1982

Effect of dietary methionine on methylmercury and atrazine toxicities

Mohsen Meydani
Iowa State University

INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While most advanced technological means to photograph and reproduce this docun have been used, the quality is heavily dependent upon the quality of the mat submitted.

The following explanation of techniques is provided to help you underst markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the documer photographed is "Missing Page(s)". If it was possible to obtain the missir page(s) or section, they are spliced into the film along with adjacent page This may have necessitated cutting through an image and duplicatir adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is a indication that the film inspector noticed either blurred copy because movement during exposure, or duplicate copy. Unless we meant to dele copyrighted materials that should not have been filmed, you will find a goc image of the page in the adjacent frame. If copyrighted materials we deleted you will find a target note listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being phot graphed the photographer has followed a definite method in "sectionin the material. It is customary to begin filming at the upper left hand corner a large sheet and to continue from left to right in equal sections with sm overlaps. If necessary, sectioning is continued again—beginning below t first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerograph photographic prints can be purchased at additional cost and tipped into yo xerographic copy. Requests can be made to our Dissertations Custom Services Department.

5. Some pages in any document may have indistinct print. In all cases we ha filmed the best available copy.

University
Microfilms
International

300 N. ZEEB RD., ANN ARBOR, MI

Meydani, Mohsen

EFFECT OF DIETARY METHIONINE ON METHYLMERCURY AND ATRAZINE TOXICITIES

Iowa State University PH.D. 1982

Effect of dietary methionine on methylmercury and atrazine toxicities

by

Mohsen Meydani

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1982

TABLE OF CONTENTS

DEDICATION

To my wife, Simin Meydani

INTRODUCTION

Dietary protein offers strong protection in some toxicoses of drugs, pesticides, minerals and natural toxicants (Hathcock, 1976). The protective effect of dietary protein is partially related to the sulfhydryl containing amino acids such as cysteine and methionine. Dietary protein deficiency may increase the toxicity of heavy metals (Hathcock, 1976). High cysteine content of intestine, liver, and kidney metallothionein, a ligand which buffers heavy metal ion concentration (Cousins, 1979) suggests that deficiency of sulfur amino acids may increase heavy metal toxicity through limitation of metallothionein synthesis.

Both cysteine and methionine are the precursors of glutathione, a non-protein thiol compound which is present in all types of cells. Besides its reducing capability as a protective mechanism against hyperoxides, and other functions in the cell, glutathione conjugates histotoxic metabolites of certain drugs and toxicants (Arias and Jakoby, 1976).

Humans are surrounded with a world of chemicals. Each year 500-1000 new compounds are produced in commercial quantities (Damstra, 1978). Increases of population, industrialization and use of pesticides in food production results in human exposure to many different chemicals. Many industrial and agricultural chemicals may enter the environment as waste products, are discharged into the air, rivers, and lakes, and some eventually find their way into humans through contaminated food, water, and air.

Because of its extreme toxicity, multiple sources and several

accidental poisonings, methylmercury has been carefully studied with regard to its effect on human health and quality of life (Clarkson, 1976). There is a progressive increase in general background level of mercury in the industrialized societies. In addition to the emission of mercury through fossil fuel burning (Joensun, 1971), the extensive use of mercury in commercial and agricultural production adds significant quantities of mercury to the human environment (Lutz, 1967). The potential health hazard of methylmercury to laboratory and industrial workers has been recognized for many years, and disastrous outbreaks of methylmercury poisoning have occurred in Japan and Iraq in recent decades (Rowland et al., 1977; Bakir et al., 1973).

Atrazine is a herbicide which, due to its low toxicity, is widely used in agriculture. Its residues persist in the environment and in plants for a long period of time (Dalgaard-Mikkelsen and Poulsen, 1962).

Methylmercury is detoxified in the body in conjugation with cysteine and glutathione. Atrazine is also detoxified by conjugation with glutathione and is excreted in urine as mercapturic acid. Since cysteine and methionine are the precursors of glutathione, their dietary content of protein would be important with regard to chronic exposure of humans to these toxicants.

In general, methionine and cysteine are high in animal proteins but plants are poor sources of these sulfur amino acids. Soybeans, which are considered an excellent source of protein, contain protein in which methionine is distinctly the first-limiting amino acid. With increasing use of soybean or other legume seed products as meat extenders or re-

placers, the sulfur amino acid content of many diets may become more marginal. Therefore, studying the role of diets containing various levels of sulfur amino acids in the detoxification of methylmercury and atrazine can provide valuable information for establishing acceptable levels of these two toxicants and for setting dietary requirements of proteins (both quality and quantity).

The purpose of this study is to investigate the effect of supplementation of diet with excess methionine on toxicity of methylmercury hydroxide and/or atrazine and also to study the synergistic effects of atrazine on methylmercury toxicity. Biochemical as well as behavioral changes due to different treatments were measured for these purposes.

REVIEW OF LITERATURE

Toxicology of Methylmercury

Methylmercury (MeHg) poisoning has occurred in two distinct situations. One has occurred as a result of industrial waste products with inorganic mercury being discharged into the sea or rivers. The inorganic mercury was then converted to organic mercury compounds by small aquatic organisms and accumulated in higher trophic levels such as fish and shellfish (Hamdy and Prabhu, 1979). The consumption of such contaminated fish and shellfish resulted in poisoning. This occurred in Minamata Bay in 1953 and in Niigata in 1965 in Japan (Rowland et al., 1977; Kutsuma, 1968; Kurland et al., 1960). The syndrome of short-chain alkylmercurials poisoning is, therefore, called the Minamata disease. MeHg and ethylmercury are often grouped together as short-chain alkylmercurials. Other large outbreaks have occurred as a result of accidental consumption of grain treated with MeHg as a fungicide. This occurred in a major episode in Iraq in 1971 (Bakir et al., 1973; Skerfving and Copplestone, 1976). The organomercurial seed-dressing compounds protect the seed from a wide range of fungi and have a low toxicity to the seed. At least 6500 people were affected with 459 recorded deaths in Iraq (Bakir et al., 1973).

The syndrome of poisoning by short-chain alkylmercurials, Minamata disease, is characterized by paresthesia of the hand, foot, lips and tongue, by ataxia and by concentric constriction of visual fields (Skerfving and Vostal, 1972; Al-Damluji, 1976).

MeHg is a potent neurotoxin; the clinical patterns of organic and inorganic mercury poisoning are very different. Organic mercury affects

mainly the nervous system and produces neurological disease. Inorganic mercury affects gastrointestinal, renal and nervous systems, stomata and gingivi (Neal and Jones, 1938).

A limited conversion of inorganic mercury to alkylmercury in the body is possible. Rowland et al. (1977) demonstrated that bacterial flora of rat gut contents synthesized MeHg from mercuric chloride in vitro, and their estimation for the total amount of MeHg synthesized for ingested inorganic mercury in man was approximately 400 ng/day. Inversely, the biotransformation of alkylmercury to inorganic mercury in the body is also possible. Jacob et al. (1975) and Magos and Butler (1976) suggested that biotransformation of some forms of organic mercury to inorganic form is responsible for the toxicity of organic mercurials, and liver is the major site. Gallagher and Lee (1980), however, found that the biotransformation is not the only mechanism by which organic mercury exerts its toxicologic effects.

The chemical properties of MeHg (CH_3Hg^+) and mercuric mercury (Hg^{++}) are quite different. MeHg is monofunctional, so that it can react with only one ligand to give CH_3Hg-ligand complex, whereas Hg^{++} is bifunctional and it can react with two ligands to form ligand-Hg-ligand complex. Therefore, their behavioral differences in absorption, transportation, translocation and accumulation, and in general, toxic effects are related to these chemical properties.

MeHg is known to have a great affinity for sulfhydryl groups (-SH) and it is causes a decrease in -SH groups in the brain and liver of the rat (Pekkanen and Sandholm, 1971). Hirayama (1975) demonstrated that

MeHg makes complexes with cysteine through the -SH group of cysteine; the amino acid portion of the structure remains intact and acts as an active carrier of MeHg across membranes, including the blood brain barrier (BBB). Therefore, MeHg can be easily absorbed, transported, and distributed. On the contrary, Hg^{++} ion not only chelates the -SH group, but it also binds to other groups like $-NH_2$ and -COOH of cysteine. Since Hg^{++} can make a complex with two ligands simultaneously, it is not easily transported across the BBB, and this is why neurological effects of inorganic mercury compounds are not predominant.

The BBB is considered to be a complex of multiple systems regulating the exchange of metabolic materials between brain and blood (Steinwall and Klatzo, 1966; Lajtha, 1962). Amino acids enter into the brain through the BBB. Oldendorf and Szabo (1976) stated that the BBB transport of amino acids has been attributed to three independent transport systems: neutral, basic and acidic systems, similar to that in the intestine. The active transport of cysteine in the BBB contributes to the uptake of MeHg-cysteine complex in the brain (Hirayama, 1980). Hirayama (1980) also studied the effect of amino acids other than cysteine on brain uptake of MeHg, and found that the brain uptake of MeHg was depressed by phenylalanine and isoleucine which are neutral amino acids and not by lysine and glutamine which are basic and acidic amino acids, respectively. Depression of MeHg uptake of the brain by phenylalanine and isoleucine was attributed to the cross inhibitory effect of amino acids, which belong to the same carrier transport system (Oldendorf and Szabo, 1976).

Hirayama (1975) found that after subcutaneous administration of MeHg chloride to rats fed on a low cysteine diet, the brain showed significantly lower mercury levels than those of controls, but liver mercury levels were significantly higher than those of controls. Chang and Hartmann (1972a,b) observed dysfunction of rat BBB in mercury toxicosis and believed it to be due to an impairment of the endothelial and glial membrane by mercury. The impairment of the BBB has also been reported in other heavy metal toxicoses such as lead poisoning (Pentschow and Carro, 1966). Chang and Hartmann (1972a) also indicated that impairment of the BBB after mercury administration is responsible for the great reduction of the brain uptake of amino acids and other metabolites. Therefore, the neurotoxic effects of mercury on the central nervous system mainly depend on BBB function and the nature of the mercury compound. It is possible that at the early stages of mercury toxicosis, the presence or absence of sulfur amino acids might affect brain uptake of mercury and that later on, after BBB impairment, its uptake is not carrier dependent and might not be affected by the changes in the level of sulfur amino acids. The effect of MeHg upon nervous tissue is associated with the disturbance of protein synthesis *in vitro* (Yoshino et al., 1966) and *in vivo* (Cavanagh and Chen, 1971). Studies have shown the impairment of the incorporation of labeled amino acids into protein. Ultrastructurally MeHg appears to have a direct effect upon ribosomes in nerve cells (Herman et al., 1973; Jacob et al., 1977). The loss of protein synthesizing capability of the nerve cell may lead to its death.

Sulfhydryl groups are essential to the structural and functional integrity of cell membranes. Disturbance in the cell membrane function

may produce biochemical changes. Because the mercurials have a high affinity to the -SH group, and membranes contain -SH groups, membrane disturbance by mercurials is more likely. It was speculated that membrane permeability and enzymes which are located in the membrane were affected by mercurials (Clarkson, 1972).

In a repeated exposure of rats to MeHg, Magos and Butler (1976) found that the accumulation of mercury in kidney and blood was distinctly higher than that of other organs. The proportion of inorganic to total mercury remained as low as 6% in whole body, whereas in the kidney with increasing doses a higher proportion of mercury was in the inorganic form. They also stated that weight loss, change in kidney function and enterohepatic circulation of MeHg might also have contributed to a redistribution of mercury.

The injection of a single dose of MeHg hydroxide in mice and rats resulted in a high accumulation of mercury in the kidney, blood, and liver respectively (Ulfvarson, 1969a; 1969b). Mercury also binds to metallothionein in the liver and kidney. Methallothionein is a low molecular weight protein which is rich in -SH groups. It has been demonstrated that mercury causes an increase of metallothionein in the liver and kidney (Piotrowski et al., 1974; Winge et al., 1975). The binding of mercury to metallothionein has been shown to be influenced by the level of selenium (Se) in the kidney but not in the liver (Piotrowski et al., 1977).

Since protein synthesis is affected by MeHg and the deprivation of protein in rats was shown to cause an increase in -SH concentration in the cytoplasm of kidney cells, the levels of mercury and -SH were found to be parallel (Clarkson, 1972). Evidence indicates that virtually all

the mercury in the kidneys is in the form of methyl and ethyl mercuric salts, and intact MeHg radical may persist in animal tissues for many weeks after a single exposure (Norseth and Clarkson, 1970).

Liver is a major site for detoxification of mercury compounds and most of the alkyl mercurials are eliminated via the bile and urine. The accumulation rate is rapid and besides binding to metallothionein and the membrane, conjugation with glutathione (GSH) and cysteine also occurs. Enterohepatic circulation of the MeHg has been identified (Norseth and Clarkson, 1971).

The major portion of the circulating blood MeHg in the rat is in the erythrocytes, bound mainly to hemoglobin (Chen, et al., 1975), however in man and the rabbit it is bound mainly to low molecular weight substances, more likely GSH rather than hemoglobin within erythrocytes (Naganumra and Imura, 1979). One possible explanation for the distributional differences of MeHg within the erythrocytes of different species is that hemoglobin of the rat contains four -SH groups per molecule whereas those of rabbit and man have two -SH groups per molecule (Snow, 1962).

Although brain accumulates mercury to a lesser degree compared to kidney, blood or liver, signs of poisoning appear when the concentration in the brain increases more than the concentration of the whole body (Magos and Butler, 1976). Estimation of mercury concentration in the blood has been used in man for the diagnosis of body burden and brain mercury concentration (Swedish Expert Group, 1971). Magos and Butler (1976) stated that blood concentration of MeHg might be used with certain limitations for the calculation of MeHg concentration in brain; half time

value, and final whole body concentration of mercury should be considered in such calculations. Butterworth et al. (1978) studied the regional distribution of MeHg in the brain following a single intravenous administration of $CH_3{}^{203}HgCl$ in rats. They found that it took four days to reach peak accumulation of ^{203}Hg by the whole brain and delay in accumulation was regionally dependent. They found that the peak levels in cerebellum, medulla oblongata and mid-brain, for example, were attained sooner than those of hippocampus or cerebral cortex of frontal or occipital lobe. Therefore, the accumulation rates for different parts of brain are different. Chang and Hartmann (1972a) indicated that in rats treated with MeHg chloride, different parts of the nervous system accumulated mercury in the following order: dorsal root ganglion, calcarine cortex of occipital lobe and cerebellum (highest).

Further biochemical analysis of nerve cells revealed that the subcellular distribution of mercury in general was mitochondrial fraction > microsomal fraction > supernatant fraction > nuclear fraction. Thomas and Smith (1979) found that in brain MeHg formed a persistent complex with GSH and this complex accounted for about 30% of the total soluble MeHg burden.

Vitamin E and Methylmercury Interaction

Welsh (1974) was the first person to show that vitamin E has a protective effect against MeHg in fowl. Later, Welsh and Soares (1976), Welsh (1976, 1977), Sunde (1976), Chang et al. (1977) demonstrated that vitamin E, when given to quail and rats, reduced the toxic effects of MeHg in these animals and rendered better growth rates

and much longer life spans as compared to those animals exposed to MeHg alone. Kasuya (1975) showed that vitamin E has a direct protective effect on the toxic effect of MeHg on nervous tissue cultures. Chang et al. (1978) demonstrated the histological evidence of the protective effect of vitamin E on nervous tissue of golden hamsters. The precise protective mechanism of vitamin E against the toxic effects of MeHg is not well established. The ability of this vitamin to prolong survival of animals given MeHg may be a significant finding in terms of understanding the mechanism of the neurotoxicity of MeHg. A new theory about the interactive mechanism of vitamin E and MeHg will be discussed later.

Selenium and Methylmercury Interaction

More than a decade ago it was reported that Se counteracts acute mercuric chloride toxicity in rats (Parizek and Ostadalova, 1967). Later, Ganther et al. (1972) showed that dietary Se delays the effect of chronic toxicity of MeHg in rats. Since then, this effect has also been demonstrated with MeHg in other animals as well as rats (Stoewsand et al., 1974; Sell and Horani, 1976; Stillings et al., 1974).

Se affects the organ distribution of MeHg. It causes increased uptake of MeHg in rat brain while exerting a protective effect (Prohaska and Ganther, 1977; Magos and Webb, 1977; Ohi et al., 1975; 1976). Se supplementation seems to increase mercury concentration in the liver, spleen (Potter and Materone, 1974) and brain and decrease its concentration in the kidney (Alexander and Norseth, 1979). Because of the above mentioned effects, Se is protective against MeHg toxicity.

Alexander and Norseth (1979) demonstrated that Se inhibits biliary excretion of mercury in rats, and increases enterohepatic circulation of MeHg. They also indicated that even a selenite to mercury ratio of 1:40 affects the biliary mercury excretion. The increase in enterohepatic circulation of mercury by Se was accompanied by a decrease in mercury concentration in the kidney.

The effect of Se on mercury concentration in the brain is dose dependent and reaches a maximum at an equimolar selenite to MeHg dose ratio (Alexander and Norseth, 1979).

The theory that Se directly binds to MeHg and alters its toxicity can not explain the relatively low level of Se required for the exertion of protective action against MeHg. Other mechanisms must be involved in mercury/Se interaction. It has been shown that MeHg excreted into the bile is mainly bound to GSH (Refsnik and Norseth, 1975). Alexander and Norseth (1979) stated that in liver MeHg exists predominantly as a MeHg-protein complex, and Se might in some way alter the transfer of MeHg from the protein complex to the GSH or inhibit MeHg and GSH conjugation. Therefore, the level of dietary Se plays an important role in the excretion of MeHg through the bile.

Another possible interaction of Se and MeHg is through its effects on the activity of seleno enzyme glutathione peroxidase (GSH-Px). Se may preferentially bind to GSH-Px and protect against MeHg toxicosis (details to be discussed later). Diplock (1976) indicated that selenite was methylated through S-adenosylmethionine (SAM) and was excreted in urine as $^{+}Se\text{-}(CH_3)_3$ and exhaled as $^{+}Se\text{-}(CH_3)_2$. Therefore, Se metabolism

produces two or three S-adenosyl-homocysteines; subsequently two or three homocysteines with free -SH groups separated from S-adenosylcysteine, which can bind MeHg. Magos and Sell also proposed (personal communication[a]) that the methyl group might bind to -SH group and form mercurymethionine which is transported to the brain and is incorporated into protein. This theory might explain the increase in mercury concentration of brain with Se supplementation. Also, it is reasonable to assume that the presence of Se prevents MeHg from binding to GSH and promotes mercury-methionine formation.

Mechanism of Action

It is often proposed that the toxic effect of mercury is due to its binding to sulfur ligands in proteins. This theory, however, has not been definitely proven. Passow et al. (1961) indicated that mercury ions probably caused damage to the membrane structure by forming cross linkage with the protein moiety of the cell membrane resulting in the formation of an abnormal structure which in tern causes the impairment of membrane functions as well as increase in permeability. Kasuya (1972) postulated that organic mercury compounds bind to the cellular membranes and result in a degeneration of the membrane structure. These presumptions of mechansim of toxic action are based on the findings that some of the phospholipids such as sphingomyelin or phosphatidyl-L-serine show an inhibitory effect on the toxic effect of ethylmercuric chloride.

---

[a]Dr. Jerry Sell, Department of Animal Science, Iowa State University, Ames, Iowa.

Also a direct protective effect of vitamin E as a membrane stabilizer against the toxic effect of MeHg on nervous tissue cells has been shown (El-Bergrami et al., 1976; Kasuya, 1975; Welsh, 1974; Lucy, 1972; Brown and Pollock, 1972; Kurokama et al., 1970; Porta et al, 1968).

Another mechanism that has been considered is that some of the toxic effects of mercury result from forming complexes with biologically active Se, such as in glutathione peroxidase (GSH-Px) (Hoekstra, 1975; Ganther, 1975). The evidence for this mechanism is derived from the findings of Forseth et al. (1974) and Welsh (1974) who showed that MeHg may induce signs of Se deficiency. Subacute doses of MeHg also caused a slight reduction in GSH-Px activity in rat brain (Prohaska and Ganther, 1977).

There is a characteristic lag period of at least one week before the manifestation of poisoning with alkylmercury compounds (Clarkson, 1972); this lag period is present regardless of whether a single dose or continuous doses are involved. No mechanism for this lag period has been established.

Recently Ganther (1978) proposed a new theory of mechanism of action of MeHg toxicity. In this theory, the possible role of free radicals in MeHg toxicity was described. Basically, the theory proposed that the CNS damages exerted by MeHg may involve free methyl radical ($\cdot CH_3$) formation by the breakdown of MeHg and may not necessarily involve the intact molecule. This radical in turn will initiate the breakdown of MeHg. Presuming that free radicals are involved in the breakdown of the MeHg, and considering the MeHg physical properties, it might be taken up by the membrane in close proximity to lipids. It would then initiate

peroxidation of various lipid constituents as a result of its chemical tendency to undergo homolytic fission, perhaps initiated by radicals produced by oxygen dependent metabolic reactions. The onset of CNS damage would be preceded by a lag phase, during which the various systems defending against lipid peroxidation would be overcome, followed by a rapid and progressive degeneration of tissue (Ganther, 1978).

The theory of Ganther (1978) also explains the protective mechanisms of Se and vitamin E, as well as other antioxidants, against MeHg toxicity. Se as a component of GSH-Px which decomposes hydrogen peroxide and hydroperoxides would slow down the process of MeHg breakdown and methyl radical production. Vitamin E would modify MeHg metabolism by acting as a radical scavenger. It might be more efficient than other antioxidants because of its location in the membrane, and stablizing the membrane by interacting with unsaturated fatty acid chains.

Although there is no conclusive evidence in support of this theory at the present time, it seems to be the most logical interpretation of the mechanism of MeHg toxicity, and the protective effects of vitamin E and Se.

Toxicology of Atrazine

One of the most commonly used herbicides and plant growth regulating compounds of the aminotriazine series is atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), (Figure 1), (Dalgaard-Mikkelsen and Poulsen, 1962).

Simazine, Propazine, Trietrazine, Ipazine, Simetone, Prometone and

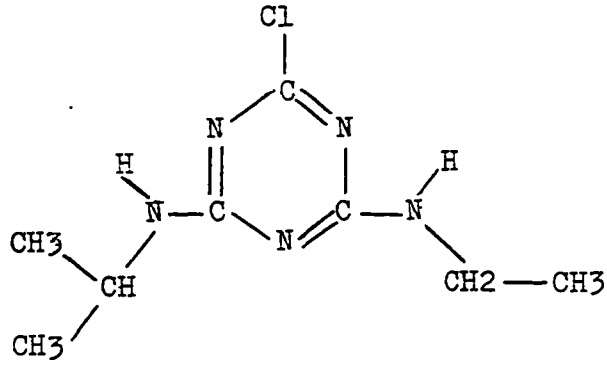

Figure 1. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5 triazine)

Atratone are the common names of triazine compounds with slight differences on the side chains of the triazine structure.

Atrazine and other similar compounds are mostly water-soluble and persist in soil (Dalgaard-Mikkelsen and Poulsen, 1962); therefore, residues of atrazine in soil may enter human and animal food. Its use with resistant crops (Shimabukuro et al., 1971) may also result in the accumulation of a residue in crops used for human and animal consumption.

Corn and sorghum metabolize atrazine to 2-hydroxyatrazine and conjugates with GSH and cysteine. The major detoxification of atrazine in corn and other plants is found to be through conjugation with GSH (Shimabukuro et al., 1970). The enzyme that catalyzes the reaction of atrazine with GSH in plants, GSH S-aryltransferase, has been partially

purified (Chasseaud, 1973). The activity of the enzyme in the plant determines its susceptibility to the herbicide atrazine (Shimabukuro et al., 1971).

Atrazine is rapidly metabolized after oral ingestion by the rat. Bakke et al. (1972) found that in the rat 65.5% of the metabolites were excreted in urine and 20.3% in feces. They identified and characterized atrazine metabolites. Dealkylation was also found to be a major detoxication mechanism in rat, rabbit, goat, sheep, and chicken (Foster and Khan, 1976). Rat liver has been shown to have glutathione-S-transferase enzyme, with a pH optimum of about pH 8, which catalyzes the conjugation of GSH with atrazine (Crayford and Hutson, 1972). Hutson et al. (1970) speculated that glutathione-S-aryltransferase could be involved in the metabolism of 2-chloro-triazines. The halogen group of the atrazine molecule was replaced by GSH and the conjugated product was excreted in the form of mercapturic acid. Crayford and Hutson (1972) indicated that the enzyme is located in hepatic cytosol. Johnson et al. (1972) indicated that this herbicide when applied to forage at the maximal recommended use rate, did not cause any adverse or toxicological effects in cattle or sheep.

Although atrazine is vastly used in agriculture, the residue alone in the food and environment is far less than apparently needed to cause any serious problem. It is possible that its toxicity would be synergized by other toxicants which deplete GSH in the body. Therefore, it can be employed in experiments as a GSH depleting agent.

Dietary Methionine and Glutathione

Methionine (Met) is an essential sulfur amino acid which is abundant in animal products such as meat, poultry, fish, milk, cheese and eggs (Mitchell et al., 1976). Met has several metabolic functions. As shown in Figure 2 (Mudd and Poole, 1975), the major functions of Met are its utilization in protein synthesis and conversion to SAM, cystathionine and cysteine.

The amount of Met required may be somewhat reduced if cysteine, a sulfur-containing nonessential amino acid, is supplied in the diet in adequate amounts (Mitchell et al., 1976). Addition of up to 1% DL-methionine in the diet was shown to improve growth rate and protein utilization of rats fed an 8% casein diet, but with the addition of 1.5-3% growth was inhibited and protein utilization was impaired (Harper et al., 1970).

Met is one of the most toxic amino acids (Hathcock, 1976). Amounts exceeding 2% of the diet will cause severe growth depression. Harper et al. (1970) stated that the inhibitory effects of Met on growth depend on type of protein in the diet. The addition of 2% Met to a diet containing 10% soy bean protein caused a 40% increase in growth, while the addition of 2% Met to diets containing 18-24% casein caused depression of growth rate to 50-80% of control value.

The major regulatory point in the metabolism of Met is the distribution of available Met between protein synthesis and SAM formation. The second control point is practically irreversible conversion to homocysteine, from which cystathionine and cysteine form by a

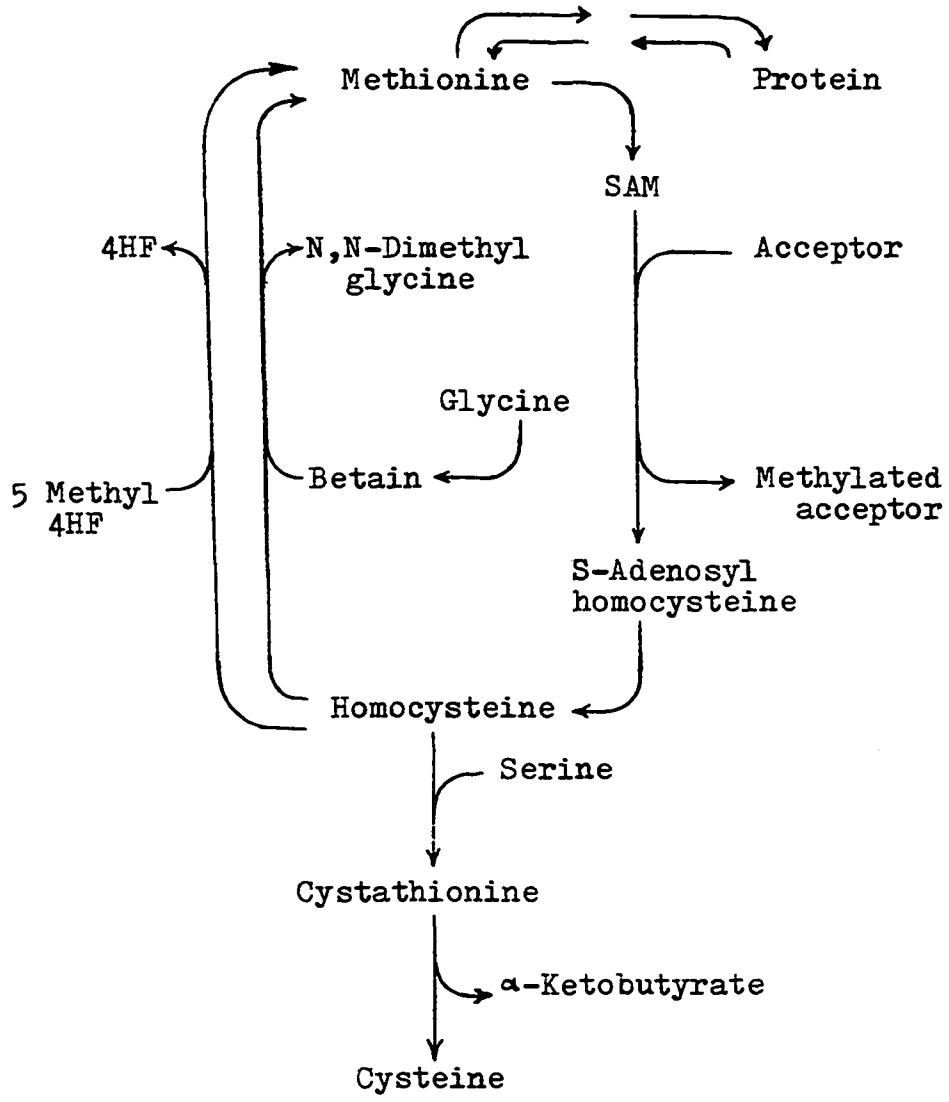

Figure 2. Metabolic pathways of methionine

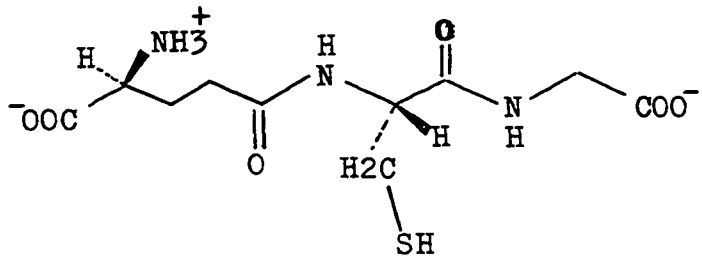

Figure 3. Glutathione (γ-L-Glutamyl-L-Cysteinylglycine)

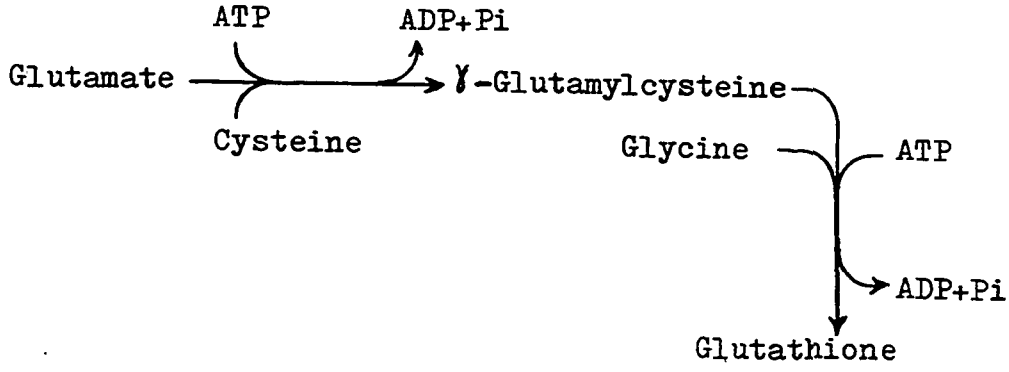

Figure 4. Biosynthesis of glutathione

transsulfuration pathway (Finkelstein et al., 1980). The activity of several of the enzymes involved in Met metabolism may be modified by changes in the concentration of sulfur containing amino acids in body fluids and by the action of hormones (Harper et al., 1970).

Met can be a precursor of GSH. GSH is an endogenous protective tripeptide (Figure 3) which is synthesized in cytosol by two ATP-dependent steps and utilizes glutamate, cysteine and glycine (Figure 4). Cysteine is an immediate precursor of tissue GSH, whereas Met is a more

distal precursor of GSH and it operates through a transsulfuration pathway.

Isolated hepatocytes have been shown to be capable of biosynthesis of GSH from exogenous methionine through the cystathionine pathway (Thor et al., 1979; Krebs et al., 1978). Campbell et al. (1978) and Poirier et al. (1977) showed that a methionine deficiency with other lipotrope deficiencies did not alter the hepatic level of GSH, while it caused a decrease in SAM in male rat liver.

The capability of isolated kidney cells in catalyzing this pathway is less than that of hepatocytes, and the cysteine generated by the cystathionine pathway in kidney cells is not sufficient to support biosynthesis of GSH (Ormestead et al., 1980).

GSH is the major nonprotein thiol compound found in all types of living cells. It occurs in a dynamic state, and there is an interorgan cycle of glutathione metabolism. Anderson and Meister (1980) indicated that glutathione is translocated to plasma in the form of GSH which constitutes the major source of plasma thiol. Griffith and Meister (1979) stated that substantial amounts of GSH are translocated from liver and probably from other tissues in the blood plasma. The thiol group (-SH) of GSH is the most chemically important active group in its biological and biochemical function. However, in the γ-glutamyl cycle for amino acid transport across the plasma membrane the -SH group is not involved.

GSH has a potent oxidation-reduction capability and it plays a protective role against hydroperoxides and prevents the formation of the hydroxy radicals (·OH) (Sunds and Hoekstra, 1980) by the formation of

glutathione disulfide (GSSG). The enzymes involved in the oxidation and reduction of GSH are glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd), respectively.

GSH plays an important role as an intracellular reducing agent and protects the -SH groups of proteins from oxidation. It also regulates mitosis, cytoskeletal structure, intermediary metabolism of enzyme activity, and also regulates the synthesis of macromolecules (Suojanen et al., 1980). GSH serves as a coenzyme for several enzymes (Metzler, 1977). Conjugation of compounds with GSH is either direct or following activation by a cytochrome P-450 dependent oxidation. Conjugation may occur spontaneously or may be catalyzed by glutathione S-transferase (GSH S-trans) (Moldeus et al., 1978). The conjugated compound then can undergo mercapturic acid biosynthesis as shown in Figure 5 (Chasseaud, 1976).

Sulfur amino acids are also important in other detoxication mechanisms such as mixed-function oxidase (MFO) enzymes which metabolize a variety of substances. Edes et al. (1979) has shown that Met and cysteine deficiency causes a reduction of intestinal and hepatic MFO enzyme activity. Therefore, dietary sulfur amino acids, in addition to their important role in growth, protein synthesis and other biological functions, play an important role in the metabolic fate of numerous toxic compounds in human and animals.

Functions of Glutathione Peroxidase and Reductase

A "glutathione peroxidase system" consists of GSH-Px, GSH-Rd, and glucose-6-phosphate dehydrogenase (G6PD) which function as a metabolic

unit in the reduction of peroxides (Reddy and Tappel, 1974). GSH-Px utilizes GSH oxidation capability and appears to function in animals as a protective enzyme that guards against peroxidative damage. The enzyme was discovered by Mills (1957). It was shown to protect the erythrocyte against hemoglobin oxidation and hemolysis (McCoy and Weswing, 1969). It has also been shown that there is a GSH-Px which is a selenoenzyme. Flohe et al. (1973), Nakamura et al. (1974), and Awasthi et al. (1975) demonstrated that GSH-Px contained 4 gram-atoms of Se bound to cysteine, and it is present as selenocysteine (Cone et al., 1976, 1977; Frostrom et al., 1978; Zakowski, 1978). Se is essential for the enzymatic activity of GSH-Px, and it goes through an oxidation reduction cycle during catalysis (Flohe et al., 1973).

Animals maintained on Se deficient diets show a rapid decline of tissue GSH-Px activity (Cantor et al., 1975). Selenite, selenomethionine, and selenocysteine generally have good biopotency for the synthesis of GSH-Px (Pierce and Tappel, 1977; Hawkes et al., 1979). However, it has been reported that selenate does not have such biopotency, and it is not the form used for GSH-Px biosynthesis (Germaine and Arneson, 1977). Hydrogen peroxide as well as organic hydroperoxides are substrates for GSH-Px (Rotruck et al., 1973; Little and O'Brien, 1868a, 1968b; Christophersen, 1968, 1969). Activated oxygen and free radicals, as products of normal cellular reactions or as products of the metabolism of toxic substances, may attack cellular components and cause hydroperoxide formation. GSH-Px together with superoxide dismutase may prevent the hydroxy radical (·OH) formation by reducing the

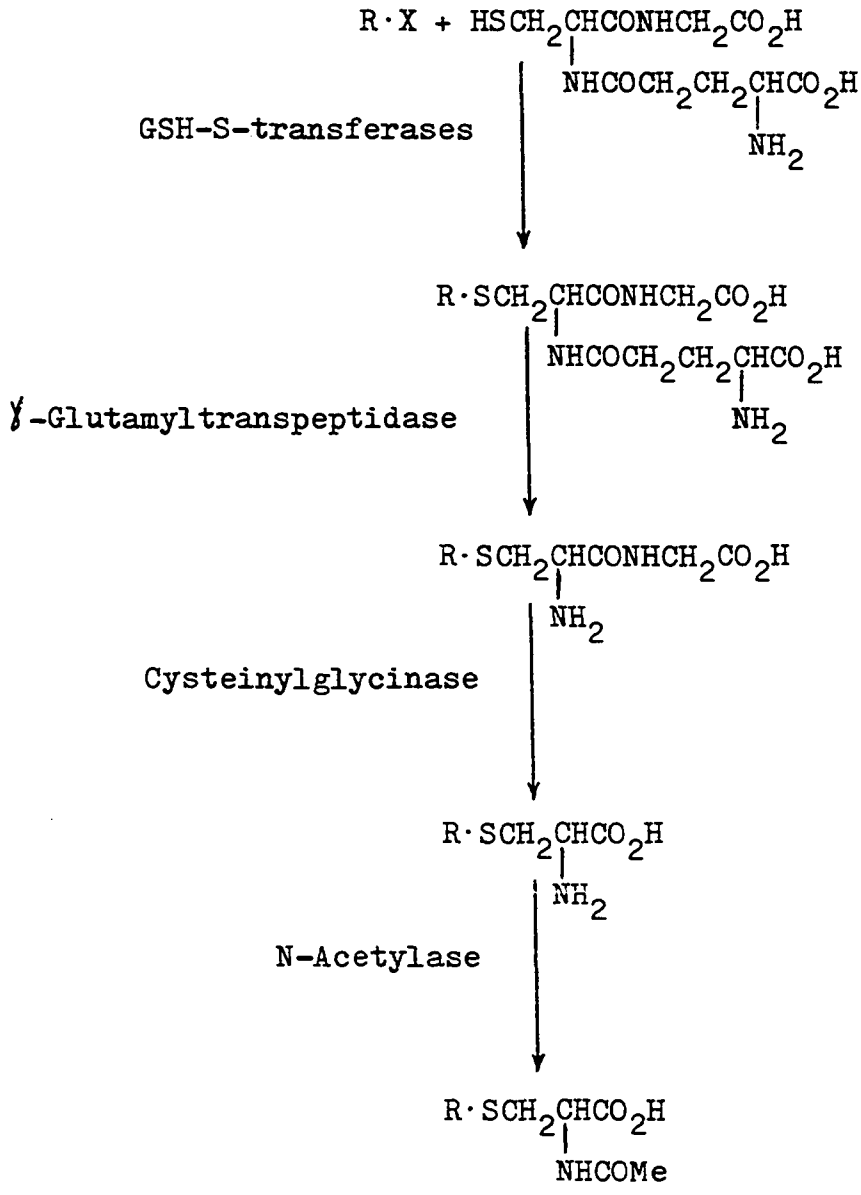

Figure 5. Glutathione conjugation and mercapturic acid synthesis

concentration of superoxide and hydrogen peroxide so they cannot react with one another (Sunde and Hoekstra, 1980).

It is known that vitamin E scavenges free radicals and possibly quenches activated oxygen. Since vitamin E is lipid soluble, it reacts on the membrane and is a lipid antioxidant (Tappel, 1962), whereas GSH-Px is located in the cytosol and mitochondria and guards cytoplasm against peroxidative damage. Sunde and Hoekstra (1980) stated that although lipid hydroperoxides are excellent substrates for GSH-Px, this soluble enzyme does not reduce lipid hydroperoxides to the corresponding alcohols in vitro. Instead GSH-Px prevents the peroxidation by destroying hydrogen peroxide, and thus preventing the formation of hydroxy radicals. When autooxidized lipids were fed to rats which were receiving diets low in Se, hepatic GSH-Px activity was shown to be increased (Reddy and Tappel, 1974).

The effects of vitamin E on the activity of GSH-Px of liver are controversial. Fukezawa and Tokumura (1976) have shown that hepatic GSH-Px activity decreased in mice with vitamin E deficiency, whereas Torstler et al. (1979) demonstrated that the activity of GSH-Px of liver increased in rats fed low vitamin E and Se diet. Recently, Lawrence and Burk (1976) reported an increase of GSH-S-transferase activities in the liver of Se deficient rats which had low levels of GSH-Px. They also showed that there are at least two GSH-Px activities in rat liver. One is Se-dependent GSH-Px which utilized hydrogen peroxide as well as many organic hydroperoxides as substrates, and the other is a

Se-independent GSH-Px which has been shown to have the activity of several GSH-S-transferases. The Se-independent enzyme cannot use hydrogen peroxide as a substrate, but protects against lipid peroxidation in the NADPH-microsomal lipid peroxidation system (Burk et al., 1980). In some tissues, the peroxidase activity from the transferase is much higher than that of GSH-Px and it has been shown that guinea pig liver has no detectable Se-dependent GSH-Px activity (Prohaska, 1980). Therefore, the GSH-S-transferase also may be important in the protection of cells against peroxidation.

It is also postulated that GSH-Px may have a specific function in the metabolism of prostaglandin hydroperoxide (Nugteren and Hazelhof, 1973; Hazelhof and Nugteren, 1978), and of arachidonic acid in platelets (Bryant and Bailey, 1980; Ohki et al., 1979).

Since there is an interaction between Se and mercury, it is possible that the enzyme activity of GSH-Px is affected by MeHg. Subacute doses of MeHg have been shown to cause a slight reduction in GSH-Px activity of rat brain (Prohaska and Ganther, 1977). Hirota et al. (1980), investigated the effect of MeHg on the activity of GSH-Px in 100,000 g supernatant of rat liver homogenate in vitro. They found marked inhibition of enzyme activity with concentrations of MeHg between 5×10^{-6}M and 5×10^{-5}M, but activity was hardly inhibited at concentrations less than 5×10^{-6}M MeHg. They also showed that inhibition was complete at concentrations greater than 5×10^{-5}M MeHg.

GSH-Rd is a flavoenzyme which catalyzes the pyridine nucleotide-dependent reduction of GSSG to reduced glutathione (GSH) and maintains

a high ratio of GSH/GSSG in cells. It is also a radical scavenger, and may regenerate other antioxidants such as α-tocopherol and serves to activate enzymes through disulfide exchange (Pryor, 1976). The activity of this enzyme is quite high in liver; thus, liver has a high capability to cope with conditions which bring about rapid oxidation of GSH (Moron et al., 1979). Kum-Tatt et al. (1975) indicated from their findings that GSSG is catalyzed by one type of GSH-Rd enzyme which is nonspecific for NADH and NADPH.

In the erythrocyte, GSH-Px, GSH-Rd and G6PD function as a single unit for peroxide reduction, and only the hexose mono-phosphate shunt produces NADPH for this unit but in the liver the G6PD serves an important role in regeneration of NADPH for fatty acid synthesis and there is a linear relationship between GSH-Rd and G6PD in mice. Thus, the maintenance of GSH-Rd need not be linked solely to peroxide reduction (Torstler et al., 1979).

Webb (1963) stated that inorganic mercury can inhibit GSH-Rd activity. Pekkanen and Sandholm (1972) found that rat liver GSH-Rd was not affected by MeHg, but brain GSH-Rd activity was decreased by MeHg administration. In addition, Mykkanen and Ganther (1974) found that blood GSH-Rd activity in rats and quail fed as high as 30 ppm MeHg were not affected. Furthermore they showed that there was a great difference between the effect of inorganic and organic mercury on the activity of rat erythrocyte GSH-Rd. A concentration of 1.6 ppm mercury as mercuric nitrate added to blood in vitro, resulted in 30-40% of decrease in activity of GSH-Rd, whereas about 200 ppm mercury as MeHg was necessary for the

same inhibition. They speculated that differences between organic and inorganic mercury in the inhibition of GSH-Rd activity might be due to structural differences which result in a preference of GSH-Rd for binding mercuric mercury or in a preference of hemoglobin in the hemolsate for binding MeHg.

It is known that GSH conjugates MeHg and atrazine as part of the detoxication mechanisms of liver. Since availability of GSH as a substrate for GSH-Px will be decreased by exposure to MeHg and atrazine, it is very likely that the activity of GSH-Px and interrelated enzymes like GSH-Rd, and glutathione-S-transferases also will be altered by exposure to these toxicants.

Functions of Glutathione-S-transferases

Conjugation with GSH is one of the biotransformation processes that generally results in less toxic products. The glutathione-S-transferases (GSH-S-trans) are a group of soluble enzymes that catalyze the conjugation of GSH with a wide range of electrophilic agents (Arias and Jakoby, 1976). Several GSH-S-transferases have been identified in the rat liver cytoplasmic fraction. They are transferases AA, A, B, C, D, and E, and they have been shown to have very broad and overlapping substrate specificities (Habig et al., 1974; Keen et al., 1976; Jakoby et al., 1976). Multiple forms of these enzymes were also found in liver of the monkey (Asaoka et al., 1977) and man as well (Kamisaka et al., 1975).

GSH-S-trans enzymes constitute about 10% of the total extractable soluble protein from rat liver (Jakoby, 1978). GSH-S-trans B has also been identified as the cytoplasmic electrophile binding protein ligand in liver (Habig et al., 1974).

There is evidence that these enzymes are present in the kidney (Kaplomitz et al., 1976), small intestine (Pinkus et al., 1977), lung (Guthenberg and Mannervik, 1979), and steroidogenic organs (Bend et al., 1977). Several studies, usually on the rodents, have shown that GSH-S-trans activities towards a range of electrophiles are generally highest in liver and steroidogenic organs such as adrenals, ovary and testis (Kraus and Kolft, 1980; Chasseaud, 1979; Datta et al., 1973). Kraus and Kolft (1980) found that activities of these enzymes in the heart, brain, spleen and lung were relatively low or nil for most substrates. Although the presence of these enzymes was determined in the other organs, the present data do not permit one to evaluate the role of different tissues in catalyzing the conjugation of GSH with electrophilic xenobiotics. Kraus and Kolft (1980) also speculated that the presence of GSH-S-trans in the other organs might protect nucleophilic components of tissue from denaturation by electrophilic reactants, or they might be needed for transport of protein. Transferase activities in the soluble fraction of liver of mice were shown to have significant differences between the male and female (Hayakawa et al., 1974). It was shown that male mice had twice as much activity as female mice with naphthalene-1,2-oxide as the substrate; however, when styrene oxide was used as the substrate, no significant differences between the sexes

were observed.

The enzyme activities also appear to be age dependent (Mukhtar et al., 1979). Hayakawa (1977) stated that livers from 17 to 18 day rat embryos showed almost no activity; however, the activity appeared and sharply increased at about three weeks of age and reached a maximum at about six weeks of age.

GSH-S-trans has other detoxification functions in addition to GSH conjugation. One is the binding of a large number of xenobiotics as well as a bilirubin, until they are converted to less toxic compounds (Wolkoff et al., 1978). GSH-S-trans also has been claimed to have activity as a scavenger protein (Smith et al., 1977). In addition, the intermediate reactive products of drugs biotransformation which are thought to play an important role in carcinogenesis, mainly bind covalently to a ligand which is identical to GSH-S-trans B.

The hepatic cytoplasmic treatments such as phenobarbital, DDT, methylcholanthrene, trans-stilbene oxide and rifampcin, as well as fasting and some other metabolic disturbances, induce these enzymes (Younes et al., 1980; Friedberg et al., 1979; Down and Chasseaud, 1979). Inducibility of GSH-S-transferases in hepatocytes of two species of non-human primates is lower than in the rodents (Down and Chasseaud, 1979).

Microsomal GSH-S-trans activity in rat liver was also reported by Booth et al. (1961). Such activity was reported also in the microsomes and mitochondria of rat liver (Kraus and Gross, 1979) and in mouse liver (Glatt and Oesch, 1977).

The catalytic characteristics of microsomal GSH-S-transferases are

quite different from cytoplasmic ones. The enzyme cannot be removed from the membrane, but the enzyme is at least partially exposed on the cytoplasmic surface of endoplasmic reticulum (Morgenstern et al., 1980). Three microsomal GSH-S-transferases were identified in the rat liver by Friedberg et al. (1979), and they found that the pattern of GSH-S-transferases in the microsomal fraction is similar to that in the cytoplasm. However, the specific activity of cytoplasmic enzymes was shown to be increased by inducer agents, but microsomal enzyme activity was not affected (Friedberg et al., 1979; Morgenstern et al., 1979). Morgenstern et al. (1979) proposed that the -SH group is involved in the activation of microsomal GSH-S-transferase activity and presented evidence that activation results from attack on the microsomal sulfhydryl group(s). Membrane bound GSH-S-transferase may be advantageous in the inactivation of reactive metabolites formed within the microsomal membrane, which are lipophilic and therefore remain within the membrane rather than diffuse into the cytoplasm (Friedberg et al., 1979).

Prostaglandins

The prostaglandins (PGs) are derivatives of essential fatty acids. Each compound has a five membered ring structure and two side chains. They seem to be synthesized by all mammalian cells with the possible exception the mature red blood cells. The PGs are realized to be involved in the fundamental responses of cells and organs (Horrobin, 1978). In PG synthesis, endoperoxide formation is the critical point. Regulation of PG synthesis may depend on regulation of essential fatty acid

metabolism (Van Drop, 1971; Ramwell et al., 1977), regulation of lipase activity (Sameulsson, 1969; Ramwell et al., 1977; Flower, 1977), cofactors, and steroid hormones (Horrobin, 1978).

Some compounds may inhibit or stimulate biosynthesis of PGs. From the view point of my interest, it was documented that GSH enhances PGE_2 production at the expense of the other PGs. Horrobin (1978) stated that omission of GSH tends to increase production of other PGs, including PGF. The omission of GSH did not alter oxygen consumption although PGE_2 formation was reduced. Ho et al. (1976) found that there was no effect of GSH on thromboxane B_2 (TXB_2) production while Tai and Yuan (1977) reported some stimulation of TXB_2 synthesis.

Since oxidation and reduction play a critical role in the formation of PGs, agents which modify redox states may modify PG synthesis (Nugteren and Hazelhof, 1973). Antioxidants such as α-tocopherol inhibited PG synthesis (Nugteren, 1970; Lands and Rome, 1976); however, at low concentrations, synthesis might be enhanced, especially the PGEs.

Morse et al. (1977) reported a stimulatory effect of hydrogen peroxide on PG synthesis in various tissues. They stated that H_2O_2 or some product derived from this molecule might act in concert with or on the prostaglandin endoperoxide forming cyclooxygenase or on some substrate formed as a consequence of activity of this enzyme. They suggested that H_2O_2 and organic peroxides derived from it serve as donors of some electronically activated species of oxygen (such as hydroperoxy free radical HOO., or the peroxy radical .OO.) that is uniquely reactive and suitable as substrate for the cyclooxygenase-

catalyzed addition of oxygen.

Horrobin et al. (1978) suggested a decrease in PGE_1 synthesis due to excess methionine. They stated that Met gives rise to taurine which decreases PGE_1 synthesis via inhibition of the prolactin effect on PGE_1 production in brain tissue. Both PGE_1 and TXA_2 were shown to be involved in anxiety and depression (Horrobin, 1978).

Although there are no reports indicating the effects of MeHg and atrazine on PG synthesis, consequent to these points it is more likely that PG synthesis could be altered indirectly by these toxicants possibly through an effect on the GSH and/or GSH-Px systems.

Significance of Behavioral Studies in Toxicant and Nutrient Interactions

Although the deleterious effects of many substances have been known for many years, their effects on behavior have not been well documented. Chronic low dose exposure of man and animal to toxicants might produce no observable pathological or biochemical changes, whereas behavioral impairment might be present. Behavioral measures might provide more sensitive indices of the consequences of exposure to the environmental toxicants than clinical measures.

The nervous system serves to maintain homeostasis under certain input/output conditions. When output is generated which involves the motor system a measurable behavior occurs (Reiter, 1978). Behavioral impairment might not necessarily result from the effect on the particular function or structure of nervous system. Reiter (1978) stated that a chemical might act to disrupt the input/output relation-

ships either by directly affecting some levels of neuronal organization or by interacting with other organ systems, thus affecting nervous system function indirectly.

The nervous system is not often identified as the primary site of damage by MeHg compounds. Behavioral and neurological symptoms are virtually the only ones detectable in very low chronic exposure. Few overt neurological signs appear before advanced stages of toxicity are manifested (Hughes et al., 1975). A wide variety of behavioral tests such as open field behavior, active avoidance, learning ability, operant reinforcement, and discrimination behavior have been employed with animals to assess MeHg effects on motor and sensory systems.

Suzuki and Miama (1971) demonstrated in mice that when mercury was accumulated slowly, symptoms were more associated with lower brain concentrations than when accumulation was rapid. Lown et al. (1977) indicated that the route of administration of 10 mg mercury as MeHg per kg of body weight is important in behavioral impairment in rat. They found that ambulation and rearing of rats were depressed in open field behavior tests when MeHg was administrated intraperitoneally whereas other routes of administration of MeHg did not affect the open field behavior of rats. They also found that behavioral alterations were not directly related to brain mercury level following acute administration of MeHg by different routes, but liver and kidney mercury levels were significant predictors of behavior. Reduced locomotion and rearing in the open field in rats exposed to high doses of MeHg were observed by Post et al. (1973). They found that rats required significantly

more trials to learn a T-maze after oral administration of MeHg in a single dose of 20 mg mercury per kg body weight.

Zenick (1974) showed that the ability of rats to swim through a water maze was impaired following an indeterminate dose of MeHg given at early stages of development. Hughes et al. (1975) demonstrated that in rats exposed to small amounts of MeHg at age 28-42 days, the ability to learn an active avoidance response as adults was impaired. Evans et al. (1975) used visual form and brightness discrimination to study chronic MeHg intoxication in monkeys and found significant alterations.

Since MeHg accumulates in brain, it may cause an alteration in the neurotransmitter synthesis. Acetylcholine (Ach) is one of the several transmitters which is synthesized in the neurons. Kobayashi et al. (1980) postulated that MeHg poisoning might reduce Ach concentration and turnover rate in brain of mice. PGE_2 and 11-thiol-11-deoxy PGE_2 were shown to possess an anticholinergic effect (Bloss and Singer, 1978). Since PGs effects on brain function might be dependent on modulation of transmitters actions (Horrobin, 1978), it is possible that changes in PG synthesis by MeHg through alteration in the GSH-Px system cause a change in neurotransmitter concentration or turnover rate which in turn alters the behavior of the animal. Possibly excess Met exerts its neurological effect through alteration in PG synthesis (Horrobin et al., 1978).

As shown by the studies cited above it is clear that behavioral alteration in animals by toxicant and nutrient interaction should be

considered closely and the mechanism of action and biochemical pathways which are involved in certain behavioral responses should be investigated.

Summary and Conclusions

Exposure to methylmercury and atrazine has deleterious effects on the physiological homeostasis of the human and animal body. Methylmercury and atrazine are both introduced into the environment and food chain through industrial and agricultural activities (Rowland et al., 1977; Shimabukuro et al., 1971). Accidental consumption of agricultural commodities containing some amount of these toxicants has resulted in some tragic human episodes (Bakir et al., 1973; Skerfving and Copplestone, 1976).

The neurotoxicity of methylmercury is well-documented (Thomas and Smith, 1979; Chang and Hartmann, 1972a, 1972b; Skerfving and Vostal, 1972). The detoxification mechanism for methylmercury and atrazine have been studied by Norseth and Clarkson (1970, 1971), and by Crayford and Hutson (1972), respectively. Both toxicants are shown to be conjugated with GSH and excreted partially as mercapturic acids in urine. The conjugation is catalyzed by GSH-S-trans, of which several have been identified (Habig et al., 1974; Keen et al., 1976; Jakoby et al., 1976).

GSH is the major nonprotein thiol compound in all types of living cells. It also plays a protective role against hydroproxides and hydroxy radicals (Sunde and Hoekstra, 1980). Since both toxicants are conjugated with GSH, they might have the capability of depleting GSH of human and

animal bodies exposed to these toxicants for long periods of time. Therefore, GSH biosynthesis and GSH-Px, and GSH-Rd activities possibly will be altered in this condition.

There are significant numbers of studies on the role of Se and vitamin E in relation to mercury toxicity (Welsh, 1976, 1977; Kasuya, 1975; Ganther, 1978; Alexander and Norseth, 1979). Since there is an interaction between Se and mercury, the activity of selenoenzyme GSH-Px may be altered through such interactions.

Synthesis of PGs might also be affected by both toxicants indirectly, probably through changes in GSH level or GSH-Px activity in the tissues (Horrobin, 1978; Tai and Yuan, 1977). Increase of endogenous hydrogen peroxide may also elevate PG synthesis (Morse et al., 1977). The role of antioxidant α-tocopherol and methionine on PG synthesis has also been studied (Nugteren, 1970; Lands and Rome, 1976; Horrobin et al., 1978).

The behavior of animals has been shown to be changed when exposed to toxicants. Several investigators have studied the behavioral effects of acute and chronic exposure to methylmercury (Suzuki and Miama, 1971; Lown et al., 1977; Post et al., 1973; Zenick, 1974; Hughes et al., 1975). The behavioral effects of atrazine alone or in combination with methylmercury in man and animals, however, have not been studied.

An hypothesis can be made based on the present information indicating that synergistic GSH depletion is possible by one exposure to methylmercury and atrazine. Since methionine is convertible to cysteine, which in turn is a substrate for GSH synthesis, an experiment with administration of low, normal, and excess dietary methionine to

animals receiving methylmercury and atrazine subacutely, will provide valuable information on the alleviation of methylmercury and atrazine toxic effects. If so, such a diet modification would be appropriate for the human population which is more likely to be exposed to both toxicants or similar toxicants with similar biochemical actions.

MATERIALS AND METHODS

Animals and Treatments

Randomly bred weanling male Wistar rats from the stock colony of the Department of Food and Nutrition at Iowa State University were used. The study was conducted in two replicates with 72 rats per replicate for a total of 144 rats. Each replicate included 18 treatments with four rats per treatment for a total of eight rats per treatment in the complete study.

Rats were randomly assigned to each treatment and were housed individually in hanging type stainless steel wire mesh rat cages (40 x 24 x 20 cm). Animals were maintained at 22^{o} with 12 hours of light and 12 hours of darkness per day. Diets and distilled water were provided ad libitum and changed twice weekly unless otherwise specified. Rats were treated in the two following phases with diets and toxicants:

Pre-exposure Phase (26-32 days) In this phase, rats were adjusted to the new environment and received basal diet with adequate methionine for 20-26 days after which they were fed the experimental diets (without atrazine) until the beginning of the next phase. On the fifth day of this phase, water was removed for 12-14 hours during the dark period of the day. Water deprivation was used to motivate rats to respond to the light stimuli in testing chambers. Animal health and weight gains were recorded every three days.

Exposure Phase In this phase, methylmercury hydroxide and/or atrazine were fed to the appropriate groups of rats (see page 45). Water was removed for 12-14 hours during the dark period of the day. Urine was collected one and three weeks after starting toxicant treatment and also

a day prior to sacrificing the rats at the end of each phase. Rats were closely observed for their general health appearances and neurological effects of methylmercury hydroxide toxicity. Onset of hind limb paralysis and gait alteration was recorded for rats receiving methylmercury hydroxide at higher dose levels. Weight was measured every other day and two choice form discrimination tests were conducted every day of this phase.

Diets

The composition of the basal diet is shown in Table 1. Isolated soy protein was used because it is deficient in the sulfur amino acids. L-methionine was used to adjust the sulfur amino acid content of diets. The methionine adequate and methionine excess diets contained 0.24% and 0.48% L-methionine, respectively. The basal diet and the diets with two levels of added methionine contained methionine and cysteine concentrations shown in Table 2. The 1:1 methionine to cysteine ratio was just at the limit of the useful cysteine replacement for methionine (National Research Council, National Academy of Sciences, 1978).

Selenium and vitamin E were added to the diet at a concentration of 0.1 mg and 30IU per kg of diet respectively. These levels of selenium and vitamin E were chosen to provide adequate nutrition under normal conditions; each level added was equal to 100% of the rat's requirements for selenium and vitamin E (National Research Council, National Academy of Sciences, 1978).

Table 1. Composition of basal diet[a]

Ingredient	% weight
Isolated soy protein[b]	15.2
Corn starch[c]	30.5
Sucrose[d]	31.0
Cellulose[e]	8.8
Lard[e]	5.0
Corn oil[f]	3.5
Salt mix[g]	5.0
Vitamin mix[h]	1.0
Total	100.0

[a]The basal diet contains 60% of NRC rat requirement for methionine/cystine.

[b]Supro 660, Ralston Purina Company, St. Louis, MO.

[c]Teklad, Madison, WI.

[d]Iowa State University, Food Service.

[e]Teklad, Madison, WI.

[f]Mazola brand from Iowa State University Food Service.

[g]William and Briggs salt mixture (modified), Tekled, Madison, WI, with Na_2SeO_3 added to provide 0.1 mg Se/kg diet.

[h]Amount in 100 kg diet: vitamin A acetate, 800,000 IU; vitamin D cholecalciferol, 200,000 IU; vitamin K_3 menadion, 10 mg; vitamin B_{12}, 25 mg; biotin, 10 mg; folic acid, 500 mg; thiamin HCl, 1 g; paraamino benzoic acid, 40 g; Ca-pantothenate, 2 g; niacin, 5 g; pyridoxin, 5 g; riboflavin, 1 g; cholin citrate, 120 g; inositol, 40 g; corn starch to 1 kg; vitamin E DL-α-tocopherol 30 IU/kg diet was added to the fat prior to mixing with the other ingredients.

Toxicants

Atrazine was mixed uniformly with the appropriate diets at a concentration of 500 mg/kg as powdered technical grade atrazine (97.2%). Atrazine was provided by the Ciba-Geigy Corporation, Greensboro, NC. Methylmercury hydroxide (Alfa Division, Ventron Corporation, Danvers, MA) was administered at two different doses (0.5 and 1.5 mg/kg body weight/day) to the appropriate groups of rats by gavage. The dose required for two days for each rat was combined and administered every other day.

Treatments

The eighteen treatments shown in Table 3, were achieved by adding atrazine to the appropriate diets (diets A, B, C, Table 2) with thorough mixing and oral administration of methylmercury hydroxide.

Behavioral Tests

Four behavioral test chambers were used to shape and train rats to respond to light stimuli. The test chamber (Figure 6) is constructed of Plexiglas^{R} and equipped with a barred floor, side door, variable intensity light, and an intelligence panel. Mounted on the intelligence panel are two IEEE in line stimulus projectors. A water reinforcement fount and response bar are located directly below each projector.

Each plexiglas chamber was mounted in a sound proof wooden enclosure with plexiglas window. A closed circuit television camera was mounted outside of the chamber in front of the chamber window. The presentation of the light stimuli and reinforcement was computer controlled:

Computer Instrumentation and Program The computer used for experimental control and data acquisition was a Digital Equipment Corporation (DEC) PDP-8/A with a dual floppy disk drive, buffered digital input/output interface. Output signals from the plugboard in each chamber were connected to the computer interface. Each chamber used two input channels, one for each response bar, a total of eight input channels were used for operating channels. A standard teletype was used to provide experimenter interaction with the computer.

A computer program written in the Program Assembly Language (Hopper, 1976) with a minor modification was used to control shaping and testing.

Shaping of Rats in the Pre-exposure Phase Shaping was carried out by reinforcing bar press responses during the light stimulus. A correct bar press response was reinforced by 0.1 ml of sweetened water (0.08 ml of 0.04 g sugar per ml of water), either by an observer or under computer control. An incorrect response was not reinforced and terminated the trial.

Each rat was trained once daily for a total of 20-26 training sessions during response shaping period. Each session lasted approximately eight minutes and consisted of 42 trials which began with an intertrial interval (ITI), during which both stimulus lights remained darkened. Following the ITI one of the lights was lighted and remained on until a correct or incorrect response was made, or until it was automatically terminated by the computer program after elapse of 10 seconds, whereupon the trial was defaulted and the next trial was started.

At the end of each training session, a quick reference data summary was printed under computer control. It included a list of delays employed during the training session, the number of correct and

Figure 6. Behavioral test chamber

[a]Sweetened water reservoir.

[b]Light stimulus display.

[c]Response keys.

[d]Reinforcement cups.

[e]Variable intensity house light.

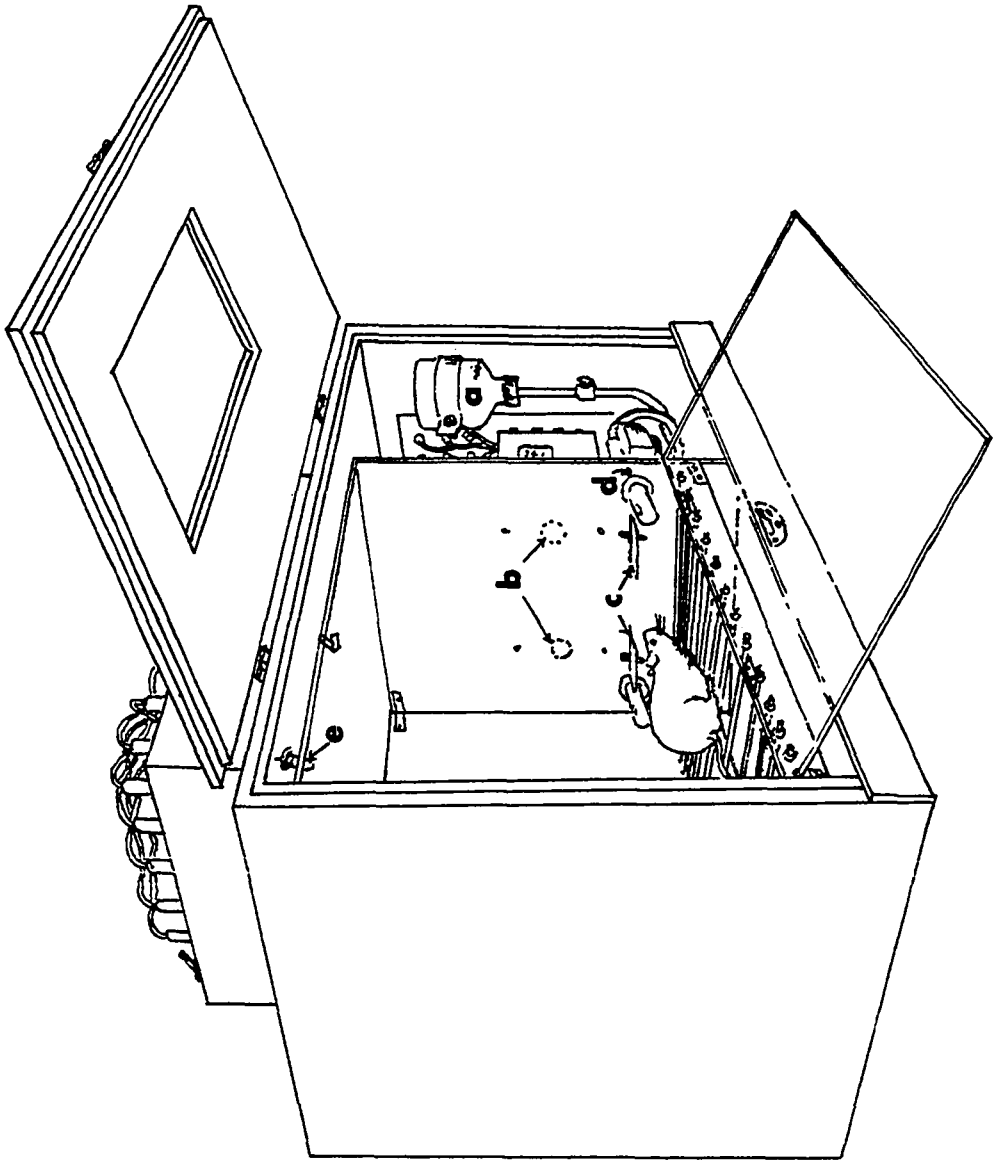
a
b
c
d
e

Table 2. Sulfur amino acid concentration of experimental diets

Diet	% sulfur amino acid concentration			% of
	methionine	cystine	total	requirement
A (basal + 0.24% L-met)	0.42	0.18	0.60	100
B (basal)	0.18	0.18	0.38	60
C (basal + 0.48% L-met)	0.66	0.18	0.84	140

Table 3. Composition of treatments

Treatment	Diet	Atrazine mg/kg diet	Hg mg/kg body wt.
1	A	0	0
2	B	0	0
3	C	0	0
4	A	0	0.5
5	B	0	0.5
6	C	0	0.5
7	A	0	1.5
8	B	0	1.5
9	C	0	1.5
10	A	500	0
11	B	500	0
12	C	500	0
13	A	500	0.5
14	B	500	0.5
15	C	500	0.5
16	A	500	1.5
17	B	500	1.5
18	C	500	1.5

incorrect responses, the number of defaults to the left and right stimulus lights, the number of spontaneous responses during stimulus presentation, and the intertrial interval.

Two-choice Form Discrimination Test in Exposure Phase Tests were started a day after exposure to toxicants. The behavioral chambers were used and random light stimuli in triangle and square patterns with equal light intensity were presented in this test.

The pattern which was rewarded by sweetened water was the triangle which appeared randomly on either the right or the left side of the intelligence panel simultaneously with the square pattern on the other side of the panel. Correct responses were recorded by pressing of the pedal under the triangle illuminated pattern. Incorrect response were recorded by pressing of the pedal under the square illuminated pattern.

The two-choice form discrimination test was conducted once a day for each rat, with a total of 25-29 sessions in the exposure phase of the experiment. Each session consisted of 42 trials. The course of each session was similar to that described for shaping sessions with one exception, two lights were presented in the form of a triangle and a square. Following each session a short data summary similar to the one described for the shaping session was printed and a longer trial-by-trial data summary was recorded in one disk and later transferred to tape.

Urine Collection

During the exposure phase of the experiment, urine of rats was collected three times, as mentioned on page 38. Urine was collected on

wrinkled heavy duty aluminum foil for 8-10 hours in the dark period (Black and Claxton, 1979). Hanging type stainless steel wire mesh rat cages (20 x 24 x 20 cm) were used for this purpose and only distilled water was available to the rats during this period. Collected urine was frozen at -20° until it was analyzed for mercapturic acid and mercury content.

Necropsy and Sample Collection

All rats were anesthetized with ether and the abdominal cavities were exposed by longitudinal incision on the midline of the abdomen. The following samples were collected:

Blood was collected from vena cava. A four ml sample was collected in a 10 ml disposable polyethylene syringe containing two drops of heparin (Sigma Company, St. Louis, MO) (1000 U/ml deionized water of Na heparin) for enzyme and mercury analysis. The blood was then delivered to polyethylene tubes containing acid-citrate-dextrose (ACD). Another 2 ml of blood was collected in a 10 ml polyethylene disposable syringe and incubated for 10 min. at 37° in a shaking waterbath. After exactly 10 min., 0.1 ml sodium salicylate solution (4.2 mM sodium salicylate in 0.1 M potassium phosphate buffer pH 7.4) per ml of whole blood was added. Serum was obtained by centrifugation at 4° and immediately frozen and kept at -20° for prostaglandin analysis.

Livers were perfused with cold 0.9% saline in situ to remove blood. The liver was excised, weighed, divided into four pieces, which were wrapped separately in aluminum foil. The samples were immediately frozen in liquid nitrogen and stored at -80° for GSH-Px, GSH-Rd, GSH-S

trans, and total glutathione analysis and at -20° for mercury analysis.

Kidneys were removed, weighed, frozen in liquid nitrogen and stored at -20°. The cortex and medulla of the kidneys were used for mercury analysis.

Whole brains were removed, frozen in liquid nitrogen and stored at -20°. The right occipital lobe was used for mercury analysis.

Analytical Methods

Tissue Composition and Enzymes

Glutathione peroxidase specific acitvity Whole blood ACD was used to measure GSH-Px by the method of Paglia and Valentine (1967). Changes in absorbance (A) per min. on the linear portion of the curve was measured and units of GSH-Px per ml of whole blood were calculated as

$$\text{GSG-Px, unit/ml blood} = \frac{(\text{A sample} - \text{A blank}) \times 0.48231}{\text{ml of sample}} \times \text{dilution factor}$$

GSH-Px of liver was measured by the modified method of Pierce and Tappel (1978) and assisted by a personal communication from Dr. Shirley C. Chen, Department of Food and Nutrition, Iowa State University, Ames, IA. Instead of using hydrogen peroxide, T-butyl hydroperoxide (0.05 ml of 1.5 mg/ml per assay) was used as a substrate.

Glutathione Reductase specific activity Activity of this enzyme in liver was measured using the method presented in Methods of Enzymatic Analysis (Bergmeyer and Gawehn, 1974).

Glutathione-S-transferase specific activity This enzyme was measured by the method of Booth et al. (1961) and by adapting $\Delta\varepsilon$ for 1,2-dichlor-4-nitrobenzene from Habig et al. (1974) for the calculation of

enzyme activity.

Total protein In order to calculate the specific activities of enzymes, the total protein of supernatants prepared from liver samples was assayed using Biuret reagent (White et al., 1976). The specific activity of enzymes was calculated using the formula described by Bergmeyer (1978).

Total glutathione Total glutathione in blood and liver was calculated by measuring GSH and GSSG by the fluorometric method of Hissin and Hilf (1976). Addition of whole blood into a tube containing meta-phosphoric acid and phosphate EDTA buffer resulted in hemolysis and breakdown of blood cells. Therefore, the homogenization step was omitted for the whole blood supernatant preparation.

Mercapturic acids Urine mercapturic acid was estimated by measuring -SH group concentration before and after alkaline hydrolysis of urine. The method of Ellman (1959) was used for the determination of -SH group concentration. The sulfhydryl bond of mercapturic acid derivatives was hydrolyzed by the addition of 0.5 ml of 2N NaOH into the diluted urine and incubated at 60° for 10 minutes.

Mercury analysis Mercury in blood, brain, liver, kidney, and urine was measured by using the modified flameless atomic absorption spectrophotometric method of Leong and Ong (1971), with assistance of personal communication from Dr. Henry M. Stahr, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA. The method involved an oxidation of a known amount of sample in nitric acid at 50-60° overnight in Hypovials^{R} sealed with a teflon disk, a silicon rubber disk, and an aluminum seal. The clear digested sample after proper dilution was placed in a sample bottle

and 10 ml of 10% stannous chloride was added to it. A closed air aspirator carried volatilized mercury to a gold screen where it was collected by amalgamation for 2½ minutes. Heat then was applied to the amalgam and the mercury was volatilized quickly and produced a sharp peak on a strip chart recorder. All glassware was acid washed in 50% nitric acid.

Mercury stock solution was prepared by dissolving methylmercury hydroxide in 50 ml redistilled nitric acid and dilution to a volume of 1 liter. Mercury intermediate solution (100 ug Hg/ml) was prepared by dilution of 10 ml of stock solution to 100 ml. Mercury working standard (1 ng Hg/ml) was prepared daily by dilution of 1 ml of intermediate standard solution to 100 ml. A series of standards (25, 50, 100, 150 ng Hg) was run with every 10 samples analyzed.

Prostaglandin analysis PGE_1 and TXB_2 in serum was measured by radioimmunoassay as described by Hwang et al. (1976) and McCosh et al. (1976). The method involved precipitation of each PG with specific antiserum and anti rabbit gamma globulin. The PGE_1 antisera had a cross reactivity of 15% with PGE_2 and TXB_2 antisera did not show any cross reactivity. Standards were a gift from the Upjohn Company (Kalamazoo, MI). Tritiated PGE_1 and TXB_2 were purchased from New England Nuclear (Boston, MA).

Statistical Analysis

Results of the clinical and biochemical studies were analyzed by factorial analysis of variances (Dr. David Cox, Statistical Laboratory, Iowa State University, Ames, Iowa), and means were compared by use of a least significant difference (LSD) test at the 5% level of probability (Snedecor and Cochran, 1967).

Seven variables from the behavioral studies, namely: number of correct responses (CR), number of incorrect responses (IR), number of defaults, total time for correct responses (TTCR), and average time of incorrect responses (ATIR), were analyzed by split plot factorial analysis of variances (Dr. David Hopper, Behavioral Toxicology Division, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa).

In the original design of the experiment, 29 testing sessions were planned for each rat during the exposure phase. Some of the rats died prior to the last testing session. Since data from sessions 1 to 27 were available for the majority of the rats, these data were used for analysis of the effect of treatments as well as testing session on the variables. Because of limitations in the capacity of computer memories, data of alternate testing sessions from session 1 to 27 for a total of 14 sessions were used and summarized in order to run split plot factorial analysis of variance. Means of different variables were compared for treatment effects by the use of the LSD test at a 5% level of probability.

RESULTS

Results of Clinical and Biochemical Studies

The treatment of groups of rats with a total of 18 different treatments which contained combinations of three levels of dietary methionine (diets A, B and C, Table 2), two levels of atrazine (-atrazine = 0.0 mg, +atrazine = 500 mg/kg of diet, Table 3), caused significant changes in the rats general appearance, and in physiological, biochemical and behavioral parameters.

The major observable drastic health alteration resulted from methylmercury hydroxide (MeHg) toxicity. Administration of high methylmercury hydroxide ($MeHg_3$) for five weeks in the group which received three different diets with or without atrazine, resulted in the occurrence of classic signs of MeHg toxicity in rats in the following progression: roughness of fur, alteration of gait, decrease of food intake, subtle widening of hindlimb placement, loss of weight, progressive ataxia, dragging and wide spacing of hindlimb during ambulation, crossing phenomenon[1], hematuria, frontlimb ataxia, and death.

A decrease of observable food intake in these groups was detected at the fourth week of exposure following alteration of gait. The onset of MeHg toxicity signs at about the fourth week of exposure was approximately 4-5 days earlier in the groups which received both atrazine and $MeHg_3$,

---

[1]Crossing phenomenon is an exaggerated adduction with crossing over the hind limb while the rat is suspended by the tail.

The groups of rats treated with low levels of methylmercury hydroxide ($MeHg_2$), except for the roughness of fur, did not show other signs of MeHg toxicity after five weeks of exposure. A decrease in food intake due to $MeHg_2$ or atrazine treatment was not detectable in these groups by daily observations.

A total of six rats died in the course of the experiment. Only one rat died due to the method of application of MeHg. The loss of rats before the termination of the experiment in the groups receiving $MeHg_3$ and atrazine was higher than that of those treated with $MeHg_3$ alone.

Table 4. The effect of dietary methionine, methylmercury hydroxide and atrazine on the percent weight gain[a] of rats (mean ± SE, $\bar{n}$=7.44, LSD=10.13)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	76.66 ± 12.50	67.37 ± 7.19	84.59 ± 6.15
+Atrazine	70.90 ± 5.53	55.17 ± 6.24	69.59 ± 8.38
$MeHg_2$			
-Atrazine	81.40 ± 6.24	85.80±13.80	80.41 ± 9.70
+Atrazine	54.21±12.90	43.39± 5.26	80.99±15.49
$MeHg_3$			
-Atrazine	4.35 ± 9.97	24.05 ± 7.24	42.53±15.34
+Atrazine	21.53±10.00	-1.54 ± 9.09	-0.63± 9.56

[a] Significant effect of Met ($P < 0.01$), MeHg ($P < 0.01$), Atrazine ($P < 0.01$) and Met x MeHg x Atrazine ($P < 0.05$).

[b] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$= 1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

There was a significant effect of dietary methionine (Met), MeHg, and atrazine treatments as well as their interactions on weight gain (Table 4). Weight gain mean comparisons indicated that administration of MeHg caused a decrease in weight gain. Animals fed diet B had a significant decrease of weight gain, but with administration of $MeHg_2$ and $MeHg_3$, diet B did not cause such weight reduction in comparison to

Table 5. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver weight (% body weight)[a] of rats (mean ± SE, $\bar{n}$=7.06, LSD=0.21)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	2.80 ± 0.10	2.94 ± 0.15	3.11 ± 0.29
+Atrazine	3.14 ± 0.14	3.38 ± 0.11	3.04 ± 0.14
$MeHg_2$			
-Atrazine	3.00 ± 0.20	3.29 ± 0.21	3.22 ± 0.19
+Atarzine	3.14 ± 0.22	3.18 ± 0.16	2.95 ± 0.19
$MeHg_3$			
-Atrazine	3.40 ± 0.25	3.47 ± 0.14	3.12 ± 0.22
+Atrazine	3.16 ± 0.26	3.59 ± 0.33	3.28 ± 0.22

[a]Significant effect of MeHg ($P < 0.10$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

the groups receiving diet A and C and MeHg. Administration of atrazine in the diet to the rats receiving diet B also resulted in a decrease in weight gain. In the groups which received $MeHg_3$, only the groups which were fed diet C without atrazine showed a greater weight gain in comparison to those which received both MeHg, atrazine and diet C.

Table 6. The effect of dietary methionine, methylmercury hydroxide, and atrazine on the kidney weight (% body weight)[a] of rats (mean ± SE, $\bar{n}$=7, LSD=0.11)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.68 ± 0.02	0.70 ± 0.02	0.72 ± 0.02
+Atrazine	0.97 ± 0.18	0.89 ± 0.04	0.81 ± 0.04
$MeHg_2$			
-Atrazine	0.81 ± 0.03	0.85 ± 0.03	0.85 ± 0.03
+Atrazine	0.91 ± 0.04	0.92 ± 0.03	0.86 ± 0.03
$MeHg_3$			
-Atrazine	1.57 ± 0.15	1.54 ± 0.13	1.40 ± 0.13
+Atrazine	1.66 ± 0.19	1.94 ± 0.13	1.69 ± 0.29

[a]Significant effect of MeHg and atrazine ($P < 0.01$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Liver and kidney weights as percent of body weight at the end of the experiment were calculated and are shown in Tables 5 and 6, respectively. There were no statistically significant differences among the means of liver weight as percent of body weight due to dietary Met and atrazine treatments (Table 5). The response of kidney to the treatments was more pronounced than that of liver. The effect of both MeHg and atrazine on excess kidney weight was highly significant (Table 6). This effect was more distinct in the groups of rats treated with 1.5 mg MeHg. Dietary methionine did not affect kidney weight.

The mean concentrations in the blood, liver, kidney and brain of treated rats are shown in Tables 7, 8, 9, and 10, respectively. When $MeHg_3$ was administered, the occipital lobe of rat brain accumulated a larger amount of mercury per gram of tissue in comparison to the other tissues.

The concentration of mercury in the blood was significantly affected by MeHg treatment as well as by its interaction with dietary Met (Table 7). The addition of atrazine to the diet did not cause a significant change in blood mercury concentration. As the dose of MeHg increased, the mean blood mercury increased greatly. The Met deficient diet (diet B) produced a significant increase of blood mercury only in the groups of rats treated with $MeHg_3$.

The concentration of mercury in the liver was significantly increased with increasing dosage of MeHg (Table 8). Mean comparisons of liver mercury concentration revealed that only those groups treated with $MeHg_3$ showed a significant increase of mercury concentration in the liver due to the presence of atrazine in the diet. There was no significant effect of

dietary methionine treatments on the accumulation of mercury in the liver.

The kidneys of groups of rats that were fed diets A, B, or C showed increases of mercury concentration in response to an increase in MeHg

Table 7. The effect of dietary methionine, methylmercury hydroxide, and atrazine on the mercury concentration[a] (ug/g) in the whole blood of rats (mean ± SE, $\bar{n}$=6.66, LSD=66.5)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	166 ± 24	161 ± 23	174 ± 29
+Atrazine	199 ± 42	182 ± 23	135 ± 26
$MeHg_3$			
-Atrazine	560 ± 123	630 ± 62	588 ± 54
+Atrazine	518 ± 97	824 ± 84	631 ± 103

[a] Significant effect of MeHg ($P < 0.01$), and MeHg x Met ($P < 0.10$).

[b] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Table 8. The effect of dietary methionine, methylmercury hydroxide, and atrazine on the mercury concentration[a] (ug/g) in the liver of rats (mean ± SE, $\bar{n}$=6.58, LSD=17.2)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	20.2 ± 1.0	31.9 ± 3.0	23.2 ± 1.7
+Atrazine	30.6 ± 7.1	28.0 ± 1.9	22.7 ± 1.9
$MeHg_3$			
-Atrazine	87.7 ±14.2	111.0 ±10.5	86.0 ±12.1
+Atrazine	128.6 ±27.9	121.6 ±26.6	158.6 ±52.0

[a]Significant effect of MeHg ($P < 0.01$), atrazine ($P < 0.05$) and MeHg x atrazine ($P < 0.10$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

dose (Table 9). The addition of atrazine to the diet had no significant effect on kidney mercury concentration. Diet B, in the groups of rats treated with $MeHg_2$, caused a significant accumulation of mercury in kidney compared to those which received $MeHg_2$ and diets A or C. However, groups of rats that were fed diet B and treated with $MeHg_3$ showed a lower accumulation of mercury in the kidney as compared with those which were treated with $MeHg_3$ and diet A or C.

Mercury concentration in the occipital lobe was increased significantly only by an increase in the dose of MeHg (Table 10); dietary methionine and atrazine did not have significant effects.

Urinary excretion of mercury for 12-14 hours at periods I, II and III (urines which were collected after 1, 3, and 5 weeks of administration of MeHg and atrazine) is shown in Tables 11, 12, and 13, respectively. At the end of period I, the excretion of mercury was significantly increased by an increase in dose of MeHg (Table 11). There were no significant effects of dietary methionine and atrazine on urine mercury excretion during this period.

Analysis of mercury content of urine collected during period II indicates the excretion of mercury was significantly increased due to an increase of MeHg dose (Table 12) except for the rats fed diet A with atrazine and treated with $MeHg_2$ and $MeHg_3$.

The administration of atrazine in the diet of rats fed diet A and treated with $MeHg_3$, caused an increase in mercury excretion in the urine during period II. Although the mean urine mercury excretion of the group fed diet C, $MeHg_3$ and atrazine was 1.5 times higher than that of the group fed diet C, $MeHg_3$ and no atrazine, this increase was not statis-

Table 9. The effect of dietary methionine, methylmercury hydroxide and atrazine on the concentration of mercury[a] (ug/g) in kidney of rats (mean ± SE, $\bar{n}$=6.67, LSD=16.5)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0.	0.0	0.0
$MeHg_2$			
-Atrazine	85.5 ± 4.4	147.2 ± 22.6	122.7 ± 12.5
+Atrazine	104.8 ± 3.7	118.7 ± 6.6	100.2 ± 11.2
$MeHg_3$			
-Atrazine	190.4 ±23.9	171.4 ± 9.9	187.5 ± 9.0
+Atrazine	181.2 ±24.9	167.6 ± 17.6	209.7 ± 11.8

[a]Significant effect of MeHg ($P < 0.01$) and MeHg x Met ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Table 10. The effect of dietary methionine, methylmercury hydroxide, and atrazine on the mercury concentration[a] (ug/g) in the occipital lobe. (mean ± SE, $\bar{n}$=6.83, LSD=55.6)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	6.2 ± 1.1	8.5 ± 0.6	9.2 ± 0.9
+Atrazine	9.4 ± 1.2	7.8 ± 1.1	9.1 ± 1.0
$MeHg_3$			
-Atrazine	46.0 ± 7.7	51.3 ± 2.0	42.6 ± 6.8
+Atrazine	52.9 ± 9.6	50.2 ± 6.6	55.7 ± 12.0

[a]Significant effect of MeHg ($P < 0.01$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg Hg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Table 11. The effect of dietary methionine, methylmercury hydroxide, and atrazine on urine mercury excretion[a] (ng/day) during period I (mean ± SE, $\bar{n}$=7.08, LSD=93.4)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	90.2 ± 31.6	63.3 ± 20.6	78.7 ± 29.7
+Atrazine	133.7 ± 34.4	100.4 ± 47.8	56.0 ± 25.2
$MeHg_3$			
-Atrazine	220.1 ± 55.3	155.4 ± 40.6	329.1 ± 70.9
+Atrazine	527.2 ±137.3	383.7 ±194.0	217.7 ± 67.4

[a]Significant effect of MeHg ($P < 0.01$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine-0.0 mg, +Atrazine=500 mg/kg diet.

Table 12. The effect of dietary methionine, methylmercury hydroxide and atrazine on urine mercury excretion[a] (ng/day) during period II (mean ± SE, $\bar{n}$=7.25, LSD=434)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	1066.0 ± 374.5	298.4 ± 135.7	501.7 ± 178.3
+Atrazine	1043.4 ± 245.1	740.4 ± 140.4	676.4 ± 136.3
$MeHg_3$			
-Atrazine	991.4 ± 388.2	1158.7 ± 349.1	1045.0 ± 342.0
+Atrazine	1691.8 ± 749.2	2725.1 ± 791.5	1456.4 ± 367.7

[a]Significant effect of MeHg ($P < 0.01$) and Atrazine ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Table 13. The effect of dietary methionine, methylmercury hydroxide, and atrazine on urine mercury excretion[a] (ng/day) during period III (mean ± SE, $\bar{n}$=6, LSD=383)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.0	0.0	0.0
+Atrazine	0.0	0.0	0.0
$MeHg_2$			
-Atrazine	434.7 ± 163.9	843.5 ± 218.2	1389.4 ± 145.3
+Atrazine	1189.0 ± 251.1	904.7 ± 120.5	812.4 ± 185.5
$MeHg_3$			
-Atrazine	1645.8 ± 420.8	1218.7 ± 436.3	1472.0 ± 436.8
+Atrazine	16.7.7 ± 337.8	1774.2 ± 514.5	1584.0 ± 514.0

[a]Significant effect of MeHg ($P < 0.01$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mgk/g diet.

cally significant. The overall effect of atrazine treatment during period II on urine mercury excretion was more pronounced in the groups which received high doses of MeHg than those which received low doses of MeHg. Dietary methionine by itself did not have a significant effect on urine mercury excretion during this period.

The mean urine mercury excretion during the period III is shown in Table 13. The administration of high doses of MeHg caused an increase in urinary excretion of mercury during this period. This effect was uniformly seen in the groups of rats fed any of the three diets with atrazine. The increase in urine mercury excretion due to the high dose of MeHg was also significant in groups of rats which were fed diet A without atrazine. Comparison of the means of urinary mercury excretion by groups of rats at different periods indicates that, generally, mercury excretion in urine increased as the number of days of exposure to MeHg increased. This trend was more consistent in the groups of rat which received atrazine in their diet.

The activity of whole blood glutathione peroxidase (GSH-Px) was significantly decreased with an increase in MeHg dose (Table 14). Mean enzyme activity (Table 14) showed an increase of activity related to atrazine treatment. Significant differences were found for rats which were fed diet C and no MeHg, and in groups fed diet B or C with $MeHg_3$.

Feeding rats with diet A, with or with atrazine, and $MeHg_3$ resulted in a highly significant increase in the specific activity of the enzyme GSH-Px of the liver in comparison with the two other dietary groups. Rats which were fed diet B showed a lower liver GSH-Px activity than did groups fed diets A or C. However, the lower activity of enzyme was statistically

Table 14. The effect of dietary methionine, methylmercury hydroxide and atrazine on whole blood glutathione peroxidase activity[a] (U/ml) (mean ± SE, ñ=7, LSD=2.7)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	31.95 ± 2.47	35.67 ± 2.32	31.75 ± 1.14
+Atrazine	31.84 ± 2.04	35.31 ± 3.08	39.34 ± 2.36
$MeHg_2$			
-Atrazine	25.70 ± 2.71	22.16 ± 2.48	26.51 ± 5.08
+Atrazine	27.66 ± 1.77	31.23 ± 2.56	30.18 ± 3.34
$MeHg_3$			
-Atrazine	24.11 ± 3.59	18.35 ± 0.93	21.85 ± 1.74
+Atrazine	24.71 ± 2.25	20.29 ± 1.20	22.75 ± 2.14

[a]Significant effect of MeHg ($P < 0.01$) and atrazine ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

significant only in the group which was also treated with atrazine or $MeHg_3$. Rats fed diet C alone had a significant increase in enzyme activity in comparison with rats fed the other diets. The rats which received diet C with $MeHg_2$ and no atrazine also showed a higher enzyme activity compared with those which received diet B with MeHg and no atrazine.

A factorial analysis of variance did not indicate any significant effects of either toxicant on liver GSH-Px. Least significant difference

Table 15. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver glutathione peroxidase activity[a] ($x10^{-4}$U/g) in rats (mean ± SE, $\bar{n}$=7.06, LSD=1.77)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	6.14 ± 1.40	6.46 ± 1.65	11.64 ± 3.32
+Atrazine	7.58 ± 1.10	5.86 ± 0.54	7.67 ± 0.89
$MeHg_2$			
-Atrazine	8.65 ± 1.29	7.98 ± 1.98	9.97 ± 1.43
+Atrazine	8.08 ± 0.93	8.47 ± 1.07	9.35 ± 1.24
$MeHg_3$			
-Atrazine	10.47 ± 1.63	6.25 ± 1.72	9.22 ± 1.22
+Atrazine	11.58 ± 2.87	8.06 ± 1.39	9.53 ± 1.50

[a]Significant effect of met ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

tests, however, showed some inconsistent and non-directional effects of atrazine and MeHg on the specific activity of this enzyme in liver.

The activity of liver glutathione reductase (GSH-Rd) which reduces oxidized glutathione, was not affected by any of the treatments (Table 16).

The measurement of the specific activities of glutathione-S-transferases (GSH-S-trans) of rat liver for conjugation of reduced glutathione and 1,2-dichloro-4-nitrobenzene revealed that there was a statistically signif-

Table 16. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver glutathione reductase activity ($x10^{-4}$ U/g) in rats (mean ± SE, $\bar{n}$=6.94, LSD=1.77)

Toxicant[a]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	3.20 ± 0.80	3.77 ± 0.98	3.92 ± 0.85
+Atrazine	4.04 ± 0.94	4.50 ± 1.29	4.34 ± 0.87
$MeHg_2$			
-Atrazine	4.40 ± 1.05	3.72 ± 0.82	3.30 ± 1.70
+Atrazine	5.37 ± 1.48	4.14 ± 1.09	4.08 ± 1.13
$MeHg_3$			
-Atrazine	4.82 ± 1.02	5.37 ± 1.58	5.19 ± 1.28
+Atrazine	4.47 ± 0.99	6.10 ± 2.69	4.95 ± 1.59

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

icant effect of MeHg and atrazine and an interaction effect of MeHg and dietary Met on the activity of the enzymes (Table 17). Administration of low or high levels of MeHg in rats fed diet A or B, caused decrease and increase of specific activity of this enzyme in liver respectively. The effects of MeHg were exaggerated in rats fed diet A with $MeHg_3$ and in rats fed diet B with $MeHg_2$. In the groups fed diet C, the administration of $MeHg_2$ and $MeHg_3$ caused an increase and a decrease of enzyme specific activity, respectively. Feeding the Met deficient diet (diet B) resulted

Table 17. The effect of dietary methionine, methylmercury hydroxide, and atrazine on the activity of liver glutathione-S-transferases[a] (U/g) in rats (mean ± SE, $\bar{n}$=6.89, LSD=2.96)

Toxicant [b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	31.00 ± 4.88	26.17 ± 2.21	33.41 ± 3.39
+Atrazine	26.74 ± 2.40	20.35 ± 3.05	26.91 ± 3.57
$MeHg_2$			
-Atrazine	30.29 ± 3.04	36.87 ± 2.12	36.83 ± 2.39
+Atrazine	24.44 ± 1.95	26.96 ± 3.31	25.27 ± 2.24
$MeHg_3$			
-Atrazine	25.03 ± 2.27	31.01 ± 2.23	27.41 ± 2.25
+Atrazine	23.15 ± 2.69	26.81 ± 2.64	21.25 ± 2.05

[a]Significant effect of MeHg ($P < 0.05$), MeHg x met ($P < 0.01$) and atrazine (P 0.01).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

in a decrease in activity of GSH-S-trans in rats treated with or without atrazine and no MeHg, i.e. $MeHg_1$. However, enzyme activity was high when $MeHg_2$ or $MeHg_3$ was administered in groups fed diet B with or without atrazine in the diet. The groups which received atrazine in their diet showed a significant decrease in liver GSH-S-trans specific activity in comparison to those which did not receive atrazine.

Table 18. The effect of dietary methionine, methylmercury hydroxide, and atrazine on prostaglandin E_1 concentration[a] (ng/ml) in serum of rats (mean ± SE, $\bar{n}$=6.72, LSD=0.86)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.88 ± 0.19	1.48 ± 0.43	1.05 ± 0.24
+Atrazine	1.29 ± 0.43	2.10 ± 0.99	2.00 ± 0.58
$MeHg_2$			
-Atrazine	1.22 ± 0.28	1.74 ± 0.37	2.28 ± 0.48
+Atrazine	1.88 ± 0.27	1.56 ± 0.21	1.29 ± 0.32
$MeHg_3$			
-Atrazine	3.32 ± 1.29	4.82 ± 1.67	4.46 ± 1.24
+Atrazine	2.59 ± 0.46	5.61 ± 1.52	3.70 ± 1.12

[a] Significant effect of MeHg ($P < 0.01$) and Met ($P < 0.10$).

[b] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, and -Atrazine=0.0 mg/kg diet, +Atrazine=500 mg/kg diet.

A significant increase in ex-vivo synthesis of prostaglandin E_1 (PGE_1) in blood caused by increasing MeHg dose is shown in Table 18. Dietary Met has a slight effect on ex-vivo synthesis of PGE_1. Although, as shown in Table 18, the rats receiving diet B showed an elevated mean serum PGE_1 concentration, only in the group which received diet B, $MeHg_3$ and atrazine did the elevation of PGE_1 in the serum reach statistical significance in comparison to the groups which were fed diet A or C with

Table 19. The effect of dietary methionine, methylmercury hydroxide, and atrazine on thromboxane B_2 concentration[a] (ng/ml) in serum of rats (mean ± SE, $\bar{n}$=5.56, LSD=31.2)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	19.1 ± 4.9	34.2 ± 12.2	27.5 ± 10.3
+Atrazine	52.7 ± 13.2	24.9 ± 9.5	48.0 ± 17.0
$MeHg_2$			
-Atrazine	42.3 ± 17.0	45.1 ± 12.5	116.4 ± 47.7
+Atrazine	24.0 ± 5.3	45.7 ± 8.3	26.5 ± 6.8
$MeHg_3$			
-Atrazine	34.9 ± 20.7	157.4 ± 55.7	180.7 ± 62.5
+Atrazine	49.4 ± 12.8	208.9 ± 47.6	172.9 ± 36.4

[a]Significant effect of MeHg, Met and MeHg x Met ($P < 0.01$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

$MeHg_3$ and atrazine. Atrazine treatment had no significant effect on the ex-vivo synthesis of PGE_1 in blood.

Overall factorial analysis of variances for thromboxane B_2 (TXB_2) concentration in serum of rats indicated a highly significant effect of MeHg, Met, as well as their interaction on the ex-vivo TXB_2 synthesis in blood (Table 19). However, the least significant mean difference test

revealed that only the rats which were fed diet B or C, with $MeHg_2$ or $MeHg_3$, had a significant increase in ex-vivo synthesis of TXB_2 in the blood. The high dose of MeHg also caused a significant increase in ex-vivo synthesis of TXB_2 in the blood of groups receiving atrazine in diets B and C. The effect of MeHg on ex-vivo synthesis of blood TXB_2 in rats fed diet A was not statistically significant.

Analysis of whole blood reduced glutathione (GSH) showed a significant effect of MeHg, Met, atrazine and also an interaction of MeHg and atrazine treatments on the concentration of whole blood GSH at the end of the experiment (Table 20). Except for the rats which were fed diet A with $MeHg_1$ and $MeHg_3$, all rats given atrazine in their diet had a significant increase of whole blood GSH after 5 weeks. The MeHg treatment effect was consistent on whole blood GSH in rats which received atrazine in the diet. $MeHg_2$ and $MeHg_3$ caused a decrease and increase in whole blood GSH respectively, in comparison to control rats. Although the overall factorial analysis of variance indicated a significant effect of dietary Met treatments on whole blood GSH, the mean comparison for least significant differences did not show any directional and meaningful trend on particular groups of rats.

MeHg had a highly significant effect on rats whole blood oxidized glutathione (GSSG) (Table 21). Administration of $MeHg_2$ in rats which were fed diet A, without atrazine, caused a significant decrease in whole blood GSSG, but such a response was not seen in the groups which received atrazine in the diet. MeHg treatments of rats receiving diet B or C without atrazine caused a significant decrease of whole blood GSSG.

Table 20. The effect of dietary methionine, methylmercury hydroxide, and atrazine on whole blood reduced glutathione[a] (ug/ml) in rats (mean ± SE, $\bar{n}$=7.17, LSD=0.12)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.69 ± 0.15	0.37 ± 0.09	0.71 ± 0.04
+Atrazine	0.69 ± 0.09	0.90 ± 0.08	0.79 ± 0.09
$MeHg_2$			
-Atrazine	0.48 ± 0.16	0.76 ± 0.11	0.35 ± 0.08
+Atrazine	0.84 ± 0.07	1.06 ± 0.06	0.89 ± 0.18
$MeHg_3$			
-Atrazine	0.47 ± 0.11	0.53 ± 0.11	0.59 ± 0.12
+Atrazine	0.49 ± 0.19	0.66 ± 0.13	0.79 ± 0.15

[a]Significant effect of atrazine ($P < 0.01$), MeHg, Met and MeHg x atrazine ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine=0.0 mg, and +Atrazine-500 mg/kg diet.

Analysis of whole blood total glutathione (TGSH=GSH+GSSG) indicated a significant effect of MeHg and Met treatments as well as MeHg and atrazine interaction effect on TGSH (Table 22). Least significant differences of means in Table 22 indicated that MeHg caused a significant decrease of whole blood TGSH in rats which were fed diet A, B, or C without atrazine. In rats fed atrazine, $MeHg_3$ caused a decrease of

Table 21. The effect of dietary methionine, methylmercury hydroxide, and atrazine on whole blood oxidized glutathione[a] (ug/ml) in rats (mean ± SE, $\bar{n}$=7.17, LSD=0.07)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.52 ± 0.06	0.76 ± 0.15	0.57 ± 0.03
+Atrazine	0.50 ± 0.05	0.68 ± 0.05	0.54 ± 0.08
$MeHg_2$			
-Atrazine	0.39 ± 0.02	0.48 ± 0.03	0.50 ± 0.14
+Atrazine	0.47 ± 0.07	0.55 ± 0.06	0.50 ± 0.05
$MeHg_3$			
-Atrazine	0.56 ± 0.05	0.45 ± 0.08	0.45 ± 0.06
+Atrazine	0.47 ± 0.08	0.43 ± 0.06	0.25 ± 0.05

[a]Significant effect of MeHg, MeHg x Met ($P < 0.05$) and MeHg x atrazine ($P < 0.10$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine-0.0 mg, +Atrazine=500 mg/kg diet.

TGSH; whereas, $MeHg_2$ caused an increase of TGSH of whole blood in the groups fed diet A or C with atrazine. Significant effects of dietary Met treatments were observed in the rats which were fed diet B, i.e., an increase of TGSH in whole blood when rats were treated with $MeHg_1$ or $MeHg_2$, with or without atrazine as compared with rats fed diet A or C. No significant effects of dietary Met were detected in the groups treated

Table 22. The effect of dietary methionine, methylmercury hydroxide, and atrazine on whole blood total glutathione[a] (ug/ml) in rats (mean ± SE, $\bar{n}$=7.17, LSD=0.15)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	1.21 ± 0.20	1.56 ± 0.23	1.28 ± 0.07
+Atrazine	1.19 ± 0.12	1.59 ± 0.12	1.33 ± 0.07
$MeHg_2$			
-Atrazine	0.88 ± 0.15	1.24 ± 0.12	0.86 ± 0.14
+Atrazine	1.31 ± 0.12	1.61 ± 0.10	1.52 ± 0.19
$MeHg_3$			
-Atrazine	1.03 ± 0.13	0.99 ± 0.12	1.05 ± 0.10
+Atrazine	0.96 ± 0.23	1.09 ± 0.12	1.04 ± 0.19

[a]Significant effect of MeHg, Met, and MeHg x atrazine ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine-0.0 mg, +Atrazine=500 mg/kg diet.

with $MeHg_2$. The elevation of TGSH in whole blood due to atrazine was significant only in the rats which were treated with $MeHg_2$. Concentration of liver GSH, GSSG, and TGSH unexpectedly were not affected by either dietary treatments or toxicants (Tables 23, 24, and 25).

Table 23. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver reduced glutathione (ug/mg) in rats (mean ± SE, $\bar{n}$=7.11, LSD=1.14)

Toxicant [a]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	2.28 ± 0.46	2.21 ± 0.31	3.01 ± 0.38
+Atrazine	2.89 ± 0.39	2.10 ± 0.21	2.59 ± 0.35
$MeHg_2$			
-Atrazine	2.24 ± 0.46	2.11 ± 0.24	2.42 ± 0.33
+Atrazine	2.48 ± 0.33	2.71 ± 0.53	2.51 ± 0.33
$MeHg_3$			
-Atrazine	2.36 ± 0.30	2.92 ± 0.40	2.67 ± 0.45
+Atrazine	3.21 ± 0.52	2.72 ± 0.62	2.24 ± 0.59

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 24. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver oxidized glutathione[a] (ug/mg) in rats (mean ± SE, $\bar{n}$=7.11, LSD=0.89)

Toxicant[a]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	0.44 ± 0.12	0.37 ± 0.09	0.55 ± 0.11
+Atrazine	0.55 ± 0.12	0.38 ± 0.09	0.48 ± 0.07
$MeHg_2$			
-Atrazine	0.43 ± 0.07	0.44 ± 0.11	0.50 ± 0.10
+Atrazine	0.34 ± 0.08	0.43 ± 0.13	0.50 ± 0.05
$MeHg_3$			
-Atrazine	0.38 ± 0.08	0.45 ± 0.08	0.45 ± 0.06
+Atrazine	0.64 ± 0.14	0.43 ± 0.06	0.38 ± 0.07

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 25. The effect of dietary methionine, methylmercury hydroxide, and atrazine on liver total glutathione[a] (ug/mg) in rats (mean ± SE, $\bar{n}$=7.11, LSD=1.28)

Toxicant[a]			
$MeHg_1$			
-Atrazine	2.72 ± 0.56	2.58 ± 0.36	3.56 ± 0.42
+Atrazine	3.42 ± 0.45	2.48 ± 0.26	3.07 ± 0.36
$MeHg_2$			
-Atrazine	2.67 ± 0.45	2.55 ± 0.31	2.93 ± 0.38
+Atrazine	2.83 ± 0.35	3.14 ± 0.64	3.01 ± 0.33
$MeHg_3$			
-Atrazine	2.74 ± 0.33	3.30 ± 0.45	3.25 ± 0.51
+Atrazine	3.90 ± 0.63	3.15 ± 0.65	2.62 ± 0.60

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Measurement of conjugated sulfhydryl groups as a crude estimator of mercapturic acids excretion in the urine across period I, II and III were also employed to obtain further information about the effects of treatments on glutathione metabolism in this experiment. The urinary excretion of mercapturic acids at different periods of time (periods I, II, and III) was affected significantly by administration of atrazine in the diet (Tables 26, 27, and 28). A significant increase of mercapturic acids excretion due to atrazine was observed in the urine collected during period I from rats treated with $MeHg_3$ and rats treated with $MeHg_2$ and fed diet A or B (Table 26). Rats fed diet B and given $MeHg_1$ also showed a significant increase of mercapturic acids excretion in urine during period I due to the presence of atrazine in the diet.

Analysis of urine collected from rats during period II revealed that both MeHg and atrazine had a significant effect on urine mercapturic acids excretion (Table 27). With the exception of the groups which were fed diet A and no atrazine, the administration of $MeHg_2$ caused a significant increase of mercapturic acid excretion in the urine of rats in comparison to the groups treated with $MeHg_1$ or $MeHg_3$ and were fed any of the three diets with or without atrazine. With respect to period I, the excretion of mercapturic acids in the urine was generally elevated during period II.

There was a significant increase in mercapturic acids excretion in the urine of rats during period III due to atrazine in rats treated with no MeHg (Table 28). The effects of atrazine in the other groups which were treated with $MeHg_2$ or $MeHg_3$ were not significant, with

Table 26. The effect of dietary methionine, methylmercury hydroxide, and atrazine on urine mercapturic acid excretion[a] (-SH x 10^{-5} mol/day) during period I (mean ± SE, N=7.61, LSD=4.35)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	17.22 ± 0.20	9.63 ± 1.22	15.33 ± 6.37
+Atrazine	14.91 ± 3.05	20.14 ± 5.71	10.58 ± 3.15
$MeHg_2$			
-Atrazine	15.17 ± 2.67	6.72 ± 1.35	11.07 ± 2.34
+Atrazine	23.59 ± 5.17	14.50 ± 4.08	11.18 ± 3.24
$MeHg_3$			
-Atrazine	8.43 ± 2.62	8.29 ± 2.11	10.05 ± 2.51
+Atrazine	20.36 ± 8.49	16.93 ± 6.35	21.04 ± 6.27

[a] Significant effect of atrazine ($P < 0.01$).

[b] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

Table 27. The effect of dietary methionine, methylmercury hydroxide, and atrazine on urine mercapturic acid excretion[a] ($-SH \times 10^{-5}$ mol/day during period II in rats (mean ± SE, n=7.44, LSD=5.48)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	27.97 ± 5.06	25.43 ± 6.16	25.36 ± 3.06
+Atrazine	25.46 ± 3.43	29.95 ± 4.39	30.80 ± 4.46
$MeHg_2$			
-Atrazine	30.02 ± 6.39	36.69 ± 8.09	30.11 ± 2.78
+Atrazine	38.80 ± 4.59	38.82 ± 3.67	35.08 ± 3.15
$MeHg_3$			
-Atrazine	29.64 ± 4.34	26.47 ± 8.63	15.20 ± 3.92
+Atrazine	25.60 ± 6.48	28.31 ± 5.79	29.67 ± 6.10

[a]Significant effect of MeHg ($P < 0.01$) and atrazine ($P < 0.10$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine=0.0 mg, +Atrazine-500 mg/kg diet.

Table 28. The effect of dietary methionine, methylmercury hydroxide, and atrazine on urine mercapturic acid excretion[a] (-SH x 10^{-5} mol/day) during period III (mean ± SE, n=6.67, LSD=5.84)

Toxicant[b]	Diet A	Diet B	Diet C
$MeHg_1$			
-Atrazine	8.14 ± 3.03	10.96 ± 2.79	8.83 ± 4.17
+Atrazine	17.33 ± 6.52	26.12 ±10.45	36.42 ± 8.51
$MeHg_2$			
-Atrazine	18.58 ± 6.41	14.12 ± 3.30	13.03 ± 3.93
+Atrazine	19.00 ± 4.20	16.18 ± 3.63	16.03 ± 2.04
$MeHg_3$			
-Atrazine	15.97 ± 4.76	10.50 ± 3.23	15.10 ± 3.96
+Atrazine	18.19 ± 2.99	16.37 ± 5.84	17.23 ± 7.87

[a]Significant effect of atrazine ($P < 0.01$) and MeHg x atrazine ($P < 0.05$).

[b]$MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine=0.0 mg, +Atrazine=500 mg/kg diet.

the exception of rats fed diet B and treated with $MeHg_3$.

In the course of the experiment, the excretion of mercapturic acids in the urine of all groups generally increased until about the third week (period II) of the exposure phase, and thereafter, it decreased. The elevation of mercapturic acids excretion at period II was also observed with the groups of rats which did not receive the toxicant.

A factorial analysis of variance for the effect of variables (MeHg, Met, and atrazine) and their interactions with each other on the clinical, and biochemical parameters are shown in Tables 29, 30, 31, and 32. The F values were tested for significance at $P<0.01$, $P<0.05$, and $P<0.10$ and these values are designated with letters a, b, and c, respectively.

Results of Behavioral Studies

After a total of 20-26 training sessions in the pre-exposure phase, the average number of correct and incorrect responses in the last two training sessions of all groups of rats in both replicates were 28.86 and 10.53, respectively. The average number of correct and incorrect responses in the last two training sessions in both replicates are shown in Table 33.

The factorial analysis of variance also indicates that none of the dietary methionine levels of toxicants alone had effects on the behavioral variables. Only the dietary methionine and atrazine interaction showed a significant effect on IR, TTCR and ATCR in one of the replicates (Tables 34 and 35). Daily testing sessions had a highly significant effect on the behavior of rats during the exposure phase, and most of the variables showed significant effects at $P<0.01$ (Tables 34 and 35).

Table 29. Analysis of variance of % weight gain, liver and kidney weight as % of body weight and urine mercapturic acid excretion

Source of variation	df	F value					
		% wt. gain	Liver wt. % of B.W.	Kidney wt. % of B.W.	Urine mercapturic acid I	II	III
Mercury (Mer)	2	67.01[a]	2.68[b]	119.00[a]	0.02	5.02[a]	0.43
Methionine (Met)	2	3.01[b]	1.78	1.03	1.03	0.55	0.22
Mer x Met	4	0.68	0.20	0.51	0.97	0.30	0.88
Atrazine (Atr)	1	16.27[a]	0.29	12.32[a]	9.08[a]	2.64	8.24[a]
Mer x Atr	2	0.21	0.81	1.42	1.38	0.08	3.42[c]
Met x Atr	2	1.03	0.43	0.28	1.03	0.73	0.76
Mer x Met x Atr	4	3.23[c]	0.75	0.70	0.61	0.63	0.59

[a]Significant effect of variation sources $P < 0.01$.

[b]Significant effect of variation sources $P < 0.10$.

[c]Significant effect of variation sources $P < 0.05$.

Table 30. Analysis of variance of blood and liver enzymes activity and prostaglandin synthesis in the blood

Source of variation	df	F value					
		Blood GSH-Px	Liver GSH-Px	Liver GSH-Rd	Liver GSH-S-Trans	TXB_2	PGE_1
Mercury (Mer)	2	35.39[a]	1.01	1.69	3.66[b]	24.97[a]	20.12[a]
Methionine (Met)	2	0.59	3.31[b]	0.11	0.22	8.52[a]	2.49[c]
Mer x Met	4	1.53	0.98	0.50	3.90[a]	5.49[a]	0.99
Atrazine (Atr)	1	5.49[b]	0.10	0.71	26.09[a]	0.00	0.04
Mer x Atr	2	0.85	0.94	0.14	1.23	2.05	0.58
Met x Atr	2	0.71	0.54	0.02	0.36	1.05	0.27
Mer x Met x Atr	4	0.99	0.17	0.08	0.30	0.80	0.47

[a]Significant effect of variation sources $P < 0.01$.

[b]Significant effect of variation sources $P < 0.05$.

[c]Significant effect of variation sources $P < 0.10$.

Table 31. Analysis of variance of liver and blood glutathione

Sources of variation	df	F value					
		Liver GSH	Liver GSSH	Total liver glutathione	Blood GSH	Blood GSSG	Total blood glutathione
Mercury (Mer)	2	0.62	0.16	0.67	3.72[a]	6.35[b]	7.58[b]
Methionine (Met)	2	0.23	1.40	0.44	3.81[a]	2.01	4.82[b]
Mer x Met	4	0.87	0.61	0.88	1.52	3.81[a]	0.93
Atrazine (Atr)	1	0.33	0.03	0.30	12.46[b]	0.13	6.77[a]
Mer x Atr	2	0.30	0.27	0.19	3.37[a]	2.83[c]	5.08[a]
Met x Atr	2	1.23	1.39	1.54	0.47	0.03	0.19
Mer x Met x Atr	4	0.43	1.06	0.59	0.17	0.52	0.27

[a]Significant effect of variation sources $P < 0.05$.

[b]Significant effect of variation sources $P < 0.01$.

[c]Significant effect of variation sources $P < 0.10$.

Table 32. Analysis of variance of mercury concentrations in blood, liver, kidney, brain and urine

Source of variation	df	F value						
		Blood mercury	Liver mercury	Kidney mercury	Brain mercury	Urine I mercury	Urine II mercury	Urine III mercury
Mercury (Mer)	1	16.75[a]	97.89[a]	66.91[a]	193.18[a]	18.00[a]	10.86[a]	10.57[a]
Methionine (Met)	2	2.29	0.20	0.99	0.02	0.83	0.67	0.16
Mer x Met	2	2.64[b]	0.31	4.00[c]	0.06	0.19	1.93	0.35
Atrazine (Atr)	1	0.98	5.73[c]	0.18	1.43	2.48	5.17[c]	0.58
Mer x Atr	1	0.72	4.75[b]	0.61	0.84	1.43	2.10	0.12
Met x Atr	2	2.10	1.13	0.64	0.57	2.15	0.91	0.98
Mer x Met x Atr	2	2.10	0.93	1.53	0.45	1.11	0.29	1.45

[a]Significant effect of variation sources $P < 0.01$

[b]Significant effect of variation sources $P < 0.10$.

[c]Significant effect of variation sources $P < 0.05$.

Table 33. The average number of correct and incorrect responses of groups of rats at the last two training sessions of the preexposure phase[a]

Toxicant	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
- Atrazine	26[b]/13[c]	23/8	28/13	32/8	30/11	25/16
+ Atrazine	29/12	26/12	28/13	27/10	32/9	29/9
$MeHg_2$						
- Atrazine	26/14	23/13	31/9	34/7	30/11	29/12
+ Atrazine	30/10	35/5	28/11	30/9	26/14	25/9
$MeHg_3$						
- Atrazine	27/14	32/8	31/8	26/12	32/9	30/10
+ Atrazine	34/8	31/7	26/15	32/9	32/9	25/12

[a]All groups were fed diet A without any toxicants during the preexposure phase for 20-26 days after which they were fed the experimental diets (without atrazine) until the exposure phase, and were assigned to the different treatments as indicated in this table during exposure phase only.

[b]Average number of correct responses.

[c]Average number of incorrect responses.

Table 34 . Factorial analysis of variance for the effects of methylmercury hydroxide, dietary methionine, atrazine, and training session on the coded behavioral variables

Treatments	df	F value					
		Correct response		Incorrect response		Default	
		I[a]	II[b]	I	II	I	II
MeHg	2	0.24	0.13	0.26	0.59	0.35	0.17
Met	2	0.66	1.33	2.18	1.91	1.34	1.07
Atr	1	1.28	0.91	0.51	0.89	0.04	1.47
MeHg x Met	4	1.50	0.39	0.02	0.65	0.74	0.71
MeHg x Atr	2	1.64	0.94	0.10	1.86	0.49	2.02
Met x Atr	2	1.18	0.56	4.97[d]	1.20	0.77	1.24
MeHg x Met x Atr	4	0.46	0.71	0.51	0.55	0.67	0.57
Training session (T)	13	3.38[c]	3.48[c]	4.49[c]	2.04[c]	1.76[d]	2.65[c]
T x MeHg	26	1.10	1.51	0.76	1.66	1.26	1.92[c]
T x Met	26	1.04	1.02	1.11	0.96	0.98	1.07
T x Atr	13	2.33[c]	0.80	0.67	0.91	1.37	0.91
T x MeHg x Met	52	0.89	1.12	0.83	1.14	0.82	0.91
T x MeHg x Atr	26	0.96	1.37[e]	1.06	1.51[d]	1.20	0.98
T x Met x Atr	26	0.77	0.95	0.87	0.65	1.80	0.80
T x MeHg x Met x Atr	52	0.69	1.09	0.76	0.98	0.78	1.10

[a]Replicate I, n=3.89.

[b]Replicate II, n=3.78.

[c]$P < 0.01$.

[d]$P < 0.05$.

[e]$P < 0.10$.

Table 35. Factorial analysis of variance for the effect of methylmercury hydroxide, dietary methionine atrazine and testing sessions on the coded variables

		F value							
		T.T. cor. res.		T.T. inc. res.		A.T. cor. res.		A.T. inc. res.	
Treatment	df	I[a]	II[b]	I	II	I	II	I	II
MeHg	2	0.27	0.74	0.19	0.17	1.24	1.42	0.67	0.93
Met	2	0.45	2.31	1.08	1.13	0.13	1.08	1.32	1.11
Atr	1	0.82	0.35	1.17	0.17	0.19	1.05	1.39	0.99
MeHg x Met	4	1.66	0.27	0.15	0.55	1.20	0.35	1.06	0.40
MeHg x Atr	2	0.66	0.34	0.13	1.64	0.25	1.19	0.46	0.92
Met x Atr	2	4.16[d]	1.02	1.68	1.52	1.89	0.45	3.27[d]	0.57
MeHg x Met x Atr	4	0.24	0.39	0.27	1.18	0.38	1.66	0.24	0.99
Testing Session (T)	13	2.33[c]	0.74	5.81[c]	5.00[c]	1.28	10.12[c]	1.42	9.30[c]
T x MeHg	26	0.87	1.41[e]	0.62	1.20	1.02	1.00	1.74	0.52
T x Met	26	0.84	1.03	1.28	1.04	1.63	0.79	1.13	0.64
T x Atr	13	1.71[e]	0.73	1.00	0.76	1.48	0.60	1.28	0.38
T x MeHg x Met	52	0.79	1.32[e]	0.91	1.20	0.55	0.74	0.95	0.80
T x MeHg x Atr	26	0.91	1.30	1.01	1.33	0.92	1.25	0.79	0.95
T x Met x Atr	26	0.96	0.91	1.19	1.00	1.52[d]	1.53[d]	1.01	1.45[e]
T x MeHg x Met x Atr	52	0.58	1.05	0.95	0.88	0.97	0.90	1.27[e]	0.74

[a]Replicate I, n=3.89.

[b]Replicate II, n=3.78.

[c]$P < 0.01$.

[d]$P < 0.05$.

[e]$P < 0.10$.

Except for the interaction of session and dietary methionine, the rest of the session interactions with other factors had significant effects on one or two of the variables in one of the replicates with the probability level of $P<0.01$ to $P<0.10$ (see Tables 34 and 35). Only the ATCR variable in both replicates showed a significant effect of session, dietary methionine and atrazine interactions (Table 35).

Since most of the session effects on variables were highly significant, the average values of variables of each session from different treatments were plotted in Figures 7 to 10. Figure 7 shows that, by increasing the number of sessions the average number of CR of rats in both replicates to the two-choice form stimuli significantly increased and that of the IR decreased.

The average number of defaults of rats in all treatments decreased until the 9th session. From the 9th to the 15th session defaults plateau, after which they increase slightly (Figure 8).

The mean TTCR and TTIR of rats to the two-choice form stimuli are shown in Figure 9. The TTCR significantly increased and the TTIR decreased as the number of sessions increased. Only in replicate II did the mean of ATCR and ATIR show significant decrease as the number of sessions increased (Figure 10).

The means of each variable across 14 sessions are shown in Tables 36 to 41. LSD for the mean CR of rats in 14 sessions revealed that in replicate I, rats fed diet A and atrazine, had a significant increase of CR as compared with rats given only diet A (Table 36). In replicate II, there was also a significantly lower CR in rats fed diet C, plus atrazine, and treated with $MeHg_3$ as compared with rats fed diet C

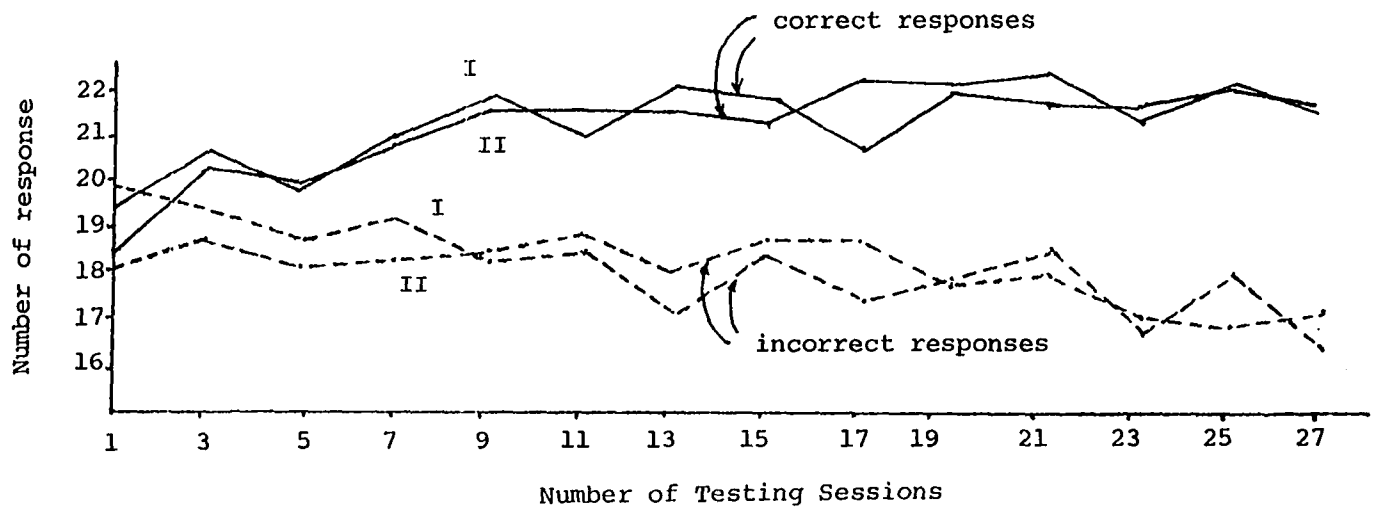

Figure 7. Change of average number of correct and incorrect responses of rats across all treatments in replicates I and II

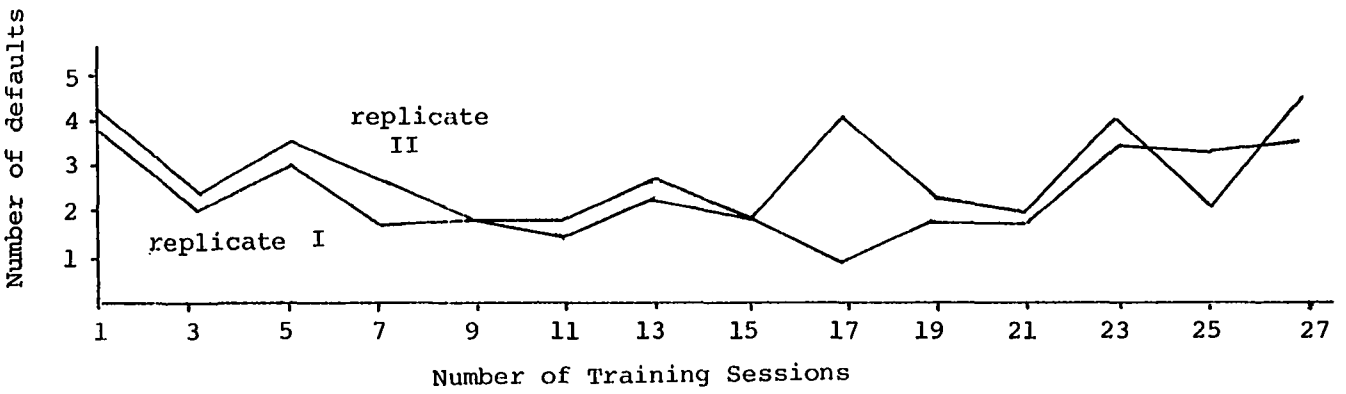

Figure 8. Change of average number of defaults of rats across all treatments in replicates I and II

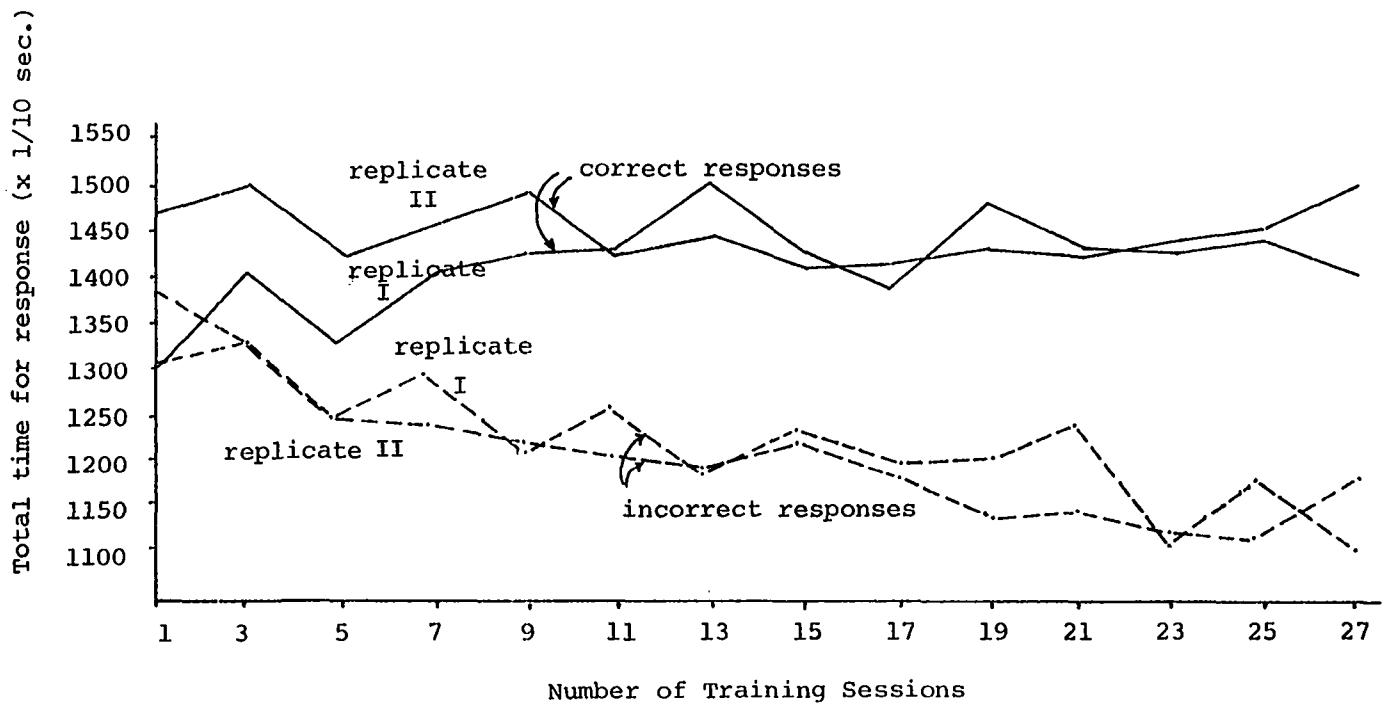

Figure 9. The change of mean of total time for correct and incorrect responses of rats across all treatments in replicate I and II

and atrazine (Table 36).

Table 37 shows that in replicate I feeding rats atrazine and diet A caused a significant decrease of IR for all doses of MeHg, and feeding groups of rats diet A without atrazine, resulted in a significantly higher IR in comparison with the groups fed diets B or C without atrazine but treated with MeHg. In replicate II, feeding atrazine to the rats resulted in a significant increase of IR in groups fed diet B and treated with either $MeHg_1$ or $MeHg_2$. There was also a significantly higher IR in comparison with groups fed alike but treated with $MeHg_1$ or $MeHg_2$. In replicate II, there was also a significant increase in IR by rats given diet A without atrazine and treated with $MeHg_3$ as compared with rats given only diet A (Table 37).

Comparisons of the mean number of defaults in replicate I indicated that there were no significant differences among the means as a result of treatments (Table 38). However, replicate II showed that the number of defaults was significantly decreased by administration of atrazine in the diet of the group which received diet B or C with no MeHg treatment. The mean number of defaults in replicate II was decreased significantly by the higher dose of MeHg in the group of rats fed diet B without atrazine, but it increased with increasing dose of MeHg in the groups receiving diet C and atrazine (Table 38).

Table 39 shows that in replicate I rats fed atrazine had a significant increase in mean TTCR when given diet A and $MeHg_1$ or $MeHg_2$. In replicate II, there was a significant increase of mean TTCR in rats fed diet B and treated with $MeHg_2$ in comparison to rats receiving diet B only.

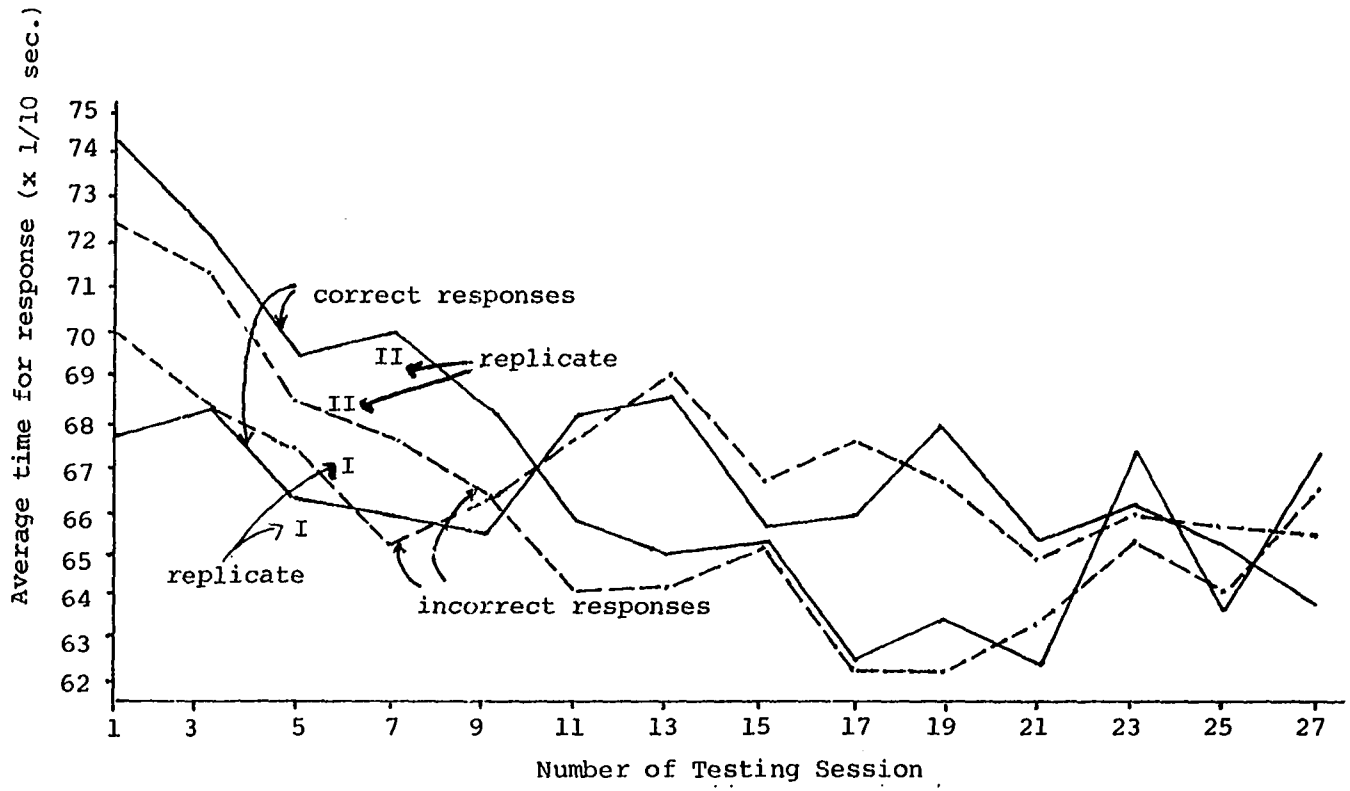

Figure 10. The change in the mean of the average total time for correct and incorrect responses of rats in replicates I and II across all treatments

Table 36. The effect of dietary methionine, methylmercury hydroxide and atrazine on the mean number of correct responses of rats in the 14 testing sessions of each replicate (replicate I: LSD=1.62, $\bar{n}$=3.89; replicate II: LSD=1.42, $\bar{n}$=3.78)

Toxicant[a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	20.73	21.67	20.50	19.33	21.19	20.98
+Atrazine	22.78	21.85	21.00	22.89	20.67	21.98
$MeHg_2$						
-Atrazine	21.39	22.30	20.83	22.33	22.30	20.67
+Atrazine	22.21	22.51	20.23	22.02	21.55	20.80
$MeHg_3$						
-Atrazine	20.46	21.33	21.14	21.64	20.76	21.58
+Atrazine	21.05	22.51	22.03	22.12	21.46	19.89

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 37. The effect of dietary methionine, methylmercury hydroxide and atrazine on the mean number of incorrect responses of rats in the 14 testing sessions of each replicate (replicate I: LSD=1.43, $\bar{n}$=3.89; replicate II: LSD=1.35, $\bar{n}$=3.78)

Toxicant[a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	19.39	17.85	17.69	15.48	17.60	18.57
+Atrazine	17.98	18.82	18.78	18.35	17.46	19.19
$MeHg_2$						
-Atrazine	19.87	18.57	18.60	16.62	17.45	18.50
+Atrazine	17.73	17.92	18.26	18.62	18.46	17.78
$MeHg_3$						
-Atrazine	19.51	19.07	17.51	18.64	17.37	18.58
+Atrazine	17.41	19.01	18.82	17.80	17.85	18.01

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 38. The effect of dietary methionine, methylmercury hydroxide and atrazine on the mean number of defaults of rats in the 14 testing sessions of each replicate (replicate I: LSD=2.14, $\bar{n}$ = 3.89; replicate II: LSD=1.93, $\bar{n}$=3.78)

Toxicant[a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	1.84	2.44	3.78	7.14	3.11	2.44
+Atrazine	1.23	1.32	2.21	0.75	3.86	0.80
$MeHg_2$						
-Atrazine	0.73	1.12	2.55	3.04	2.24	2.80
+Atrazine	2.07	1.55	3.56	1.31	1.98	3.30
$MeHg_3$						
-Atrazine	1.87	1.57	3.21	1.69	3.86	1.78
+Atrazine	3.52	0.42	1.11	2.07	2.64	4.07

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 39. The effect of dietary methionine, methylmercury hydroxide and atrazine on mean total time (x 1/10 sec.) for correct response of rats in the 14 sessions of each replicate (replicate I: LSD=110.7, $\bar{n}$=3.89; replicate II: LSD=103.4, n=3.78)

Toxicant [a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	1371	1460	1411	1349	1431	1423
+Atrazine	1494	1527	1403	1505	1417	1385
$MeHg_2$						
-Atrazine	1362	1507	1402	1512	1534	1399
+Atrazine	1478	1494	1336	1531	1435	1425
$MeHg_3$						
-Atrazine	1364	1420	1424	1434	1361	1430
+Atrazine	1467	1469	1435	1492	1381	1300

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Table 40. The effect of dietary methionine, methylmercury hydroxide and atrazine on the mean average time (x 1/10 sec.) for correct response of rats in the 14 testing sessions of each replicate (replicate I: LSD=3.73, $\bar{n}$=3.89; replicate II: LSD=3.40, $\bar{n}$=3.78)

Toxicant[a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	66.45	66.53	69.12	71.99	68.61	68.63
+Atrazine	65.78	69.83	67.10	65.83	68.05	63.19
$MeHg_2$						
-Atrazine	63.72	67.66	66.33	68.34	68.78	66.74
+Atrazine	67.05	66.84	64.25	69.42	65.99	70.24
$MeHg_3$						
-Atrazine	65.51	66.92	66.76	66.85	65.28	67.02
+Atrazine	69.07	65.19	65.50	67.67	64.24	62.15

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

Because the split plot factorial analysis of variance indicated no significant effect of treatments on TTIR (Table 35), mean comparisons on TTIR were not conducted.

In replicate I, rats fed diet C and treated with $MeHg_2$ had a significant increase of mean ATCR in comparison with those fed diet A and treated with $MeHg_2$ (Table 40). In replicate I, rats treated with diet C, atrazine and $MeHg_3$ also showed a significant decrease in mean ATCR in comparison with rats fed diet A, atrazine and treated with $MeHg_3$. The mean ATCR in replicate II, was significantly higher for rats receiving diet B without atrazine and MeHg in comparison with those fed diets A or C (Table 40). In replicate II, rats fed diet A and atrazine showed a significantly higher ATCR compared with groups treated alike and given either diet B or C. In replicate II, also, groups given diet C, atrazine and $MeHg_2$ showed a significantly higher mean ATCR in comparison with rats fed diet A, atrazine and treated with $MeHg_2$ (see Table 40).

Rats receiving diet B, atrazine and $MeHg_3$ in replicate II showed a significantly higher mean ATCR in comparison to those treated with diet C, atrazine and $MeHg_3$ (see Table 40). Atrazine caused an increase of ATCR in rats treated with diets B or C and resulted in a decrease of ATCR in those fed diet A (see Table 40). Atrazine in replicate II also resulted in a decrease of ATCR in the group fed diet C and treated with $MeHg_3$.

In replicate I, there was a significant decrease of mean ATIR due to atrazine in rats which were fed diet B with no MeHg treatment (Table 41). The $MeHg_2$ treatment in replicate I resulted in a decrease of mean ATIR in the rats fed diet C (Table 41). Rats fed diet C without atrazine

Table 41: The effects of dietary methionine, methylmercury hydroxide and atrazine on the mean average time (x 1/10 sec.) for the incorrect responses of rats in the 14 testing sessions of each replicate (replicate I: LSD=3.60, $\bar{n}$=3.89; replicate II: LSD=3.05, $\bar{n}$=3.78)

Toxicant[a]	Diet A		Diet B		Diet C	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
$MeHg_1$						
-Atrazine	66.77	66.15	69.58	71.67	69.20	67.69
+Atrazine	66.80	69.00	65.92	65.41	67.41	63.07
$MeHg_2$						
-Atrazine	64.07	65.95	65.86	66.95	69.72	65.77
+Atrazine	67.52	65.86	64.26	67.38	67.78	67.93
$MeHg_3$						
-Atrazine	66.05	64.84	68.04	67.34	68.25	66.13
+Atrazine	68.14	63.63	66.46	66.62	65.22	63.99

[a] $MeHg_1$=0.0 mg, $MeHg_2$=0.5 mg, $MeHg_3$=1.5 mg/kg body weight, -Atrazine= 0.0 mg, +Atrazine=500 mg/kg diet.

but treated with $MeHg_2$ showed a significantly higher mean ATIR in comparison with those treated with diet A or C, $MeHg_2$ and no atrazine (Table 41). In replicate II, atrazine caused a decrease of mean ATIR in rats receiving diet B or C (Table 41). In the same replicate, rats fed only diet B showed a significantly higher mean ATIR compared to those fed only diet A or C (see Table 41). In replicate II, the groups treated with diet A, atrazine and no MeHg showed significantly higher mean ATIR than those treated with diet B or C and atrazine. A higher mean ATIR was observed for rats given diet B, atrazine, and $MeHg_3$ compared to groups fed diet A, atrazine, and $MeHg_3$ (see Table 41).

DISCUSSION

Since methionine (Met) is one of the essential sulfur amino acids which has an important role in protein, S-adenosylmethionone, cystathionine, cysteine, and glutathione synthesis, its dietary supplementation might play an important role in the metabolism of nutrients and toxicants. It has been shown that addition of small amounts of DL-Met into a casein-based diet improves growth rate, while further addition results in inhibition of protein utilization in rats (Harper et al., 1970). Thus, dietary Met has a narrow margin of safety and it is known to be one of the most toxic amino acids.

In our experiment, the results of weight gains of rats in response to different diets and toxicants indicates a role of detoxification capability for excess dietary Met on MeHg and atrazine toxicity. Low weight gain due to the consumption of Met deficient diet (diet B) occurred as expected. This is in agreement with the finding of Edes et al. (1979), who reported no weight gain in the rats fed a diet free of Met and cysteine. However, in this experiment the excess Met at the level of 24% above the requirement (diet C) did not cause toxicosis or beneficial weight gain increases in comparison to adequate Met diet (diet A). Both the MeHg and atrazine caused lower weight gains. Similar results following the repeated administration of MeHg have been reported by Mivakawa and Deshimaru (1969). For MeHg toxicosis, the excess Met in the diet showed some protective effect. Improved weight gains in rats fed excessive Met in the diet and treated with atrazine or $MeHg_3$ alone or atrazine and $MeHg_2$, are to some degree supportive of the protective effect of excess Met against atrazine as well as against MeHg. When

atrazine and $MeHg_3$ were administered to the rats the excess Met diet did not protect animals from the weight loss of rats fed the Met deficient diet with atrazine and MeHg. The high Met rats lost more weight than those fed the Met adequate diet and both toxicants

Glutathione conjugation is probably one of the important mechanisms of detoxification of both MeHg and atrazine in the rat. The glutathione conjugation process does not require the initial formation of high energy intermediate involving ATP, the synthesis of glutathione (GSH) from its component amino acids and N-acetylation of cysteine conjugate does utilize ATP (Chasseaud, 1976). In this experiment, in the course of repeated exposure of rats to the toxicants, conversion of Met to cysteine through the cystathionine pathway and biosynthesis of GSH are possibly stimulated. Thus, disruption of normal Met and cysteine metabolism and increased utilization of energy and the sulfur amino acids for glutathione synthesis and excretion of toxicants are possible explanations for the limited protective effect of excess Met in the diet and the loss of body weight in rats due to toxicants and/or the Met deficient diet.

The liver and kidney are the major sites of detoxification for most xenobiotics. Atrazine in rats has been shown to be conjugated with GSH and excreted through urine or bile (Dauterman and Muecke, 1974; Climie and Hutson, 1979). The conjugation of MeHg with GSH and cysteine also occurs in liver and the conjugate is excreted either through bile or urine (Norseth and Clarkson, 1971). The liver and kidney also accumulate mercury to a great extent by conjugation to metallothionein, membranes

or other available -SH groups (Piotrowski et al., 1974; Winge et al., 1975). Thus, increases in the activity and induction of enzymes or metallothionein or proliferation of membranes or cells in response to atrazine and MeHg toxicity might result in weight change of these organs. A change in proportional liver weight due to feeding a Met deficient diet alone was not observed in this experiment. Similar results were obtained by Edes et al. (1979). The increase of liver weight due to MeHg toxicity has been demonstrated previously by Chang and Desnoyers (1978). Considering the liver weight increases as a response of rats to both toxicants, alone or in combination, the groups fed Met deficient or adequate diets showed significant increases in liver weight, whereas the groups fed Met excess diet did not show such a significant increase. Again such results support the protective effect of excess dietary Met against liver damage by MeHg and atrazine.

Ormestead et al. (1980) stated that drugs conjugated with GSH in the liver are further metabolized to the corresponding cysteine derivatives in the kidney. In this experiment, the response of the kidney to detoxification of atrazine and MeHg was manifested by an increase in kidney weight. The drastic increase of kidney weight due to the high level of mercury ($MeHg_3$) may be partly due to the induction of metallothionein after the biotransformation of MeHg to inorganic mercury. The increase of inorganic mercury and induction of metallothionein in kidney after MeHg exposure to rats has been shown previously in the rat (Chmielnicka and Brzeznicka, 1978). In the kidney, the protective effect of excess Met was not as clear as in the liver. This might be due to the fact that the kidney was more responsible for the detoxification of both

toxicants, compared to the liver. Only the Met excess diet caused a significant small increase in kidney weight in rats treated with $MeHg_3$ when compared to the other two levels of Met in the diet. Dietary Met alone had no effect on kidney weight.

The accumulation and concentration of mercury in the organs and tissues depends on the type of mercury compound, dose, duration of exposure, age, health of the rat and the time of sampling after the last administration of mercury. Injection of a single dose of MeHg in mice and rats has been shown to result in an accumulation of mercury in kidney, blood, and liver in that order (Ulfvarson, 1969a; 1969b). In addition, the administration of a total dose of 40 mg MeHg per kg of body weight in three days to rats resulted in an accumulation of mercury after 19 days in blood, kidney, liver and brain. Since longer exposure (five weeks) of MeHg were used in our experiment, and mercury concentrations were measured in the samples prepared a day after the last exposure, the order of mercury concentration in the tissues is in agreement with those of Ulfvarson (1969b). The blood had the highest and the brain had the lowest concentration of mercury.

In rats, a major portion of the circulating blood MeHg is bound in erythrocytes, mainly to hemoglobin (Chen et al., 1975). In humans and rabbits, however, it is mainly bound to glutathione rather than to hemoglobin (Naganumra and Imura, 1979). Therefore, the change of blood GHS in the rat should not affect the blood mercury concentration extensively. Also, we did not find any correlation between blood mercury concentration and blood GSH in this experiment.

Nevertheless, the data on organ and tissue concentrations of mercury are rather difficult to interpret. Obviously, the concentration of mercury in the organs is highly influenced by the dose of MeHg; an increase of mercury concentration in the organs resulted from administration of high dose of MeHg. Also, administration of atrazine caused more accumulation of mercury in the liver of rats treated with $MeHg_3$, but it is not possible to draw any conclusion regarding the influence of dietary Met on mercury deposition in the organs.

From the works of Ulfvarson (1969b) and Cember and Donagi (1964) on the distribution and elimination of organic mercury compounds by rats, calculations can be made based on the two following criteria which will help to evaluate the effect of dietary Met on MeHg deposition in the organs.

1. The concentration of mercury in the kidney at all dose levels is a constant fraction of mercury retained in the body (Cember and Donagi, 1964). Therefore, concentrations of mercury in blood, liver and brain relative to kidney mercury, indicate the degree of toxicity or accumulation of mercury in the organ. This is calculated as: Ratio A = (Hg conc. tissue) ÷ (Hg conc. kidney).
2. The ratio of relative concentrations of mercury in the organs should be similar among the groups treated alike. This was calculated as: Ratio B = (Ratio A for $MeHg_3$ treated animals) ÷ (Ratio A for $MeHg_2$ treated animals). Therefore, any differences seen in the present study would be either due to dietary

methionine or atrazine treatments.

The relative concentrations of Hg in the blood, liver, and brain to that of the kidney (Ratio A) are shown in Tables 42 and 43. Dividing data from Table 42 by data in Table 43 gives the ratios in Table 44 (Ratio B).

Relatively more mercury was accumulated in the blood and brain than in the liver with either dose of mercury (Tables 42 and 43). The effect of dietary Met is shown clearly in Table 44. Feeding the Met deficient diet resulted in a distinctly greater accumulation of mercury in the organs. The Met deficient diet tended to accentuate the accumulation of mercury in the brains of rats fed either $MeHg_3$ or $MeHg_3$ levels. The protective effects of excess Met in the diet were not distinguishable from the effect of the adequate diet with regard to the accumulation of mercury in the organs. There was no consistent effect of atrazine on these values. The effects shown in Table 44 are illustrated in Figure 11.

Although brain accumulated a higher amount of mercury in the group fed the Met deficient diet, the onset of clinical manifestations of MeHg neurotoxicity in this group was not different from those of the rats fed the adequate or excess Met diet. This might be due to the fact that in the rat the first observable damage occurs in the peripheral nervous system and in cerebellum rather than in sensory cerebral cortex (Evans et al., 1975). In this experiment, despite the effect of atrazine on liver mercury concentration, it did not affect the brain mercury concentration. Clinically, however, the onset of MeHg neurotoxicity signs started earlier in the groups fed atrazine in the diet. This discrepancy might also be explained in the same way as the effect of mercury on peripheral

Table 42. Relative mercury concentration of blood, liver, and brain to kidney in the rats treated with low mercury dose ($MeHg_2$= 0.5 mg/kg)

	Diet A		Diet B		Diet C	
	-Atr.	+Atr.	-Atr.	+Atr.	-Atr.	+Atr.
Hg blood/ Hg kidney	1.94	1.90	1.09	1.53	1.42	1.35
Hg liver/ Hg kidney	0.24	0.29	0.22	0.24	0.19	0.23
Hg brain/ Hg kidney	0.07	0.09	0.09	0.6	0.07	0.09

Table 43. Relative mercury concentration of blood, liver, and brain to kidney in rats treated with high mercury dose ($MeHg_3$= 1.5 mg/kg)

	Diet A		Diet B		Diet C	
	-Atr.	+Atr.	-Atr.	+Atr.	-Atr.	+Atr.
Hg blood/ Hg kidney	2.94	2.86	3.67	4.92	3.14	3.01
Hg liver/ Hg kidney	0.46	0.70	0.65	0.72	0.46	0.76
Hg brain/ Hg kidney	0.24	0.29	0.30	0.30	0.23	0.26

Table 44. Ratio of the relative mercury concentration of tissues to kidney at the high mercury ($MeHg_3$) treatment to the low mercury ($MeHg_2$) treatment

	Diet A		Diet B		Diet C	
	-Atr.	+Atr.	-Atr.	+Atr.	-Atr.	+Atr.
Hg blood/ Hg kidney	1.51	1.50	3.37	3.21	2.21	2.23
Hg liver/ Hg kidney	1.92	2.41	2.95	3.00	2.42	3.30
Hg liver/ Hg kidney	3.31	3.24	5.24	4.60	3.02	2.91

nervous system rather than on the cerebral cortex.

The excretion of mercury in urine increased as the cumulative dose of MeHg (total dose of exposure at the time of urine collection) increased. The results of this study showed that toxicity of atrazine in rats had no effect on urine excretion of mercury after stimultaneous treatment of rats with atrazine and MeHg for a week. However, atrazine significantly affected mercury excretion in urine collected during period II (after 3 weeks of treatments). No effect of atrazine on urine mercury excretion was seen during period III. This might be due to the cumulative toxicity of MeHg which overcame the effect of atrazine toxicity and, consequently an atrazine effect was not detected during period III.

Mercury content of urine samples might also be subjected to the errors of the urine collection method. Technically the collection of urine by the method of Black and Claxton (1979), in aluminum foil may be only applicable

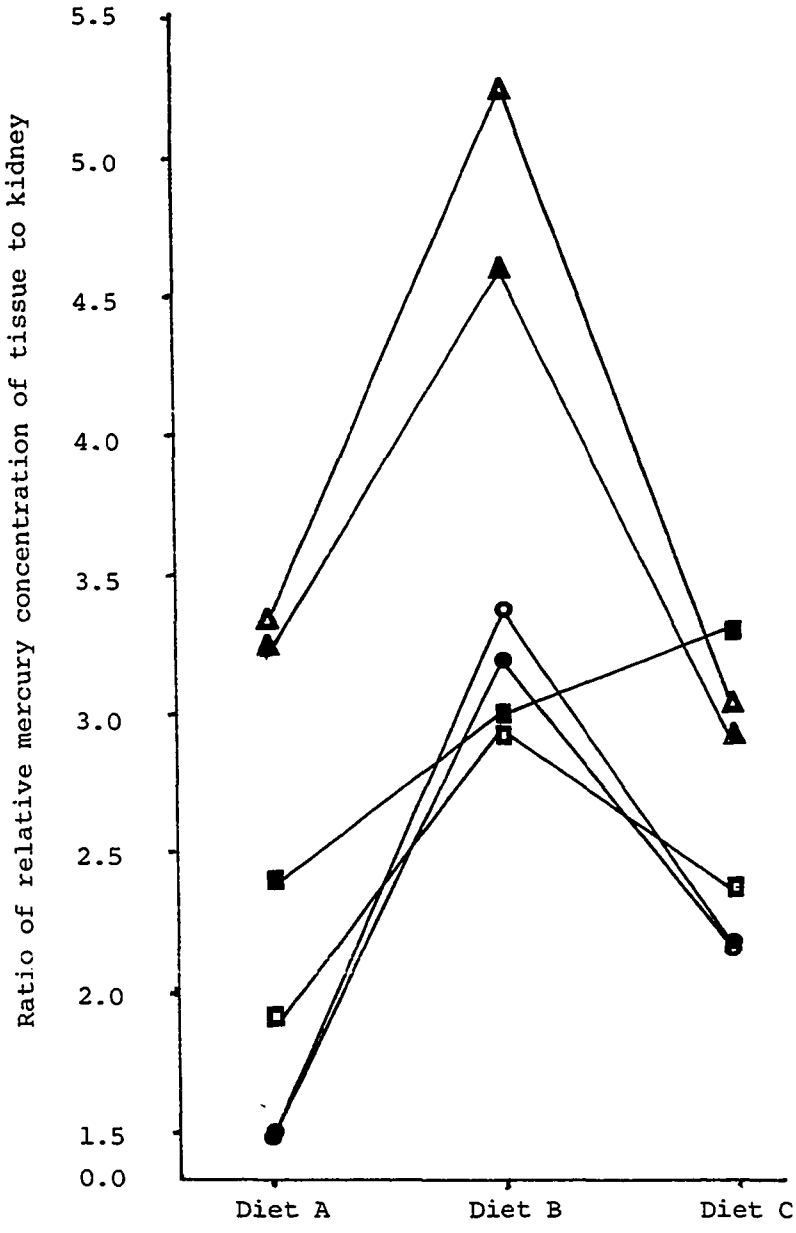

Figure 11. The effects of dietary met on the ratio of relative mercury concentration of tissues to kidney at $MeHg_3$ treatment (● and o=Hg blood/Hg kidney, ■ and □ =Hg liver/Hg kidney, ▲ and △ =Hg brain/Hg kidney. Solid and hollow symbols are related to groups with and without atrazine treatments respectively)

to a short time urine collection and may not be suitable for the long period (12-14 hours) collection as was done in this experiment. Although, as suggested in the method, many wrinkles were made in the aluminum foil, and food was not provided uring urine collection, the evaporation of urine and contamination with feces and food particles carried on the fur of the rat remain major problems associated with this method. Since mercury is also excreted through the feces, the contamination of urine with feces in the collecting cages might contribute to the urine mercury concentration. Use of metabolic cages, if available, would be desirable for large numbers of animals and long term urine collection.

The interaction between selenium and mercury has been shown in rats and other animals. The possible interaction between Se and Hg through the effect of selenium on the activity of the selenoenzyme, GSH-Px, was of particular interest in this experiment. The biochemical function of GSH-Px in reduction of hydrogen peroxide as well as organic hydroperoxides is of critical importance in the erythrocyte (Sunde and Hoekstra, 1980). This enzyme may be required in the metabolism of arachidonate and in prostaglandin synthesis (Anonymous, 1981). In this study, administration of MeHg caused a significant reduction in the activity of whole blood GSH-Px of rats. This reduction of activity was related directly to the dose of MeHg administered. The enzyme activity reduction by MeHg might be related to the complex formation of MeHg with the biologically active SE of GSH-Px as has been speculated by Hoekstra (1975) and Ganther (1975).

In the present study, the activity of whole blood GSH-Px in the rat declined as the concentration of mercury in the blood increased as a result of high MeHg treatment. Such Hg and GSH-Px relationship existed

in the blood of rats fed either of the dietary Met levels. Although statistical analysis did not show any significant influence of dietary Met on the whole blood GSH-Px; however, considering Table 44, one can infer some effect of low Met diet (B) on the increase of blood mercury concentration, and consequently its effect on the depression of whole blood GSH-Px activity.

It is rather difficult to draw a clear conclusion on the overall effect of treatments on liver GSH-Px activity. Despite the increase in mercury concentration of liver by the higher dose of MeHg, the specific activity of Se-dependent GSH-Px remained unchanged. The inhibitory effect of MeHg observed with blood GSH-Px was not seen with liver GSH-Px. To calculate the specific activity of GSH-Px of liver, the total liver protein was measured. The inhibitory effect of mercury on protein synthesis in liver might have caused the above mentioned unexpected results. The activity of the enzyme was also decreased by the Met deficient diet. However, this kind of effect was not significant for all groups receiving Met deficient diets.

The specific activity of GSH-Rd in liver did not change significantly in any of the treatments. This confirms the finding of Pekkanen and Sandholm (1972) that showed liver GSH-Rd activity was not affected by MeHg.

Use of 1,2-dichloro-4-nitrobenzene conjugation with GSH is a valid model to evaluate liver competence for the initial step in the mercapturic acid synthesis for atrazine detoxification. Therefore, the cytosolic glutathione-S-transferase of liver was investigated. It was found that long term exposure of atrazine causes reduction of specific activity of this enzyme in rat liver. It seems that increasing MeHg dose causes

a decrease of specific activity of GSH-S-trans in rats fed Met deficient diets, but an increase of specific activity was caused in the rats fed Met imbalanced diets (Met deficient or Met excess). The overall picture does not indicate that feeding rats Met excess diet will result in a greater liver competence with respect to conjugation of atrazine with GSH, by GSH-S-trans. The biochemical inhibitory effects of atrazine and MeHg on specific activity of liver GSH-S-trans need further investigation.

Prostaglandins (PGs) are fundamental response metabolites of cells or organisms. Synthesis of PGs depends on regulation of essential fatty acid metabolism, lipase activity, cofactors, hormones and other factors. Metabolism of glutathione, vitamin E, Met, and GSH-Px activity may influence the PGs synthesis (Tai and Yuan, 1977; Nugteren, 1970; Lands and Rome, 1976; Horrobin et al., 1978; Morse et al., 1977). In this experiment, ex-vivo synthesis of PGE_1 and TXB_2 by platelets from clotted blood were higher in the groups treated with MeHg. There are no previous reports of effect of MeHg on synthesis of PGs. Several factors might be responsible for the increase of PG synthesis in blood. With the available data, the most logical speculation might be the possible reduction of activity of GSH-Px of blood by MeHg. Since hydrogen peroxide and organic hydroperoxides have stimulatory effects on PG synthesis, the increase of PGs in this experiment might have resulted from a build up of some endogenous hydroperoxides by the decrease of GSH-Px activity in the blood. The effects of hydroperoxy free radicals or peroxy radicals on endoperoxide formation and PG synthesis are described by Morse et al. (1977). A

decrease in availability of vitamin E might also have contributed to increased PG synthesis. Vitamin E as an antioxidant plays an important role in membrane stabilization. Vitamin E has also been shown to have a protective effect against MeHg toxicity (Welsh, 1974; Sunde, 1976; Chang et al., 1977). The inhibitory effect of vitamin E on biosynthesis of PGs has been established (Nugteren, 1970; Lands and Rome, 1976). Such an inhibitory effect might result from its antioxidant activity. Considering the above effects, MeHg toxicity in the rats might have resulted in a decline of vitamin E concentration which consequently caused an enhancement of biosynthesis of PGs. The level of vitamin E in the diet of rats in this experiment was at the minimum requirement for the rat as indicated by NRC/NAS (1978). Decrease of PGE_1 synthesis in brain by excess Met through taurine metabolism has been suggested (Horrobin et al., 1978). In this experiment, although dietary Met showed an influence on both PGE_1 and TXB_2 ex-vivo synthesis in clotted blood, the effects were not clear enough to be in agreement with the suggestions of Horrobin et al. (1978).

Administration of xenobiotics subject to GSH conjugation may lead to extensive depletion of cellular GSH (Wood, 1970; Chasseaud, 1973; Gillette, 1977). It has been shown that deposition of MeHg in liver was not affected when liver GSH was depressed to 16% of control after 30 min. of administration of diethylmaleate in rat (Richardson and Murphy, 1975). But depletion of blood and kidney GSH were correlated to lower depositions of mercury in the same experiment. Congiu et al. (1979) found that administration of MeHg chloride to rats resulted in an increase of liver GSH and a decrease of kidney GSH, without any increase in mercury concentration in liver. The results of our experiment are somewhat different

from those. Although it has been shown that atrazine is conjugated with GSH by the enzyme GSH-S-trans (Dauterman and Muecke, 1974; Climie and Hutson, 1978), feeding rats with atrazine in the amount of 500 mg/kg of diet ad libitum for five weeks, did not cause any change in liver GSH levels. However, atrazine caused an increase in the mercury deposition with high level of MeHg administration. Atrazine also caused a significant increase of GSH in blood, but blood mercury concentration was not significantly affected. MeHg deposition in kidney and brain also did not change significantly with atrazine treatment. Administration of MeHg alone produced a slight numerical increase of GSH of liver by increasing the dose from $MeHg_2$ to $MeHg_3$, but it did not reach statistical significance. However, liver and kidney mercury deposition were greatly increased. These results also are not in agreement with Congui et al. (1979).

Since GSH measurements of the organs in most experiments were conducted within short periods of time after exposure to GSH depleting agents (Griffith and Meister, 1979; Mitchell et al., 1973; James et al., 1971; Boyland and Chasseaud, 1979), the development of tolerance by repeated exposure, presence of alternative detoxifying pathways and interorgan translocation of GSH might be the reason for lack of effect of atrazine on liver GSH concentration in this experiment. Development of tolerance to sodium maleate which decreases GSH has been demonstrated in rats by Richardson and Murphy (1975). Although Refsvik (1978) interpreted that a high concentration of liver GSH is requisite for the normal translocation of MeHg from liver to bile, the result of the present experiment indicates that in long term exposure to MeHg other sulfhydryl containing compounds such as cysteine may be involved in

translocation because liver mercury concentration increased with increasing MeHg dose in this experiment.

Dietary Met with or without administration of both toxicants had no effect on liver glutathione status, but Met deficient diet alone caused a decrease of GSH and increase of GSSG and TGSH in blood. Met deficient diet together with atrazine also caused increases of blood GSH, GSSG and TGSH. However, when both toxicants were administered, the effect of dietary Met on blood glutathione was not clear.

The mercapturic acids in urine were estimated by measuring conjugated sulfhydryl groups after alkaline hydrolysis. Conjugated cysteine or any other conjugated -SH groups were not excluded by this measurement. Therefore, the measurement was a crude estimation of mercapturic acid derivatives in the urine. Thus, the effect of diet and toxicants on mercapturic acids excretion must be evaluated conservatively. In this study, the most predominant influence on urine mercapturic acids excretion was through atrazine treatment, which caused an increase in excretion. This finding is in accord with the view that atrazine is a percursor of mercapturic acids in the rat (Climie and Hutson, 1978). However, the increase of mercapturic acids excretion in urine was not accompanied by liver or blood glutathione decreases, as stated before.

The effect of atrazine on increasing mercapturic acids excretion was also accompanied by increased excretion of mercury in the urine. During period II, the administration of low dose of mercury ($MeHg_2$) with or without atrazine in the diet generally resulted in higher excretion of mercapturic acid in urine in comparison with $MeHg_1$ (no mercury) and

$MeHg_3$ (high mercury) treatments. During period III, the effects of low and high doses of MeHg with or without atrazine are not distinguishable from each other. The excretion of mercapturic acids in urine is accompanied by increase of mercury excretion until period II, but during period III, the mercapturic acids excretion declined while mercury excretion increased and became independent from mercapturic acids excretion. This is possibly due to the fact that between the third and fifth week of administration of toxicants, the kidney had developed a dysfunction. The increase in kidney weight might also be such dysfunction. Therefore, mercury leakage into the urine without prior conjugation may have occurred.

The increase of mercapturic acids excretion during period II was seen in the urine in all treatments. Such an increase might be related partially to the excretion of endogenous -SH conjugated metabolites, such as steroids, prostaglandins, and quinones (Chasseaud, 1976; Chaudhari et al., 1978).

Discussion of Behavioral Studies

Performance of a rat in the exposure phase in response to the two-choice light stimuli is highly dependent on its previous experience with light stimuli in the pre-exposure phase. The unsatisfactory responses of rats to the two-choice form light stimuli in the exposure phase might be due to the fact that the correct responses of rats to the light stimuli during the last two sessions in the pre-exposure phase were only 55-81% (23-34 correct responses from 42 trials; see Table 33).

Daily treatments of rats with dietary Met and toxicants had no meaningful effect on performances of rats as measured by number of, or time for, correct and incorrect responses, and number of defaults in response to the two-choice form light stimulus. Despite significant clinical and biochemical effects of treatments, performances in response to the visual stimulus were not significant after 27 daily testing sessions. Nevertheless, analysis of variance and mean comparisons revealed that, except ATCR variable which was affected in both replicates, the other variables were only affected in one of the two replicates (see Tables 34 and 35). Thus, combining of data from two replicates of behavioral studies might give a fallacious result.

Although ATCR in both replicates was significantly ($P<0.10$) affected by testing sessions and interaction of Met and atrazine factors, the mean comparison revealed that the effects were different in the two replicates. Therefore, they were not combinable and inconclusive. The overall session effects on the performance of all groups of rats are shown in Figures 7 to 10. The performance of rats in response to the stimulus in general improves significantly with the repetition of session. The average time for both CR and IR decreases until the 9th session (see Figure 10). This might indicate that all of the rats in different groups were learning the two-choice form of light stimulus and were responding at a fast rate. However, after the 9th session the improvement in learning was gradually diminished. Similar trends are observable in Figure 8 for the number of defaults which decreases until about the 9th session.

Since MeHg accumulated to a great extent in CNS, the effect of two doses of MeHg (0.5 and 1.5 mg/kg body weight) was the major consideration among the other treatments for the behavioral study. Although the occipital lobe of rats brain did accumulate a high concentration of mercury (see Table 10), visual pathways probably were not affected in this experiment because MeHg in rat mainly effects the cerebellum and peripheral nervous system rather than the cerebral cortex (Evans et al., 1975). This might be an important explanation for the no-effect findings. Thus, more probably the visual pathway of the rats was less affected during the course of repeated exposures, or the effect was not detected by the two-choice form discrimination test. Ataxia, the leg-crossing phenomenon and other clinical signs of MeHg toxicity were predominant in high exposure (1.5 mg/kg) animals. A replication of this study with pathological investigation would give greater information about the defects in the visual pathway in this study.

Perhaps it would have been possible to see the effects of diets and toxicants by the two-choice form light discrimination test if the number of training sessions in the pre-exposure phase had been extended from 20-26 sessions to 40-50 sessions and the number of testing sessions in the exposure phase had been extended from 25-29 to 90-120 sessions. However, such extensions were not possible in our experiment. Since extension of pre-exposure days would have caused the rats to be significantly older, achieving a Met deficient condition would have been less likely during the short exposure phase. Increasing the exposure days, at least for the rats treated with high level of MeHg, probably would have resulted in high mortality (death of rats in these groups started after

30 days of exposure of high level of MeHg).

An alternative explanation for the no effect findings would be that the subtle visual effect of MeHg toxicity was masked by a high motivation of rats by water deprivation, i.e., water deprivation resulted in a high motivation of the rats in both control and treatment groups to respond to the light stimulus. Such speculation might be repudiated by the evidence that the mean CR increased while the IR decreased in all treatments as the session number increased (Figure 7).

The findings suggest that the two-choice form discrimination is not the prime choice of behavioral studies in rats for a short period of time. Other behavioral tests such as ambulation, rearing (Lown et al., 1977), T-maze learning (Post et al., 1973), swimming ability (Zenick, 1974), and active avoidance learning (Hughes et al., 1975) might be more suitable for the investigation of MeHg effect on the rat and its interaction with nutrients and other toxicants in short term studies.

SUMMARY

The effect of dietary methionine (Met), a percursor of cysteine and glutathione, on the toxicity of methylmercury hydroxide (MeHg) and atrazine in male Wistar rats was investigated. Eighteen daily treatments were arranged from combinations of three levels of dietary Met (diet A = 0.42%, diet B = 0.18%, and diet C = 0.66%), three levels of MeHg ($MeHg_1$ = 0.0 mg, $MeHg_2$ = 0.5 mg, $MeHg_3$ = 1.5 mg/kg of body weight) and two levels of atrazine (-Atrazine = 0.0 mg and +Atrazine = 500 mg/kg diet) to which a total of 144 rats in two replicates (72 rats/replicate containing 4 rats/treatment) were randomly assigned.

Rats of each replicate were trained for 20-26 sessions to respond to the computer generated light stimulus in the pre-exposure phase of the study (26-32 days). A two-choice form light discrimination test was conducted for 25-29 sessions to investigate behavioral effects of diets and toxicants on rats in the exposure phase (32-35 days).

Atrazine was administered in the diets which were provided to the rats ad libitum, and a combined two-day dose of MeHg was administered to the rats by gavage every other day. For mercapturic acids and mercury analyses, urine of rats was collected three times, in two-week intervals between collection, beginning a week after the treatments were started.

At the end of the experiment, rats were anesthetized with ether; samples of blood, liver, kidney, and brain were collected for glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), glutathione-

S-transferase (GSH-S-trans), total glutathione (GSH and GSSG), prostaglandin (PG), and mercury assays.

Excess dietary Met had a protective effect on MeHg and atrazine toxicity in rats using weight gain as the index of toxicity. An increased weight loss was observed in the rats fed diet B and treated with MeHg and atrazine in this experiment. The onset of MeHg toxicity signs started earlier in the groups fed atrazine in the diet. Liver weight was increased in response to the toxicants in the groups of rats fed diet A or B but liver weight was not changed by exposure to toxicant in the groups fed diet C. The protective effect of excess Met content of diet C caused a significantly lower increase of kidney weight in the rats which were treated with $MeHg_3$, in contrast with the two other diets.

Blood had the highest and liver the lowest concentrations of mercury. Kidney accumulated more mercury than did brain in rats which were treated with $MeHg_2$. The reverse effect was found when $MeHg_3$ was administered. There was no correlation between blood mercury concentration and status of blood GSH. Feeding the Met deficient diet (B) resulted in a greater accumulation of mercury in blood, liver and brain. This effect was most pronounced in brain tissue. Atrazine treatment caused a significant increase in mercury excretion in urine after three weeks of exposure but its effect at the end of the experiment was not significant, suggesting adaptation.

Activities of whole blood GSH-Px declined as the concentration of mercury in blood increased. The deficiency of Met in the diet may have caused the decline of GSH-Px through increases in blood mercury concentrations. Despite an increase in mercury concentration in liver,

liver GSH-Px and GSH-Rd were unchanged. The long-term exposure of rats to atrazine in this experiment caused reductions of liver GSH-S-trans activity toward 1,2-dichloro-4-nitrobenzene. Increase in MeHg dose caused a decrease in GSH-S-trans activity in the groups fed diet B while GSH-S-trans activity increased in rats fed Met imbalanced diets (B and C). Feeding rats with diet C did not result in a greater liver competence for atrazine and MeHg detoxification.

Treatments with $MeHg_2$ and $MeHg_3$ resulted in increases in ex-vivo synthesis of PGE_1 and TXB_2 by platelets from clotted blood. The increase in PGs might have resulted from a buildup of some endogenous hydroperoxides because of a decrease of GSH-Px activity, or a decrease in the availability of vitamin E.

Although it has been shown that atrazine can deplete the GSH reserves of the body, feeding rats 500 mg of atrazine/kg diet ad libitum for 5 weeks did not cause persistent changes in liver total GSH, but there was an increase in blood GSH. Administration of MeHg alone caused a slight increase of GSH in liver. The development of tolerance after repeated exposure and the presence of alternative detoxifying pathways and interorgan translocation of GSH might be the reasons for no effect of atrazine on liver GSH status.

Dietary Met caused no effect on liver GSH and an increase in GSSG (oxidized glutathione) and total GSH in blood. It also caused an increase in GSH, GSSG and total GSH in blood when atrazine was present. Atrazine increased urinary mercapturic acids excretion, and the excretion was also particularly affected by the low dose of MeHg after three weeks of repeated exposure. The increased mercapturic acid excretion may have been related

to the endogenous conjugated metabolites such as steroids, prostaglandins, and quinones.

Despite significant clinical and biochemical effects of treatments on rats, performance of rats after 27 trials with two-choice form light stimulus as measured by number of, or time for correct and incorrect responses and number of defaults was not significantly affected by the treatments. Although the occipital lobe of rats brains did accumulate a high concentration of mercury, an effect of MeHg on the visual pathway of rats was not detected by the two-choice form discrimination test after 27 testing sessions. Instead peripheral nervous system and cerebellum were highly affected, as indicated by the presence of ataxia and the leg-crossing phenomenon.

ACKNOWLEDGEMENTS

I would like to express my thanks to my major professor, Dr. John Hathcock, for his helpful advice. His careful thoughts on the design of this experiment, his introduction and guidance to nutritional toxicology are greatly appreciated.

Advice and assistance from Dr. Jacqueline Dupont, Dr. Robert E. Serfass, Dr. William E. Lloyd and Dr. Jerry L. Sell were helpful and constructive on numerous occasions.

I wish to thank Dr. David L. Hopper who introduced me to behavioral toxicology research and provided me with behavioral toxicology laboratory facilities and for his suggestions that guided this research. Beyond this, his assistance on statistical analysis of the behavioral data is greatly appreciated.

I would like to thank Dr. Henry M. Stahr for his technical advice and for offering me facilities of the Chemistry Laboratory in the Veterinary Diagnostic Laboratory.

I am also grateful to Mr. Darrel Stearns from the behavioral toxicology laboratory, and Mrs. Cynthia Shriver and Miss Mary Remmes from the laboratories of the Food and Nutrition Department, for their skillful assistance.

And finally, I would like to express my special thanks to my wife, Dr. Simin Meydnai, whose technical assistance and encouragement have always been supportive.

LITERATURE CITED

Al-Damluji, S. F. 1976. The Clinical Committee on Mercury Poisoning. WHO Bull. 53 (Suppl.):65-82.

Alexander, J. and T. Norseth. 1979. The effect of selenium on the biliary excretion and organ distribution of mercury in the rat after exposure to methylmercury chloride. Acta Pharmacol Toxicol. 44: 168-176.

Anderson, M. E. and A. Meister. 1980. Dynamic state of glutathione in blood plasma. J. Biol. Chem. 225:9533.

Anonymous. 1981. Selenium-containing glutathione peroxidase: its synthesis and function of arachidonate metabolism. Nutr. Rev. 39: 21-23.

Arias, I. M. and W. B. Jakoby. 1976. Glutathione: Metabolism and Function. Raven Press, New York.

Asaoka, K., H. Ito, and K. Takahashi. 1977. Monkey glutathione-S-transferases. J. Biochem. 82:973-981.

Awasthi, Y. C., E. Beutler, and S. K. Srivastava. 1975. Purification and properties of human erythrocyte glutathione peroxidase. J. Biol. Chem. 250:5144-5149.

Bakir, F., S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N. Y. Al-Pami, S. Tikriti, H. I. Dhahir, T. W. Clarkson, J. S. Smith, and R. A. Doherty. 1973. Methylmercury poisoning in Iraq. An Inter-university Report. Science 181:230-241.

Bakke, J. E., J. D. Larson, and C. E. Price. 1972. Metabolism of atrazine on 2-hydroxyatrazine by the rat. J. Agric. Food Chem. 20:602-607.

Bend, J. R., H. Mukhtar, G. L. Fourman, and I. P. Lee. 1977. Epoxide metabolizing enzyme activities in steroidogenic tissues of the rat. Fed. Proc. 36:961.

Bergmeyer, H. U. 1978. Formulas. Pages 236-239 in H. U. Bergmeyer, ed. Principles of Enzyamatic Analysis. Verlag Chemie, Weinheim, New York.

Bergmeyer, H. U., and K. Gawehn (eds). 1974. Methods of Enzymatic Analysis. Vol. I, 2nd ed. Academic Press, New York.

Black, W. D. and M. J. Claxton. 1979. A simple, reliable and inexpensive method for the collection of rat urine. Lab. Anim. Sci. 29:253-254.

Bloss, J. L. and G. H. Singer. 1978. Neuropharmacological and behavioral evaluation of prostaglandin E_2 and 11-thiol-11-desoxy prostaglandin E_2. Psychopharmacology 57:295-302.

Booth, J., E. Boyland, and P. Sims. 1961. An enzyme from rat liver catalyzing conjugations with glutathione. Biochem. J. 79:516-524.

Boyland, E. and L. F. Chasseaud. 1970. The effect of some carbonyl compounds on rat liver glutathione levels. Biochem. Pharmacol. 19:1526-1528.

Brown, J. H. and S. H. Pollock. 1972. Stabilization of hepatic lysosomes of rats by vitamin E and selenium in vivo as indicated by thermal labilization of isolated lysosomes. J. Nutr. 102:1413-1419.

Bryant, R. W. and J. M. Bailey. 1980. Altered lipoxygenase metabolism and decreased glutathione peroxidase activity in platelets from selenium-deficient rats. Biochem. Biophys. Res. Commun. 92:268-276.

Burk, R. F., M. J. Trumble, and R. A. Lawrance. 1980. Rat hepatic glutathione-dependent enzyme protection against lipid peroxidation in the NADPH-microsomal lipid peroxidation system. Biochim. Biophys. Acta 618:35-41.

Butterworth, R. F., M. Gonce, and A. Barbeau. 1978. Accumulation and removal of ^{203}Hg in different region of the rat brain. Can. J. Neurol. Sci. 5:397-400.

Campbell, I. C., J. R. Hayes, and P. M. Newberne. 1978. Dietary lipotrops, hepatic microsomal mixed-function oxidase activities, an in vivo covalent binding of aflatoxin B_1 in rats. Cancer Res. 38: 4569-4573.

Cantor, A. H., M. L. Scott, and T. Noguchi. 1975. Biological availibility of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. J. Nutr. 105:96-105.

Cavanagh, J. B. and F. C-K. Chen. 1971. Amino acid incorporation in protein during the "silent phase" before organomercury and p-bromophenylacetylurea neuropathy in the rat. Acta Neuropathol. 19:216-224.

Cember, H. and H. Donagi. 1964. The influence of dose level and chemical form on the dynamics of mercury elimination. Pp. 440-442 in Proc. 14th Intern. Congr. Occupational Health, Intern. Congr. Ser. 62. Vol. II. Experta Medica Foundation, Amsterdam.

Chang, L. W. and P. A. Desnoyers. 1978. Methylmercury induced biochemical and microsomal changes in the rat liver. J. Environ. Pathol. Toxicol. 1:569-579.

Chang, L. W. and H. A. Hartmann. 1972a. Blood brain barrier dysfunction in experimental mercury intoxication. Acta Neuropathol. (Berl.) 21:179-184.

Chang, L. W. and H. A. Hartmann. 1972b. Electron microscopic histachemical study of the localization and distribution of mercury in nervous system after mercury intoxication. Exp. Neurology 35:122-137.

Chang, K, W, M. Gilbert, and J. Sprecher. 1978. Modification of methylmercury neurotoxicity by vitamin E. Environ. Res. 17:356-366.

Chang, L. W., M. M. Gilbert, and J. Sprecher. 1977. Morphological evidence on the protective effects of vitamin E against methylmercury toxicity in nervous system. Fed. Proc. 36:404.

Chasseaud, L. F. 1979. The role of glutathione and glutathione-S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res. 29:175-184.

Chasseaud, L. F. 1976. Conjugation with glutathione and mercapturic acid excretion. In J. M. Arias and W. B. Jackoby, eds. Glutathione: Metabolism and Function. Raven Press, New York.

Chasseaud, L. F. 1973. The nature and distribution of enzymes catalyzing the conjugation of glutathione with foreign compounds. Drug Met. Rev. 2:185-220.

Chaudhari, A., M. W. Anderson, and T. E. Eling. 1978. Conjugation of 15-keto-prostaglandins by glutathione-S-transferases. Biochim. Biophys. Acta 531:56-64.

Chen, R. W., V. L. Lacy, and P. D. Whanger. 1975. Effect of selenium on methylmercury binding to subcellular and soluble protein in rat tissues. Res. Commun. Chem. Pathol. Pharmacol. 12:297-308.

Chmielnicka, J. and E. A. Brzeznicka. 1978. The influence of selenium on the level of mercury and metallothionine in rat kidneys in prolonged exposure to different mercury compounds. Bull. Environ. Contam. Toxicol. 19:183-190.

Christophersen, B. O. 1969. Reduction of linolenic acid hydroperoxide by a glutathione peroxidase. Biochim. Biophys. Acta 176:463-470.

Christopherson, B. O. 1968. Formation of monohydroxy-polyemic fatty acids from lipid peroxides by a glutathione peroxidase. Biochim. Biophys. Acta 164:35-46.

Clarkson, T. W. 1976. Quantitative measures of toxicity of mercury in man. Pages 433-475 in A. S. Prasad, ed. Trace Elements in Human Health and Disease. Academic Press, New York.

Clarkson, T. W. 1972. Recent advances in the toxicology of mercury with emphasis on the alkylmercurials. Pages 203-234 in CRC Critical Reviews in Toxicology. Chemical Rubber Publ. Co., Cleveland, OH.

Climie, I. J. G. and D. Hutson. 1979. Conjugation reactions with amino acids including glutathione. Pages 537-547 in H. Geissbuhler. ed. Advances in Pesticide Science, Part 3. Pergamon Press, New York.

Cone, J. E., R. M. Del Rio, and T. C. Stadtman. 1977. Clostridial glycine reductase complex. Purification and characterization of the selenoprotein component. J. Biol. Chem. 252:5337-5343.

Cone, J. E., R. M. Del Rio, J. N. Davis, and T. C. Stadtman. 1976. Chemical characterization of the selenoprotein component of chostridial glycine reductase: Identification of selenocystein as the organaselenium moiety. Proc. Natl. Acad. Sci. USA 73:2659-2663.

Congiu, L, F. P. Corongiu, M. Dore, C. Montaldo, S. Vargiolu, D. Casula, and G. Spiga. 1979. The effect of lead nitrate on the tissue distribution of mercury in rats treated with methylmercury chloride. Toxicol. Appl. Pharmacol. 51:363-366.

Cousins, R. J. 1979. Regulatory aspects of zinc metabolism in liver and intestine. Nutr. Rev. 37:97-103.

Crayford, J. V. and D. M. Hutson. 1972. The metabolism of the herbicide, 2-chloro-4-(ethylamino)-6-(1-cyano-1-methylethylamino)-s-triazine in rat. Pest. Biochem. Physiol. 2:295-307.

Dalgaard-Mikkelsen, S. V. and E. Poulsen. 1962. Toxicology of herbicides. Pharmacol. Rev. 14:225-250.

Damstra, T. 1978. Environmental chemicals and nervous system dysfunction. Yale J. Biol. Med. 51:457-468.

Datta, D. V., S. Singh, and P. N. Chhuttani. 1973. Role of liver in the release of plasma bromsulphathalein-glutathione conjugating enzyme in acute hepatocellular injury. Indian J. Med. Res. 61:1351-1359.

Dauterman, W. C. and W. Muecke. 1974. In-vitro metabolism of atrazine by rat liver. Pest. Biochem. Physiol. 4:212-219.

Diplock, A. T. 1976. Metabolic aspects of selenium action and toxicity. CRC, Critical Rev. Toxicol. 4:271-329.

Down, W. H. and L. F. Chasseaud. 1979. Effect of repeated oral administration of phenobarbitone or DDT on hepatic glutathione-S-transferase activity in non-human primates: Comparison with rat. Biochem. Pharmacol. 28:2525-2528.

Edes, T. E., S. K. Clinton, C. R. Truex, and W. J. Visek. 1979. Intestinal and hepatic mixed function oxidase activity in rats fed methionine and cystine-free diets (40620). Soc. Exp. Biol. Med. 162:71-74.

El-Begrami, M. M., H. E. Ganther, and M. L. Sunde. 1976. Vitamin E decreases methylmercury toxicity. Poult. Sci. 55:2033.

Ellman, G. L. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82:70-77.

Evans, H. L., V. G. Laties, and B. Weiss. 1975. Behavioral effects of mercury and methylmercury. Fed. Proc. 34:1858-1867.

Finkelstein, J. D., J. J. Martin, W. E. Kyle, and B. J. Harris. 1980. Enzyme of methionine metabolism in regenerating rat liver (40906). Proc. Soc. Exp. Biol. Med. 164:510-513.

Flohe, L., W. A. Gunzler, and H. H. Schock. 1973. Glutathione peroxidase: a selenoenzyme. FEBS Letters 32:132-134.

Flower, R. J. 1977. Prostaglandins and related compounds. Agent Actions Suppl. 3:99-105.

Forseth, J. A., R. C. Piper, and R. Carlsonn. 1974. Relationship of dietary selenium and methylmercury to blood and tissue selenium and mercury concentrations deficiency-toxicity signs in swine. Fed. Proc. 33:660.

Foster, T. S. and S. U. Khan. 1976. Metabolism of atrazine by the chicken. J. Agric. Food Chem. 24:566-570.

Friedberg, T., P. Bently, P. Stasiecki, H. R. Glatt, D. Raphael, and F. Oesch. 1979. The identification, solubilization, and characterization of microsome-associated glutathione-S-transferases. J. Biol. Chem. 254:12028-12033.

Frostrom, J. W., J. J. Zakowski, and A. L. Tappel. 1978. Identification of the catalytic site of rat liver glutathione peroxidase selenocysteine. Biochemistry 17:2639-2644.

Fukezawa, K. and A. Tokumura. 1976. Gultathione peroxidase activity in tissues of vitamin E deficient mice. J. Nutr. Sci. Vitaminol. 22:405-407.

Gallagher, P. J. and R. L. Lee. 1980. The role of biotransformation in organic mercury neurotoxicity. Toxicology 15:129-134.

Ganther, H. E. 1978. Modification of methylmercury toxicity and metabolism by selenium and vitamin E: possible mechanisms. Environ. Health Perspect. 25:71-76.

Ganther, H. G. 1975. Selenoproteins. Chem. Sci. 8A:79.

Ganther, H. E., C. Goudi, M. L. Sunde, M. J. Kopecky, P. Wagner, S. H. Oh, and W. G. Hoekstra. 1972. Selenium: Relation to decreased toxicity of methylmercury added to diets containing tuna. Science 175:1124-1125.

Germaine, G. S. and R. M. Arneson. 1977. Selenium induced glutathione peroxidade activity in mouse neuroblastoma cells. Biochem. Biophys. Res. Commun. 79:119-123.

Gillette, J. R. 1977. Formation of reactive metabolites of foreign compounds and their covalent binding to cellular constituents. Pages 577-589 in D. H. K. Lea, ed. Handbook of Physiology: Reactions to Environmental Agents. Section A. American Physiological Society, Bethesda, MD.

Glatt, H. and F. Oesch. 1977. Inactivation of electrophilic metabolites by glutathione-S-transferases and limitation of the system due to subcellular localization. Arch. Toxicol. 39:87-96.

Griffith, O. W. and A. Meister. 1979. Glutathione: interorgan translocation, turnover, and metabolism. Proc. Natl. Acad. Sci. USA 76: 5606-5610.

Guthenberg, C. and B. Mannervik. 1979. Purification of glutathione-S-transferases from rat lung by affinity chromatography. Biochem. Biophys. Res. Commun. 86:1304-1310.

Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione-S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139.

Hafeman, D. G., R. A. Sunde, and W. G. Hoekstra. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 104:580-587.

Hamdy, M. K. and N. V. Prabhu. 1979. Behavior of mercury in biosystems. III. Biotransference of mercury through food chains. Bull. Environ. Contam. Toxicol. 21:170-178.

Harper, A. E., N. J. Benenenge, and R. M. Wohlhueter. 1970. Effect of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428-558.

Hathcock, J. N. 1976. Nutrition: toxicology and pharmacology. Pages 504-515 in D. M. Hegsted et al., eds. Present Knowledge in Nutrition. The Nutrition Foundation, Inc., New York, Washington.

Hawkes, W. C., D. E. Lyons, and A. L. Tappel. 1979. Identification and purification of rat liver selenocysteine specific transfer RNA. Fed. Proc. 38:820.

Hayakawa, T. 1977. Glutathione-S-transferases in metabolism of foreign compounds. Ecotoxicol. Environ. Safety 1:305-309.

Hayakawa, T., R. A. LeMahifu, and S. Udenfriend. 1974. Studies on glutathione-S-arene oxide transferase - A sensitive assay and partial purification of the enzyme from sheep liver. Arch. Biochem. Biophys. 162:223-230.

Hazelhof, E. and D. H. Nugteren. 1978. Pages 201-206 in H. Sies and A. Wendel, eds. Functions of Glutathione in Liver and Kidney. Springer-Verlag, Berlin.

Herman, S. P., R. Klen, F. A. Talley, and M. R. Krigman. 1973. An ultrastructural study of methylmercury induced primary sensory neuropathy in the rat. Lab. Invest. 28:104-118.

Hirayama, K. 1980. Effect of amino acids on brain uptake of methylmercury. Toxicol. Appl. Pharmacol. 55:318-323.

Hirayama, K. 1975. Transport mechanism of methylmercury: intestinal absorption, biliary excretion and distribution of methylmercury. Kumamoto Med. J. 28:151-153.

Hirota, Y., S. Yamaguchi, N. Shimajoh, and K. Sano. 1980. Inhibitory effects of methylmercury on the activity of glutathione peroxidase. Toxicol. Appl. Pharmacol. 53:174-176.

Hissin P. J. and R. Hilf. 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74: 214-226.

Ho, P. P. K., P. Walters, and H. R. Sullivan. 1976. Biosynthesis of thromboxane B_2: assay, isolation and properties of the enzyme system in human platelets. Prostaglandins 12:951-970.

Hoekstra, W. G. 1975. Biochemical function of selenium and its relation to vitamin E. Fed. Proc. 34:2083.

Hopper, D. L. 1976. Delayed matching to sample performance and parathion toxicity in the squirrel monkey (Saimiri sciureus). Ph.D. Thesis. Library, Iowa State University, Ames, IA. 189 pp.

Horrobin, D. F. 1978. Prostaglandins - physiology, pharmacology and clinical significance. Eden Press Inc., Montreal.

Horrobin, D. R., A. I. Ally, R. A. Karmali, M. Karmazyn, M. S. Manku, and R. O. Morgan. 1978. Prostaglandins and schizophrenia: further discussion of the evidence. Psychol. Med. 8:43-48.

Hughes, R., R. Belser, and C. W. Brett. 1975. Behavioral impairment produced by exposure to subclinical amounts of methylmercury chloride. Environ. Res. 10:54-58.

Hutson, D. H., L. C. Hoadley, M. H. Giffins, and C. Donniger. 1970. Mercapturic acid formation in the metabolism of 2-chloro-4-ethylamino-6-(1-methyl-1-cyanoethylamino)-s-triazine in rat. J. Agric. Food Chem. 18:507-512.

Hwang, D. H., M. M. Mathias, J. Dupont, and D. L. Meyer. 1976. Linolate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 105:995-1002.

Jacob, J. M., N. Carmichael, and J. B. Cavanagh. 1977. Ultrastructural changes in the nervous system of rabbits poisoned with methylmercury. Toxicol. Appl. Pharmacol. 39:249-261.

Jacob, J. M., J. B. Cavanagh, and N. Carmichael. 1975. Effects of chronic dosing with mercuric-chloride on dorsal root and trigeminal ganglia of rats. Neuropathol. Appl. Neurobiol. 1:321-337.

Jakoby. W. B. 1978. Page 157 in H. Sies and A. Wendel, eds. Function of Glutathione in Liver and Kidney. Springer-Verlag, Berlin, Heidelberg, New York.

Jakoby, W. B., W. H. Habig, J. H. Keen, J. N. Ketley, and M. J. Pabst. 1976. Pages 189-211 in L. Arias and W. B. Jakoby, eds. Glutathione: Metabolism and Function. Raven Press, New York.

James, S. P., D. J. Jeffery, R. H. Waring, and D. A. White. 1971. Reaction of monobromo derivatives of cyclopentant, cyclohexane and cyclohepatane and of related compounds with glutathione in vivo and nature of the sulphur-containing metabolites excreted. Biochem. Pharmacol. 20:897-907.

Joensun, O. I. 1971. Fossil fuels as a source of mercury pollution. Science 172:1027-1028.

Johnson, E. E., K. R. Kampen, and W. Binns. 1972. Effects on cattle and sheep of eating hay treated with triazine herbicides, trazine and prometon. Am. J. Vet. Res. 33:1433-1438.

Kamisaka, K., W. H. Habig, J. N. Ketley, I. M. Arias, and W. B. Jakoby. 1975. Multiple form of renal and hepatic glutathione-S-transferases. Eur. J. Biochem. 60:153-161.

Kaplomitz, N., G. Clifton, J. Kuhlenkamp, and J. D. Wallin. 1976. Comparison of renal and hepatic glutathione-S-transferases. Biochem. J. 158:243-248.

Kasuya, M. 1975. The effect of vitamin E on the toxicity of alkyl mercurials on nervous tissue in culture. Toxicol. Appl. Pharmacol. 32:347-354.

Kasuya, M. 1972. Effects of inorganic, aryl, alkyl and other mercury compounds on the outgrowth of cells and fibers from dorsal root ganglia in tissue culture. Toxicol. Appl. Pharmacol. 23:136-146.

Keen, J. H., W. H. Habig, and W. B. Jakoby. 1976. Mechanism for the several activities of glutathione-S-transferases. J. Bio. Chem. 251:6183-6188.

Kobayashi, H., A. Yayama, N. Matsusaka, K. Takeno, and I. Yanaguju. 1980. Effect of methylmercury on brain acethyl choline concentration and turnover in mice. Toxicol. Appl. Pharmacol. 54:1-8.

Kraus, P. and B. Gross. 1979. Particle bound glutathione-S-transferases. Enzymes 24:205-208.

Kraus, P. and H. D. Kolft. 1980. The activity of glutathione-S-Transferases in various organs of the rat. Enzymes25:158-160.

Krebs, H. A., R. Hems, and J. Vina. 1978. Pages 8-12 in H. Sies and A. Wendel, eds. Function of Glutathione in Liver and Kidney. Springer-Verlag, New York.

Kum-Tatt, L., I. Tan, and A. Seet. 1975. A new colorimetric method for the determination of NADH/NADPH dependent glutathione reductase in erythrocytes in plasma. Clin. Chim. Acta 58:101-108.

Kurland, L. F., S. N. Faris, and H. Siedler. 1960. Minamata disease. World Neurol. 1:370-381.

Kurokama, I., T. Kimura, Y. Ueno, Y. Suzuki, T. Nagai, and M. Kamamura. 1970. The effect of vitamin E on the preservation of blood. J. Vitaminol. 16:180-189.

Kutsuma, M., ed. 1968. Minamata Disease. Kumamoto University, Japan.

Lajtha, A. 1962. The brain barrier system. Pages 399-430 in K. A. C. Elliot, I. H. Page, and J. H. Quastel, eds. Neurochemistry. 2nd ed. C. Thomas, Springfield, IL.

Lands, W. E. M. and L. H. Rome. 1976. Inhibition of prostaglandin biosynthesis. Pages 87-137 in S. M. M. Karim, ed. Prostaglandins: Chemical and Biochemical Aspects. University Park Press, Baltimore.

Lawrence, R. A. and R. F. Burk. 1976. Glutathione peroxidase activity in selenium deficient rat liver. Biochem. Biophys. Res. Commun. 71:952-958.

Leong, P. C. and H. P. Ong. 1971. Determination of mercury by using a gold trap in samples containing considerable sulfide minerals. Anal. Chem. 43:940-941.

Little, C. and P. J. O'Brien. 1968 a. The effectiveness of a lipid peroxide in oxidizing protein and non-protein thiols. Biochem. J. 106:419-423.

Little, C. and P. J. O'Brien. 1968 b. An intercellular glutathione peroxidase with a lipid peroxide substrate. Biochem. Biophys. Res. Commun. 31:145-150.

Lown, B. A., J. B. Morganti, C. H. Stineman, and E. J. Massaro. 1977. Differential effects of acute methylmercury poisoning in the mouse arising from route of administration: LD50, tissue distribution and behavior. Gen. Pharmacol. 8:97-101.

Lucy, J. A. 1972. Functional and structural aspects of biological membranes: A suggested structural role for vitamin E in the control of membrane permability and stability. Ann. N. Y. Acad. Sci. 203: 4-11.

Lutz, G. A. 1967. Design of overview system for evaluating the public health hazards of chemicals in the environment. Battelle Memorial Institute, Columbus, OH.

Magos, L. and W. H. Butler. 1976. The kinetics of methylmercury administered repeatedly to rats. Arch. Toxicol. 35:25-39.

Magos, L. and M. Webb. 1977. The effect of selenium on the brain uptake of methylmercury. Arch. Toxicol. 38:201-207.

McCosh, E. J., D. L. Meyer, and J. Dupont. 1976. Radioimmunoassay of prostaglandins E_1, E_2, $F_{2\alpha}$ in unextracted plasma, serum and myocardium. Prostaglandins 12:476-486.

McCoy, K. E. M. and P. H. Weswing. 1969. Some selenium responses in the rat related to vitamin E. J. Nutr. 98:383-389.

Metzler, D. E. 1977. Page 1129 in Biochemistry. The Chemical Reactions of Living Cells. Academic Press, New York.

Mills, G. C. 1957. Glutathione peroxidase; an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. 229:189-197.

Mitchell, H. S., H. J. Rynbergen, L. Anderson, and M. V. Dibble, eds. 1976. Nutrition in Health and Disease. J. B. Lippincott Co., Philadelphia. 652 pp.

Mitchell, J. R., D. J. Jollow, W. Z. Potter, J. R. Gillette, and B. B. Brodie. 1973. Acetaminophen-induced hepatic necrosis. IV. protective role of glutathione. J. Pharmacol. Exp. Ther. 187:211-217.

Miyakawa, T. and M. Deshimaru. 1969. Electron microscopical study of experimentally induced poisoning due to organic mercury compound. Mechanism of development of the morbid change. Acta Neuropathol. (Berl.) 14:126-136.

Moldeus, P., D. P. Jones, K. Ormstad, and S. Orrenius. 1978. Formation and metabolism of a glutathione-S-conjugate in isolated rat liver and kidney cells. Biochem. Biophys. Res. Commun. 83:195-200.

Morgenstern, R., J. Meijer, J. W. DePierre, and L. Franster. 1980. Characterization of rat liver microsomal glutathione-S-transferase activity. Eur. J. Biochem. 104:167-174.

Morgenstern, R., J. W. DePierre, and L. Franster. 1979. Activation of microsomal glutathione-S-tranferase activity by sulfhydryl reagents. Biochem. Biophys. Res. Commun. 87:657-663.

Moron, M. S., J. W. DePierre, and B. Mannervik. 1979. Level of glutathione, glutathione reductase, and glutathione-S-tranferase activities in rat lung and liver. Biochim. Biophys. Acta 582:67-78.

Morse, E. E., H. Duncan, N. Hooker, and H. Morse. 1977. Hydrogen peroxide induces spauning with activation of prostaglandin endoperoxide synthesis. Science 196:298-300.

Mudd, S. H. and J. R. Poole. 1975. Labile methyl balances for normal humans on various dietary regimens. Metabolism 24:721-735.

Mukhtar, H. J., E. A. Leakey, T. H. Elmamlouk, J. R. Fouts, and J. R. Bend. 1979. Precocious development of hepatic glutathione-S-transferase activity with glueocorticoid administration in neonatal rat. Biochem. Pharmacol. 28:1801-1803.

Mykkanen, H. M. and H. E. Ganther. 1974. Effect of mercury on erythrocyte glutathione reductase activity in vivo and in vitro studies. Bull. Environ. Contam. Toxicol. 12:10-16.

Naganumra, A. and N. Imura. 1979. Methylmercury binds to a low molecular weight substance in rabbit and human erythocytes. Toxicol. Appl. Pharmacol. 47:613-616.

Nakamura. W., S. Hosoda, and K. Hayashi. 1974. Purification and properties of rat liver glutathione peroxidase. Biochim. Biophys. Acta 358:251-261.

National Research Council, National Academy of Sciences. 1978. Nutrient requirements of the laboratory rat. National Academy of Sciences, Washington, D. C.

Neal, P. A. and R. R. Jones. 1938. Chronic Mercurialism in the hatters' fur-cutting industry. J. Am. Med. Assoc. 110:337-343.

Norseth, T. and T. W. Clarkson. 1971. Intestinal transport of ^{203}Hg-labeled methylmercury chloride. Role of biotransformation in rats. Arch. Environ. Hlth. 22:568-577.

Norseth, T. and T. W. Clarkson. 1970. Studies on the biotransformation of ^{203}Hg-labeled methylmercury chloride. Role of biotransformation in rats. Arch. Environ. Hlth. 21:717-727.

Nugteren, D. H. 1970. Inhibition of prostaglandin biosynthesis by 8 cis, 12 trans, 14 cis-eicasatrienoic acid and 5 cis, 8 cis, 12 trans, 14 cis eicasatetraenoic acid. Biochim. Biophys. Acta 210:171-176.

Nugteren, D. H. and E. Hazelhof. 1973. Isolation and properties of intermediates in prostaglandin biosynthesis. Biochim. Biophys. Acta 326:448-463.

Ohi, G., S. Nishigaki, H. Seki, Y. Tamura, T, Maki, H. Konno, S. Ochiai, H. Yamada, Y. Shimamura, I. Mizoguchi, and H. Yagyu. 1976. Efficiency of selenium in tuna fish and selenite in modifying methylmercury intoxication. Environ. Res. 12:49-58.

Ohi, G., S. Nishigaki, H. Seki, Y. Tamura, T. Maki, H. Maeda, S. Ochiai, H. Yamada, Y. Shimamura, and H. Yagyu. 1975. Interaction of dietary methylmercury and selenium on accumulation and retention of these substances in rat organs. Toxicol. Appl. Pharmacol. 32: 527-533.

Ohki, S., N. Ogino, S. Yamamoto, and O. Hayaishi. 1979. Prostaglandin hydroperoxidase on intergral part of prostaglandin endoperoxidase synthetase from bovine vesicular gland microsomes. J. Biol. Chem. 254:829-836.

Oldendorf, W. H. and J. Szabo. 1976. Amino acid assignment to one of three blood-brain barrier amino acid carriers. Am. J. Physiol. 230:94-98.

Ormestead, K., D. P. Jones, and S. Orrenius. 1980. Characteristics of glutathione biosynthesis by freshly isolated rat kidney cells. J. Biol. Chem. 255:175-181.

Paglia, D. E. and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

Parizek, J. and I. Ostadalova. 1967. The protective effect of small amounts of selenite in sublimate intoxication. Experimentia 23: 142-143.

Passow, H., A. Rothstein, and J. W. Clarkson. 1961. The general pharmacology of the heavy metals. Pharmacol. Rev. 13:185-224.

Pekkanen, T. J. and M. Sandholm. 1972. The effect of experimental methylmercury poisoning on the activity of the TPNH-specific glutathione reductase of rat brain and liver. Acta Vet. Scand. 13:14-19.

Pekkanen, T. J. and M. Sandholm. 1971. The effect of experimental methylmercury poisoning on the number of sulfhydryl (SH) groups in the brain, liver and muscle of rat. Acta Vet. Scand. 12:551-560.

Pentschow, A. and F. Carro. 1966. Lead encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous system's capillaries. Acta Neuropathol. 6:266-278.

Pierce, S. and A. L. Tappel. 1978. Glutathione peroxidase activities from rat liver. Biochim. Biophys. Acta 523:27-36.

Pierce, S. and A. L. Tappel. 1977. Effect of selenite and seleno-methionine on glutathione peroxidase in the rat. J. Nutr. 107:475-479.

Pinkus, L. M., J. N. Ketley, and W. B. Jakoby. 1977. The glutathione-S-transferases as possible detoxification system of rat intestinal epithelium. Biochem. Pharmacol. 26:2359-2363.

Piotrowski, J. K., E. M. Bem, and A. Werner. 1977. Cadmium and mercury binding to metallothionein as influenced by selenium. Biochem. Pharmacol. 26:2191-2192.

Piotrowski, J. K., B. Trojanowska, J. Wisnoeoska-Knyple, and W. Bolonowska. 1974. Mercury binding in the kidney and liver of rats repeatedly exposed to meruric chloride: Introduction of methallothionein by mercury and cadmium. Toxicol. Appl. Pharmacol. 27:11-19.

Poirier, L. A., P. H. Grantharn, and A. E. Rogers. 1977. The effect of marginally lipotrope-deficient diet on the hepatic levles of S-adonosylmethionine and on the urinary metabolites of 2-acetyl aminofluorin in rats. Cancer Res. 37:744-748.

Porta, E. A., F. A. DeLaIglesia and W. S. Hartcroft. 1968. Studies on dietary hepatic necrosis. Lab. Invest. 18:283-297.

Post, E. M., M. G. Yang, J. A. King, and V. L. Sanger. 1973. Behavioral changes of young rats force-fed methylmercury chloride. Proc. Soc. Exp. Biol. Med. 143:1113-1116.

Potter, S. and G. Materone. 1974. Effect of selenite on the toxicity of dietary methylmercury and mercuric chloride in the rat. J. Nutr. 104:638-647.

Prohaska, J. R. 1980. The glutathione peroxidase activity of glutathione-S-transferases. Biochim. Biophys. Acta 611:87-98.

Prohaska, J. R. and H. E. Ganther. 1977. Interactions between selenium and methylmercury in rat brain. Chem-Biol. Interactions 16:155-167.

Pryor, W. A. 1976. Free Radicals in Biology. Vol. I. Academic Press, New York. 49 pp.

Ramwell, P. W. E. M. K. Leoney, and A. L. Sintetos. 1977. Regulation of the arachidonic acid cascade. Biol. Reprod. 16:70-87.

Reddy, K. and A. L. Tappel. 1974. Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of gastrointestinal tract and other tissues in the rat. J. Nutr. 104: 1069-1078.

Refsvik, T. 1978. Excretion of methylmercury in rat bile: The effect of diethylmaleate, cyclohexene oxide and acrylamide. Acta Pharmacol. Toxicol. 42:135-141.

Refsvik, T. and T. Norseth. 1975. Methylmercuric compounds in rat bile. Acta Pharmacol. Toxicol. 36:67-78.

Reiter, L. 1978. An introduction to nuerobehavioral toxicology. Environ. Health Perspect. 26:5-7.

Richardson, R. J. and S. D. Murphy. 1975. Effect of glutathione depletion on tissue deposition of methylmercury in rats. Toxicol. Appl. Pharmacol. 31:505-519.

Rotruck, J. T., A. L. Pope and W. G. Hoekstra. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-590.

Rowland, I., M. Davies, and P. Grasso. 1977. Biosynthesis of methylmercury compounds by the intestinal flora of the rat. Arch. Environ. Health 32:24-28.

Sameulsson, B. 1969. Biosynthesis of prostaglandins. Progr. Biochem. Pharmacol. 5:109-128.

Sell, J. L. and F. G. Horani. 1976. Influence of selenium on toxicity and metabolism of methylmercury in chicks and quail. Nutr. Rep. Int. 14:439-447.

Shimabukuro, R. H., D. S. Frear, H. R. Swanson, and W. C. Walsh. 1971. Glutathione Conjugation. Plant Physiol. 47:10-14.

Shimabukuro, R. H., H. R. Swanson, and W. C. Walsh. 1970. Glutathione conjugation. Plant Physiol. 46:103-107.

Skerfving, S. B. and J. F. Copplestone. 1976. Poisoning caused by consumption of organomercury-dressed seed in Iraq. Bull. W.H.O. 54:101-112.

Skerfving, S. and J. Vostal. 1972. Symptoms and signs of intoxication. Pages 93-108 in L. Friberg and J. Vostal, eds. Mercury In The Environment - An Epidemiological and Toxicological Appraisal. CRC Press, Cleveland, OH.

Smith, G. J., V. S. Ohl, and G. Litwach. 1977. Ligandin, the glutathione-S-tranferases and chemistry induced hepatocarcinogenesis: A review. Cancer Res. 37:8-14.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. The Iowa State University Press, Ames, Iowa. 593 pp.

Snow, N. S. 1962. Some observation on the reactive sulphydryl groups in haemoglobin. Biochem. J. 84:360-364.

Steinwall, O., and I. Klatzo. 1966. Selective vulnerability of the blood-brain barrier in chemically induced lesions. J. Neuropathol. Exp. Neurol. 25:542-559.

Stillings, B. R., H. Lagally, P. Baversfeld, and J. Soares. 1974. Effects of cystein, selenium and fish protein on the toxicity and metabolism of methylmercury in rats. Toxicol. Appl. Pharmacol. 30:243-254.

Stoewsand, G. S., C. A. Bache, and D. J. Lisk. 1974. Dietary selenium protection of methylmercury intoxication of Japanese quail. Bull. Environ. Contam. Toxicol. 11:152-156.

Sunde, M. L. 1976. Effect of vitamin E on mercury toxicity. Page 384 in Annual Report. Food Research Institute, University of Wisconsin.

Sunde, R. A. and W. G. Hoekstra. 1980. Structure, synthesis and function of glutathione peroxidase. Nutr. Rev. 38:265-273.

Suojanen, A., R. J. Gay, and R. Hilf. 1980. Influence of estrogen on glutathione levels and glutathione-metabolizing enzymes in uterine and R3230 AC mammary tumors of rats. Biochim. Biophys. Acta 630:485-496.

Suzuki, T. and T. Miama. 1971. Neurological symptoms and mercury concentration in the brain of mice fed with methylmercury salts. Ind. Health 9:51-58.

Swedish Expert Group. 1971. Methylmercury in fish. A toxicologic-epidemiologic evaluation of risks. Nord. Hyg. Tidskr. Suppl. 4.

Tai, H. H. and B. Yuan. 1977. Development of radioimmunoassay for thromboxane B_2 and its application to the characterization of thromboxane synthetase from platelets. Prostaglandins 13: 1018. (Abstr.)

Tapple, A. L. 1962. Vitamin E as the biological lipid antioxidant. Vitam. Horm. 20:493-510.

Thomas, D. J. and J. C. Smith. 1979. Partial characterization of a low molecular weight methylmercury complex in rat cerebrum. Toxicol. Appl. Pharmacol. 47:547-556.

Thor, H., P. Moldius, and S. Orrenius. 1979. Metatolic activiation and hepatotoxicity: Effect of cysteine, N-acetylcysteine, and methionine on glutathione biosynthesis and bromobenzene toxicity in isolated rat hepatocytes. Arch. Biochem. Biophys. 192:405-413.

Torstler, N., P. S. Brady, D. R. Romsos, and G. A. Leveille. 1979. Influence of dietary vitamin E on malondialdhyde levels in liver and adipose tissue and on glutathione peroxidase and reductase activities in liver and erythrocytes of lean and obese (ob/ob) mice. J. Nutr. 109:345-352.

Ulfvarson, U. 1969a. The absorption and distribution of mercury in rats fed organs from rats injected with various mercury compounds. Toxicol. Appl. Pharmacol. 15:525-534.

Ulfvarson, U. 1969b. The effect of the size of the dose on the distribution and excretion of mercury in rats after single intravenous injection of various mercury compounds. Toxicol. Appl. Pharmacol. 15:517-524.

Van Drop, D. A. 1971. Recent developments in the biosynthesis and the analysis of prostaglandins. Ann. N. Y. Acad. Sci. 180:181-199.

Webb, J. L. 1963. Enzyme and Metabolic Inhibitors. Vol. II. Academic Press, New York.

Welsh, S. O. 1977. Contrasting effects of vitamin A and E on mercury poisoning. Fed. Proc. 36:1146.

Welsh, S. O. 1976. Influence of vitamin E on mercury poisoning in rats. Fed. Proc. 35:761.

Welsh, S. O. 1974. Physiological effects of methylmercury toxicity: Interaction of methylmercury with selenium, tellurium and vitamin E. Ph.D. Thesis. University of Maryland, College Park, MD.

Welsh, S. O. and J. H. Soares, Jr. 1976. The protective effect of vitamin E and selenium against methylmercury toxicity in Japanese quail. Nutr. Repts. Int. 13:43-51.

White, W. L., M. M. Erickson, and S. C. Stevens, eds. 1976. Chemistry for the Clinical Laboratory. Mosby Company, St. Louis, MO. 184 pp.

Winge, D., R. Premakuma, and K. Rajogopalan. 1975. Metal-induced formation of metallothionein in rat liver. Arch. Biochem. Biophys. 170:242-252.

Wolkoff, A. W., J. N. Ketley, J. G. Waggoner, P. D. Berk, and W. B. Jakoby. 1978. Hepatic accumulation and intracellular binding of conjugated bilirubin. J. Clin. Invest. 61:142-149.

Wood, J. L. 1970. Biochemistry of mecapturic acid formation. Pages 261-299 in W. H. Fisherman, ed. Metabolic Conjugation and Metabolic Hydrolysis. Vol. II. Academic Press, New York.

Yoshino, Y., T. Zozai, and K. Nakao. 1966. Biochemical changes in rat brain poisoned with alkyl mercury compound with special reference to inhibition of protein synthesis in brain cortex slices. J. Nuerochem. Biochem. 13:1223-1235.

Younes, M., R. Schlichting, and C. P. Siegers. 1980. Glutathione-S-transferase activity in rat liver: effect of some factors influencing the metabolism of xenobiotics. Pharmacol. Res. Commun. 12:115-129.

Zakowski, A. L. 1978. Attachment of selenocysteine in the catalytic site of GSH-Px. Biochem. Biophys. Res. Commun. 84:248-253.

Zenick, H. 1974. Behavioral and biochemical consequences of methylmercury chloride toxicity. Pharmacol. Biochem. Behav. 2:709-713.