Available [http://www.atsdr.cdc.gov/toppro2.html].
26. Mendola, P.: Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health. *Environmental Health Perspectives,* **2003***; 111:* 1939-1946.
27. Bradman, A.; Barr, D. B.; Claus Henn, B. G.; Brumheller, T.; Curry, C.; Eskenazi, B.: Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: A validation study. *Environmental Health Perspectives,* **2003**; *111*: 1779-1782.
28. Whyatt, B. M.; Barr, D. B.; Camann, D. E.; Kinney, P. L.; Barr, J. R.; Andrews, H. F.: Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. **2001**; *111*: 749-756.
29. Barr, D. B.: Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: A validation study. *Environmental Health Perspectives*, **2001**; *109*: 417-420.
30. Jirachaiyabhas, V.; Visuthismajarn, P.; Hore, P.; Robson, M. G.: Organophosphate pesticide exposures of traditional and integrated pest management farmers from working air conditions: A case study in Thailand. *International Journal of Occupational and Environmental Health,* **2004***; 10(3)*: 289-295.
31. Sakellarides, T. M.; Siskos, M. G.; Albanis, T. A.: Photodegradation of selected organophosphorus insecticides under sunlight in different natural waters and soils. *International Journal of Environmental Analytical Chemistry,* **2002***; 83(1)*: 33-50.
32. World Health Organization (WHO). Methyl parathion: *Environmental health criteria, 145***, 1993**. Geneva, World Health Organization. NLM Classification: WA 240.
33. Barr, D. B.; Turner, W. E.; DiPietro, E.; McClure, P. C.; Baker, S. E.; Barr, J. R.; Gehle, K, Grissom, Jr, RE, Bravo, R, Driskell, WJ, Patterson, Jr, DG, Hill, Jr, RH, Needham, LL, Pirkle, JL, Sampson, EJ. Measurement of p-nitrophenol in the urine of residents whose homes were contaminated with methyl parathion. *Environmental Health Perspectives,* **2002***; 110(6)*: 1085-1091.
34. Athanasopoulos, P. E.; Kyriakidis, N. V.; Stavropoulos, P. A study of the environmental degradation of pesticides azinphos methyl and parathion methyl. *Journal of Environmental Science Health,* **2004;** *39(2)*: 297-309.
35. Zhu, H.; Rockhold, R. W.; Baker, R. E.; Kramer, R. E.: Effects of single or repeated dermal exposure to methyl parathion on behavior and blood cholinesterase activity in rats. *Journal of Biomedical Science,* **2001***; 8:*467-474.
36. Sultatos, L. G.; Kim, B.; Woods, L.: Evaluation of estimations *in vitro* of tissue/blood distribution coefficients for organophosphate insecticides. *Toxicology and Applied Pharmacology*, **1990**; *103:* 52-55.
37. Extension Toxicology Network (EXTOXNET). Cholinesterase inhibition. *Toxicology Information Briefs*. Revised September **1993**. Available [http://extoxnet.orst.edu/tibs/cholines.htm].
38. Cecchine, G.; Golomb, B. A.; Hilborne, L. H.; Spektor, D. M.; Anthony, C. R.: A review of the scientific literature as it pertains to Gulf War illnesses; Volume 8. Santa Monica, CA: *RAND, MR-1018/8-OSD*, **2000**.
39. Slapper, D.: Toxicity, organophosphate and carbamate. *eMedicine*. Updated 29 December **1999**. Available [http://members.aol.com/DonationDrive/OrganphosToxemedicine.html].
40. Chambers, J. E. Carr, R. L.: Inhibition patterns of brain acetylcholinesterase and hepatic and plasma aliesterases following exposure to the phosphorothionate insecticides and their oxons in rats. *Fundamental and Applied Toxicology,* **1993**; *21:* 111-119.
41. Reddy, M. S.; Rao, K. V.: *In vitro* inhibition of Ca^{+2} ATPase by methyl parathion in prawn: A kinetic approach. *Biochemistry International,* **1990***; 22:* 1053-1058.
42. Hollingworth, R. M.; Metcalf, R. L.; Fukuto, T. R.: The selecticity of sumithion compared with methyl parathion. Metabolism in the white mouse. *Journal of Agricultural and Food Chemistry,* **1967***; 15*: 242-249.
43. Abu-Qare, A. W.; Abou-Donia, M. B.: Urinary excretion of metabolites following a single dermal dose of [^{14}C] methyl parathion in pregnant rats. *Toxicology,* **2000***; 150*: 119-127.
44. Hryhorczuk, D. O.; Moomey, M.; Burton, A.; Runkel, K.; Chen, E.; Saxer, T.; Slightom, J, Dimos, J.; McCann, K.; Barr, D.: Urinary p-nitrophenol as a biomarker of household exposure to methyl

parathion. *Environmental Health Perspectives,* **2002***; 110(6*):1041-1046.
45. California Department of Pesticide Regulation (CDPR). Evaluation of methyl parathion as a toxic air contaminant. Part C. *Human Health Assessment*, **1999**. TAC 99-02C.
46. Sanghi, R.; Pillai, M. K. K.; Jayelekshmi, T. R.; Nair, A.: Organochlorine and organophosphorus pesticide residues in breast mil from Bhopal, Madhya Pradesh, India. *Human & Experimental Toxicology,* **2003***: 22*:73-76
47. Rubin, C.; Esteban, E.; Kieszak, S.; Hill, Jr. R. H.; Dunlop, B.; Yacovac, R.; Trottier, J.; Boylan, K.; Tomasewski T.; Pearce, K.: Assessment of human exposure and human health effects after indoor application of methyl parathion in Lorain County, Ohio, 1995-1996. *Environmental Health Perspectives,* **2002***; 110(6)*:1047-1051.
48. Simpson, Jr, W. M.; Schuman, S. H.: Recognition and management of acute pesticide poisoning. *American Family Physician.* **2002***; 65(8*): 1599-1604.
49. Kamel, F.; Hoppin, J. A.: Association of pesticide exposure with neurologic dysfunction and disease. *Environmental Health Perspectives* **2004***; 112(9)*:950-958.
50. Nelson, L. M.: Epidemiology of ALS. *Clinical Neuroscience,* **1996***; 3:* 327-331.
51. Singh, B.; Dogra, T. D.; Tripathi, C. B.: A study of serum cholinesterase activity in agricultural and industrial workers occupationally exposed to organophosphates insecticides. *International Journal of Medical Toxicology,* **2002**; *5(2)*. Available http://www.ijmt.net/5_2/5_2_9.htm.
52. Fazekas, G. I.: Macroscopic and microscopic changes in Wofatox (methyl parathion) poisoning. *Zeitschift fur Rechtsmedizin (Journal of Legal Medicine)* **1971***; 68*: 189-194.
53. Fazekas, I. G.; Rengei, B.: Lethal Wofatox intoxication. *Orvosi Hetilap,* **1964**; *105*: 2335-2335.
54. Undeger, U; Basaran, N.: Effects of pesticides on human peripheral lymphocytes *in vitro*: induction of DNA damage. *Archives of Toxicology,* **2004***; 79(3)*: 169-176.
55. Czeizel, A. E.: Phenotypic and cytogenic studies in self-poisoned patients. *Mutation Research,* **1994***; 313*: 175-181.
56. Van Bao, T.; Bzabo, I.; Ruzicska, P.; Czeizel, R.: Chromosome aberrations in patients suffering acute organic phosphate insecticide intoxication. *Humangenetik,* **1974**; *24*: 33-57.
57. Cebrian, M. E.: Organophosphorus pesticide exposure increases the frequency of sperm sex null aneuploidy. *Environmental Health Perspectives*, **2001**; *109*: 1237-1240.
58. Padungtod, C.; Hassold, T. J.; Millie, E.; Ryan, L. M.; Savitz, D. A.; Christiani, D. C.; Xu, X.: Sperm aneuploidy among Chinese pesticide factory workers: Scoring by the FISH method. *American Journal of Indian Medicine*, **1999**; *36*: 230-238.
59. Zhu, H.; Rockhold, R. W.; Baker, R. E.; Kramer, R. E.: Effects of single or repeated dermal exposure to methyl parathion on behavior and blood cholinesterase activity in rats. *Journal of Biomedical Science,* **2001***; 8*:467-474.
60. Galal, E. E.; Latif, M. A.; Kandil, A.: The percutaneous cardiac toxicokinesis of anticholinesterase insecticides. *Journal of Drug Research,* **1975**; *7*: 20-43.
61. Suba, L. A.: Additional information to support the registration of methyl parathion: Two year chronic feeding study of methyl parathion in rats. *Monsanto Agricultural Products Company, St. Loius, MO.* **1984**.
62. Nehez, M.; Toth, C.; Desi, I.: The effect of dimethoate, dichlorvos, and parathion-methyl on bone marrow cell chromosomes of rats in subchronic experiments *in vivo*. *Ecotoxicology and Environmental Safety,* **1994***; 29(3*): 365-371.
63. Vijayaraghavan, M.; Nagarajan, B.: Mutagenic potential of acute exposure to organophosphorus and organochlorine compounds. *Mutation Research,* **1994***; 321(1-2):* 103-111.
64. Grover, I. S.; Malhi, P. K.: Genotoxic effects of some organophosphorus pesticides. I. Induction of micronuclei in bone marrow cells in rat. *Mutation Research,* **1985***; 155(3):* 131-134.
65. Sun, T.; Ma, T. Ho, I. K.: Differential modulation of muscarinic receptors in the rat brain by repeated exposure to methyl parathion. T*he Journal of Toxicological Sciences*, **2003**; *28(5)*: 427-438.
66. Ma, T.; Kramer, R. E.; Baker, R. C.; Fan, L. W.; Ho, I. K.: Effects of chronic dermal exposure to nonlethal doses of methyl parathion on brain regional acetylcholinesterase and muscarinic cholinergic receptors in female rats. *Journal of Neuroscience Research,* **2003***; 71(1)*: 138-145.
67. Kramer, R. E.; Wellman, S. E.; Zhu, H.; Rockhold, R. W.; Baker, R. C.: A comparison of cholinesterase activity after intravenous, oral or dermal administration of methyl parathion. *Journal of Biomedical Science,* **2002b***; 9(2)*: 140-148.
68. Liu, J.; Olivier, K.; Pope, C. N.: Comparative neurochemical effects of repeated methyl parathion or chlorpyrifos exposures in neonatal and adult rats. *Toxicology and Applied Pharmacology,* **1999**; *158(2)*: 186-196.
69. Levario-Carrilo, M.; Olave, M. E.; Corral, D. C.; Alderete, J. G.; Gagioti, S. M.; Bevilacqua, E.: Placental morphology of rats prenatally exposed to methyl parathion. *Experimental Toxicological Pathology,* **2004***; 55(6)*: 489-496.
70. Sortur, S. M.; Kaliwal, B. B.: Effect of methyl parathion formulation of estrous cycle and reproductive performance in albino rats. *Indian Journal of Experimental Biology,* **1999***; 37(2)*: 176-178.
71. National Cancer Institute. Bioassay of methyl parathion for possible carcinogenicity. Technical Report Series No. 157. Washington, DC, USA: U.S. Department of Health, Education, and Welfare, *Public Health Service*, **1979**.
72. International Agency for Research on Cancer. Methyl parathion: *Summary of data reported 1983*; 30. Updated 16 April **1998**. Available [http://www-cie.iarc.fr/htdocs/monographs/vol30/methylparathion.html].

73. Occupational Safety and Health Administration (OSHA). Sampling and analytical methods. Updated July **1987**. Available: http://www.osha.gov/dts/slts/methods/partial/t-pv-2112-01-8707-ch/t-pv2112-01-8701-ch.htm].
74. Occupational Safety and Health Administration (OSHA). *U.S. Department of Health Administration. Federal Register,* **1989**; *54*: 2923-2960.
75. National Institute for Occupational Safety and Health (NIOSH). *Pocket Guide to Chemical Hazards*. U.S. Department of Health and Human Services, Public Health Service, *Centers for Disease Control and Prevention Cincinnati, OH*, **2001**.
76. World Health Organization (WHO). Methyl parathion in drinking-water: Background document for development of WHO Guidelines for drinking-water quality. *WHO/SDE/WSH/03.04/106,* **2004**.