The Relationship Between Insect
Mineral Content and Radiation Sensitivity

By

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THE RELATIONSHIP BETWEEN INSECT
MINERAL CONTENT AND RADIATION SENSITIVITY

By

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Parts per million insect total body copper, iron, magnesium, sodium, and potassium were analyzed by atomic absorption spectrophotometry to determine if mineral content could be used as an effective biological indicator for a species-specific dose of ionizing radiation.

Simple and multiple regression analysis of 22 species of adult insects representing Anoplura, Coleoptera, Diptera, Hemiptera, Homoptera, Lepidoptera, Orthoptera and Thysanura indicated that there was a direct relationship between tissue-organ cation content and x-ray or gamma radiation sensitivity as defined by the $LD_{50/24 \text{ hour}}$, $LD_{50/28 \text{ day}}$, and $LD_{50/\text{mean-mortality}}$ dose parameters.

$LD_{50/24 \text{ hour}}$ regression models were shown to be highly correlated to insect feeding preferences. Mechanisms of death were postulated as being activated by oxidation and binding of mineral cations from high doses of ionizing radiation, with the subsequent inhibition of the CNS, and/or the inhibition of respiratory metabolism.

Analysis of $LD_{50/28 \text{ day}}$ and $LD_{50/\text{mean-mortality}}$ regression models appeared to indicate a species response to low doses of ionizing radiation via free radical inhibition of an integrated series of biological functions involving the CNS, respiratory system, circulatory system, buffer systems, tyrosinase enzyme system, RNA synthesizing system, and digestive-excretory systems. Several mechanisms for radiation-induced delayed death were postulated.

Although no LD_{50} values were available for developing regression models based on larvae, pupae, or nymphs, their mineral content did demonstrate characteristic differences when compared to the adult stages. Therefore, it would seem that specific mechanisms for characterizing their radiation sensitivity according to life cycle stage could be developed.

In all, 39 species of adults, 12 species of larvae, 5 species of pupae, and 3 species of nymphs were analyzed by atomic absorption spectrophotometry for insect total body Cu, Fe, Mg, Na, and K content in parts per million.

CHAPTER I

INTRODUCTION

Since 1935, when ionizing radiation was shown to produce mutations in fruit flies, there has been considerable interest in the effects of ionizing radiation on insects (74). In recent years, the successful uses of ionizing radiation in the control of insect pests, as well as the concern over the possible contamination of the biosphere by radiation from nuclear reactors and possible nuclear disasters have further stimulated study on radiation effects on insects (25-28).

Many attempts have been made to find biological indicators to explain the nuclear and cytoplasmic effects of ionizing radiation (12,79), but these have failed to account for the great variation in the radiosensitivities across insect orders (25-29, 61, 87).

This research is aimed at determining whether a correlation exists between the total body iron (Fe), copper (Cu), magnesium (Mg), sodium (Na), and potassium (K) content of an insect species and the radiation LD₅₀ (dose required to kill 50% of a given population) of that same species. The mineral content would, therefore, serve as an effective biological predictor of radiosensitivity. The rationale for the selection of these minerals was based on their important physiological

and biochemical roles in maintaining the normal homeostasis of organisms (16,20,90). These cations were generally essential to the insects' diet (39,51,52,96) and have been shown to be extremely important in regulating several metabolic and nervous system functions (39,41,42, 101-105).

If a relationship between mineral content and LD_{50} could be established, then the effect of a given radiation dose on the insect fraction of an ecological food chain could be predicted. In addition, tentative explanations of the mechanisms causing radiation protection and/or radiation death in insects could be postulated.

CHAPTER II

LITERATURE REVIEW

Principles of Atomic Absorption Spectroscopy

Since the development of commercial atomic absorption (= flame photometry) instrumentation in the early 1960's, atomic absorption spectroscopy has occupied an ever-increasing role in the clinical and biological laboratory (16).

Atomic absorption spectroscopy can be simply defined as the absorption of radiant energy by atoms (16). This absorption and its quantitative correlation with the concentration of metal ions originally present in a sample solution served as the basis of analytical atomic absorption spectrophotometry. The physical principles governing the use of atomic absorption spectrophotometry can be briefly described (16): Atoms of every element can absorb radiation at extremely narrow wavelength bands which are characteristically different for every element. For an element to be in a condition to absorb, its atoms must be chemically unbound and in their minimum energy or ground state. This condition is generally achieved by vaporizing the sample in a flame. The source of radiation is generally a hollow-cathode lamp,

whose cathode is made of the element being determined. The lamp emits the characteristic line spectra of that element while the sample absorbs energy only at a specific resonance line. The resultant effect is a diminished resonance line caused by the absorption of the vaporized sample in the flame.

The remaining spectral lines are removed by a monochromator which has been adjusted to select a band of wavelengths around the resonance line, and to reject all others. The photodetector would then "see" only the diminished resonance line. A schematic of the optical system is presented in Figure 1.

The results from an atomic absorption analysis would therefore be consistent with Beer's Law which states that "the concentration of the element in a sample is proportional to the absorbance (optical density) in the flame" (16).

Analytical precision or reproducibility of $\frac{1}{2}$ - 1% has been reported for the atomic absorption technique when well-designed equipment was used and elements could be determined in the optimum analytical range (16).

Atomic Absorption Analysis of Insect Tissue

Relatively few researchers have used atomic absorption spectroscopy for insect mineral determinations, although this technique has been extensively used for many other organisms (16). Nation and Robinson (71), using atomic absorption spectroscopy, have quantitatively determined the parts per million Fe, Cu, Na, K, Mg, Ca, Zn, and Mn in whole adult honey bees (Apis mellifera). They also determined the mineral concentrations

Figure 1. Optical system of Techtron Type AA-100 Atomic Absorption Spectrophotometer (57). S_1 , S_2 = optical slits.

OPTICAL SYSTEM

in the separated body regions: head, thorax, and abdomen. In a mineral salt requirement study, Stevens (96) determined the percent Na, K, Mg, Fe, Cu, Ca, Zn, and Mn in the ash of whole adult and larvae almond moths (Cadra cautella) with atomic absorption spectrophotometry. No other literature was found using the atomic absorption technique for insect mineral analysis.

Radiation Sensitivity

General

Two of the major parameters used in determining the effects of ionizing radiation in multicellular organisms have been lethality and life-span shortening (74,79). In practice, one can usually distinguish between rapid killing (lethality) from massive radiation doses and a delayed mortality (life-shortening) from substantially lower doses. It is the belief of many researchers in the field of radiation biology that the specific dose and dose-rate responses are related to nuclear and/or cytoplasmic factors (12,79). Pizzarello and Witcofski (79, p. 216) have stated that "any discussion of radiation lethality in cells must compare the roles of the nucleus and cytoplasm as they contribute to that end point. Since the metabolic activities of the cell occur primarily in the cytoplasm, extensive damage there might be expected to cause cellular death. But permanent lethal changes (serious damage to genetic function and consequently to metabolism) must occur in the nucleus. It must, therefore, be questioned: When the cell as a whole is irradiated with enough energy to kill it, where has the immediate

lethal damage actually been produced -- in the nucleus or in the cytoplasm, or in both?" An answer to this question would help determine the cause(s) of death to the total organism and therefore relate cellular and animal radiosensitivity.

Insect

Changes in an insect population following irradiation have most frequently been expressed in terms of mortality and reproduction (14,15,22,37,113,116). Most of the radiation sensitivity research on insects has involved single species with such variable experimental conditions, for example, dose, dose-rate, etc., that a useful comparison of radiation doses in the literature has been difficult.

Considerable information is available on the radiation effects on mammalian systems (7,12,79). Very little research on the physiological effects of radiation on insect systems has been done, although it is a well-known fact that many adult insects can withstand radiation levels hundreds of times greater than the doses required to produce death in mammals (74). Figure 2 illustrates comparative doses to kill insects and other organisms, while Figure 3 compares the radiosensitivity between insects.

The increased knowledge of the histopathology of vertebrate irradiation has answered several questions on why adult insects are so radiation tolerant (97). An adult vertebrate is dependent upon mitotically active cells and relatively primitive tissue, whereas an adult insect has no replaceable epithelium, no complicated mucous membranes, and no true hematopoietic or lymphatic systems (97). The insect's body surface, or cuticle, is also known to be composed of

Figure 2. LD₅₀ doses for adult vertebrates, insects, and unicellular organisms (3,24,46,47,74,113).

Figure 3. Effects of ionizing radiation on insects (4,22,44,45,47,74,97,114) .

<u>LETHAL DOSE</u>	Log Dose	<u>OTHER EFFECTS</u>
Bracon adults —	R	— halves Bracon life
Bracon, old pupae —	10^5 —	— reduces Tribolium life
Drosophila adults —		— prevents Tribolium reproduction
	10^4 —	— sterilizes Bracon
		— increases Tribolium life
Drosophila, 7.5 hr. eggs —	10^3 —	— inhibits cockroach ovulation
Melanoplus eggs —		
Drosophila, 3 hr. eggs —		
Bracon embryos —	10^2 —	

non-living tissue. In addition, the internal structure of the adult insect is chiefly composed of fat, muscle, and nerve tissue which are known to be fairly radioresistant in mammals (97).

The only tissues known to undergo rapid division in insects are the gonads, which are very sensitive to low doses of ionizing radiation (74,97). DNA turnover in the insect midgut may also be affected by ionizing radiation (28). Although much higher doses of radiation are required to produce death, there is irreversible damage to the insect's ability to reproduce (74).

The most generally accepted explanation for the radiation tolerance of insects is based on the law of Bergonie and Tribondeau which states that the sensitivity of cells to ionizing radiation is directly proportional to their reproductive rate and inversely proportional to their degree of differentiation (74,97).

After the insect hatches from the egg, very little cell division occurs during larval life (74). Cell division and differentiation of insect tissues occur mainly during embryonic development in the egg, so that as a larva, growth occurs primarily by the enlargement of the cell volume without an increase in cell numbers (74). Short bursts of mitotic activity occur before molting and in some insects in the later stages of pupation (74). Therefore, the adult insect owes its radiation tolerance to its scarcity of mitotically active cells, with the immature stages showing a much smaller degree of radiotolerance.

Nuclear Indicators of Radiosensitivity

Many nuclear and cytoplasmic parameters have been studied in an attempt to locate the site(s) of radiation damage in organisms (12,79). The following nuclear parameters or indicators for determining radiosensitivity have been most widely used: nuclear volume, interphase chromosome volume, DNA content, and ploidy (12).

Plant Nuclear Parameters

Plants, rather than animals, have been used most extensively as test systems for many of the studies on nuclear parameters which affect radiosensitivity (12). This is mostly due to the availability of plants with wide ranges of meristematic nuclear volumes, chromosome numbers, and ploidy (12). It can be presumed that similar factors are controlling the radiosensitivity in both plants and animals (12).

The radiosensitivity of many species of plants has been determined by Sparrow and co-workers (12,79,91-95). Using a regression analysis, Sparrow has found marked correlations in plants between cellular sensitivity based on LD_{50} and nuclear parameters such as nuclear volume, interphase chromosome volume ($\frac{\text{Nuclear volume}}{\text{Chromosome number}}$), chromosome numbers, and DNA content. These factors indicated that the nucleus was a major site of radiation injury. Cromroy (25) has presented an excellent review of the research dealing with nuclear indicators of radiosensitivity in plants.

Animal Nuclear Parameters

Mammalian radiation sensitivity studies have dealt primarily with cellular factors (7,25-28, 33,34). Bond and Sugahara (7) have presented an excellent review of the literature concerning radiation LD_{50} doses for numerous mammalian species.

Cromroy and co-workers (25-29) have used the nuclear volume and interphase chromosome volume of the columnar epithelial cells of the duodenal intestinal mucosa as a biological indicator to predict an $LD_{50}/30$ day radiation dose. Their research on 11 species of mammals indicated that the larger the interphase chromosome volume the more resistant the mammal was to ionizing radiation, and consequently the higher its LD_{50} . This showed an inverse relationship to that established for plants. A similar experiment relating interphase chromosome volume to mean survival time was reported by Dunaway, et al. (33,34). Dunaway, et al. (33) have also suggested a possible correlation between the red blood cell count divided by the diameter of the intestine ($2\pi r$) and $LD_{50}/30$ day radiation dose as a rough predictor of mammalian radiosensitivity.

A relationship has been demonstrated between the nuclear volume and the interphase chromosome volume of liver cells for three species of amphibians to their LD_{50} radiation dose (7). Their results indicated a linear relationship between the interphase chromosome volume and the LD_{50} parameter.

Schubert (87) has reported several impressive correlations between the LD_{50} radiation dose and the DNA content of mammals. Relationships have also been shown to exist between sensitivity to ionizing radiation

and DNA content in avian, and yeast cells (79). In addition, nuclear parameters such as nucleoli numbers, amount and distribution of heterochromatin, and the location of the centromeres on chromosomes have been correlated with the radiosensitivity of other organisms (12).

Insect Nuclear Parameters

Nuclear parameters have also been used in the determination of insect radiosensitivity (12,25-29,61,79,91-95). A classical series of experiments which demonstrated nuclear radiosensitivity in insects was done by von Borstel and Rogers (8) and Whitney (115), who used the eggs of the wasp Habrobracon. By separately irradiating the characteristically polar nucleus and the cytoplasm of the wasp eggs, they were able to determine the relative radiosensitivities of the two areas. These comparative cytoplasmic and nuclear studies showed that significantly lower doses were able to inactivate the nucleus when compared to the cytoplasm.

The research of Brown and Nelson-Rees (11) on the mealybug (Planococcus citri) further substantiated the importance of nuclear damage, with specific reference to the role of the chromosomes. Their research indicated that the genetic material in the nucleus was the primary site of the fatal lesion.

The genome number (number of sets of chromosomes per cell) has been extensively studied by Clark and Rubin (18) as a factor influencing the radiosensitivity of insects. Based on a life-shortening parameter for Hymenoptera, they found that haploid males which were produced from unfertilized eggs were more radiosensitive than diploid females which were produced from fertilized eggs. This was called the phenomenon of haplo-diploidy.

Cromroy and co-workers (28,29) made an extensive study on 21 species of insects using the interphase chromosome volume of the midgut epithelial cells as a biological indicator of radiosensitivity. The results were similar to plants and indicated that the larger the interphase chromosome volume the more sensitive the insect species was to ionizing radiation.

Levy and Cromroy (61) have also attempted to relate the nuclear volume of the ovarian nurse cells to the radiation dose required to induce sterility in blood-feeding Dipterans.

Several cytogenetic studies have shown the existence of unique chromosome structures in different orders of insects and have indicated a possible correlation between the location of the centromere and radiosensitivity (28). Cromroy (28) has stated that the rate of DNA turnover in the midgut of insects may be another factor to consider.

Cytoplasmic Indicators of Radiosensitivity

Animal Cytoplasmic Parameters

Numerous authors (12,79) have indicated the significance of certain cytoplasmic characteristics in determining the relative radiosensitivities of organisms. Duryee (35) determined that cytoplasmic factors were correlated to the radiation-induced lethality of cells. His classical transfer studies with amphibian eggs indicated that the irradiated cytoplasm played an important role in the production of nuclear damage.

Research on the irradiation of Arabacia sperm and eggs (79) has indicated the presence of certain cytoplasmic repair mechanisms.

In this study, repair of the radiation-damaged sperm only occurred inside the egg cytoplasm.

Further evidence for cytoplasmic repair has been supplied by experiments on multinucleated amoebae (12,79). These transplantation experiments indicated that the cytoplasm, specifically the mitochondria, was correlated to radiation sensitivity.

Additional research has indicated that cytoplasmic membranes may be involved in radiation sensitivity (12,79). At high radiation doses, membrane permeability of the mitochondria, endoplasmic reticulum, and lysozymes has been shown to be inhibited with the resultant death of the cell.

Specific cytoplasmic factors such as mitochondria number (79,87), copper content (86-88), cytochrome oxidase levels (87), total cytoplasmic cell volume (12), and the ratio of nuclear to cytoplasmic volume (12) have also been shown to be directly related to the radiosensitivity of mammalian species.

Insect Cytoplasmic Parameters

Very little research has been done on cytoplasmic indicators of radiosensitivity in insects. Research similar to the Habrobracon experiments was performed by Nakao (70) who irradiated silkworm eggs. His work indicated a dose effect on the paternal chromosomes in the egg cytoplasm.

Schubert (86,87) and Schubert and Westfast (88) have proposed an interesting theory for determining the radiosensitivity of multicellular organisms, including insects. In his Cu (I, II) peroxy theory Schubert

suggested that there should be a correlation between the radiosensitivity of aerobic multicellular organisms to acutely lethal doses of radiation and their total body copper content. The mechanism of damage is as follows: Ionizing radiation produces organic peroxides which inhibit the oxidative processes in the cytoplasm. This cytoplasmic effect is assumed to involve an interference with the ability of the copper oxidase enzymes from interacting with molecular oxygen due to the radiation-induced oxidation of the cuprous component. Adverse reactions in the cytoplasm then interact with the radiation-induced nuclear damage to produce lethal effects.

Non-Cellular Parameters of Radiosensitivity

Although the reviewed results remain inconclusive, the effect of radiation upon mammals, as well as insects, has been related to age, stage of development, irradiation time, nutritional status, oxygen tension, life-span, temperature (74), moisture content (12,79), weight (28,79), phylogenetic classification, physical activity (28), concentrations of protective agents, size, sex, and specific target organs (79).

Interactions of Ionizing Radiation in Biological Systems

Ionizing radiation may be produced by alpha particles or helium nuclei, beta particles or high-speed electrons, neutrons, and electromagnetic gamma or short-wave x-rays. Alpha particles, which

are too highly charged to penetrate tissues, and neutrons, which, although extremely penetrating, have been shown to produce undesirable chemical reactions in most biological substances. Neither has proven useful for insect control (69). High-energy beta particles have been used experimentally to kill powder post beetles in wood, confused flour beetles in flour, and codling moth and potato tuberworm larvae in plant tissues, but penetration was limited (69). Gamma and x-irradiations are extremely penetrating but produce few ionizations per centimeter of track, and insects are surprisingly resistant (69). Such radiation can be produced from cobalt-60 or from waste fission products such as cesium-137, etc. (69).

Radiations produce their effects principally by the process of ionization (the ejection of outer orbital electrons from atoms) (12,79). Gamma and x-irradiation lose or transfer their energy in matter by photoelectric absorption and Compton scattering (12,79), both of which result in the ejection of an orbital electron or by pair production (12,79) which involves a change of the photon energy into mass (a positive and a negative electron are formed).

Free-Radical Mechanism of Radiation Damage

Biological systems contain about 70-80% water. When their cells and tissues are irradiated most of the energy will be transferred to the water due to the greater number of target molecules encountered, in contrast to the relatively few solute molecules. Chemical changes can then occur if the energy from the ionizing ray or particle is transferred from the water to the important molecules within a cell (79).

Pizzarello and Witcofski (79, p. 85) have stated that "it is obvious with respect to changes brought about in the molecules of which cellular constituents are composed, that the interaction of ionizing radiation and water, and the chemistry (the reactions possible) of irradiated water, will be very important. The degree of change brought about by radiation, in the constituents of the cell depend upon that brought about in the molecules of which the cell is composed. The number of aberrations, for example, in the structure or function of chromosomes, of mitochondria, or any organelle, depends upon how many molecules within the organelle have been changed. The changes in these molecules following irradiation are dependent primarily upon their interaction with the products of the irradiated water, and, to a lesser but by no means insignificant extent, upon the direct interaction of the molecules with radiation." Therefore, the radiochemistry of water can be used to illustrate some of the generalizations for the possible interactions of ionizing radiation in biological systems.

The interaction of ionizing radiation with biological systems forms free radicals by the dissociation of water (12,79,85,112). The following processes are involved in the initial interaction of ionizing radiation with water (3,60): (e = electron; · = unpaired electron of free radical)

Excitation

Ionization

Followed by

or

and possibly

Combination can result in the following postulated reactions (3,60):

or

and

and with the presence of dissolved oxygen

and possibly

Reactions 6 and 7 remove reducing agents while 8-13 produce oxidizing agents. These oxidizing agents or the OH radical itself can oxidize inorganic ions and/or organic compounds that are present in the aqueous biological system (60).

Free radicals may interact with inorganic ions and produce changes (12). With inorganic solutions, the primary reactions are reduction of cations and anions by $\text{H} \cdot$ radicals and oxidation of cations by $\text{OH} \cdot$

and $\text{HO}_2 \cdot$ radicals and by H_2O_2 . The general reactions for cations (12) are: (C = cation; N = charge)

and/or

The general reactions for anions (12) are: (A = anion; N = charge)

and/or

In the presence of O_2 , the $\text{H} \cdot$ radicals will be converted to $\text{HO}_2 \cdot$ radicals and will oxidize rather than reduce materials (12). Therefore, the net effect in a system containing oxygen is oxidation of molecules. The extent of the reaction will depend upon the concentration of the solute, the amount of oxygen in the solution, and the presence of additional materials in solution (12). Since most inorganic molecules are small, the indirect effect from ionizing radiation will predominate (12).

Free radicals may also interact with the organic molecules in cells and tissues and alter them (79). This can be illustrated by the following reactions (79): (R = organic compound; $\cdot$ = unpaired electron of free radical)

and/or

A drastic change in the chemistry of metabolism of the cell would be expected if H_2O_2 were an organic molecule essential to normal cellular metabolism (79). In addition, H_2O_2 could act as a metabolic inhibitor if present in sufficient concentration (79).

It can generally be stated that the biological effects of ionizing radiation on an organism will represent the efforts of that organism to cope with the energy transferred to it after an interaction of one of their atoms with an ionizing ray or particle. For any living organism, this energy will be greater (79) than the system's normal requirement for homeostasis and therefore will cause the standard energy relationships to become unstable.

Animal and Plant Minerals

New methods for trace metal isolation and analysis have enabled researchers to demonstrate the presence of a large number of elements in plants and mammals (16). A number of these metals have been known for several decades to possess biological activity, but the vast majority have only recently received attention.

Elements are usually classified as essential or nonessential although some metals such as Fe have been known to be considered in both (16). Those present in mammals have been divided between macro and trace elements (16). This division is somewhat arbitrary and would depend on the sensitivity of the analytical methods used to detect the minerals.

One of the most reliable techniques employed in much of the pioneering research on trace elements has been emission spectroscopy (16).

Neutron activation analysis has extended the detection limits for many metals to a few nanograms and anodic stripping voltammetry has permitted the detection of 10^{-9} M concentrations of certain metals (16). No single analytical technique has been preferred for all analysis. This is also true for atomic absorption spectrophotometry. Some elements are ideally suited for atomic absorption analysis because of the high sensitivity and freedom of interference from other elements. However, others do not possess the required sensitivity for the analysis of naturally occurring concentrations (16). In spite of all the analytical advances for mineral analysis, there are still numerous cases of disagreement as to the levels of metals present in biological tissues (16). This has made it difficult to assign specific or even general roles to these minerals.

Minerals do not always act alone in performing their biological functions. There is sometimes an interelement dependence, and the effects of one element may be dependent on the presence and concentrations of another (16,20). The simultaneous actions of Na and K, as well as other metals such as the alkaline earths, have been known to be responsible for maintaining a proper balance in cellular metabolism (16). These alkali metals have been said to exhibit an "ionic strength effect" on enzyme systems containing other cations (16).

The mode of action of some minerals has been known, at least to a certain extent. The most important role of minerals has been experimentally shown to be as activators or deactivators of specific enzyme systems (16,20,89,90). Some were also shown to be non-enzymatic in function, either in addition to or instead of being enzyme-oriented (16,20,89,90). Although many minerals have been shown to possess

in vitro activity toward enzyme or other systems, no known in vivo activity or essentiality has been demonstrated (16).

The Significance of Minerals in Enzyme Systems

The entire physiology of an organism has been known to be dependent on the complex biochemical changes which occur in their cells (89). The large amounts of biochemical reactions occurring at the cellular level are regulated by the 2,000 plus enzymes present in each cell (89). These enzymes have been experimentally shown to be regulated or controlled by one or more cations, especially the metallic ones (89).

Schütte (89) has discussed the roles played by metallic ions in living cells and has emphasized the fact that they form a "cationic climate" in which interactions take place. He has also pointed out the competition for specific sites on enzymes and substates which take place at the sub-cellular level, and how a preferential absorption of certain elements can either inhibit or induce enzyme activity, and, therefore, alter the entire biochemistry of the cell or organism.

The numerous and complex ways in which metallic ions can influence cellular metabolism were stated by Schütte (89, p. 33-34):

. . . around all the macromolecular structures in the living cell hovers a cloud of metal ions, jostling for position on the surface of the large molecules and according to their numbers and characters, in the case of enzymes, helping or hindering the movement of molecules -- substates or products -- to and fro.

The wealth of living forms is reflected at the cellular and sub-cellular level by a vast number of possible molecular interactions, among which the relatively indestructible metal ions appear to have been exploited fully in a directive capacity quickening or slowing the rate of structural change of the more evanescent carbon compounds, helping to provide these metabolic bridges and feed-backs.

Non-Enzymatic Functions of Minerals

The most important role of metallic ions is obviously in controlling many enzyme systems, although these elements can also function in other ways. Metal ions have been shown to function as catalysts without being associated with enzyme systems (16,20,89,90). In addition, metallic ions have been shown to be capable of catalyzing reactions in organic systems, and are definitely known to exist in living tissue, but there is virtually no available information to show the extent these catalytic reactions take place in living cells (16,20,89,90). The catalytic properties of metallic ions have been shown to be increased and made more specific when they were bound to certain proteins, specifically the metallo-enzyme complexes (89). However, these ions were shown to be capable of catalyzing the reactions by themselves (89).

The following general discussion on the importance and distribution of mineral cations in plants and animals will be based entirely on the 5 elements studied in this dissertation.

Iron

Fe was the first trace or minor element recognized as being necessary for both plants and animals (16). Most of the Fe in mammalian systems has been found in complex form bound to proteins, either as porphyrin or heme compounds, especially hemoglobin of blood erythrocytes or myoglobin of muscles (16,20,90). The remaining concentration was shown to be stored in the liver, parts of the cytochrome and catalase

enzyme systems, and in low concentrations in blood plasma where it was bound to transferrin (16,20,90). Free, inorganic Fe was negligible (16).

Among organs and tissues of the body, the liver and spleen usually rank highest in Fe content, followed by the kidney, heart, skeletal muscles, pancreas, and brain (16,20,90).

Copper

Cu was first identified in biological material by Bucholtz in 1816 and Meissner in 1817 who found it in plants (20). It was found to be a normal constituent of human organs by Devergie in 1840 (16). Since then scientists and physiologists have been investigating its function in brain, kidney, liver, heart, blood, and other tissues, and have found it to play a complex role in many body functions (16,20,90). So far as is known, Cu can be found in every form of animal or plant life in minute amounts (16,20).

In man, Cu has been found to be equally divided in blood between cells and plasma (16,20,90). Investigations of the Cu content in the organs of several animals have shown that the metal was located in all parts of the animals, but mainly concentrated in the brain, kidney, liver, and heart (16,20,90).

A variety of Cu-protein compounds have been isolated from plant and animal tissues, several of which were enzymes with oxidative functions. Tyrosinase, laccase, uricase, cytochrome oxidase, ascorbic acid oxidase, and others, are believed to be Cu compounds (16). Cu, in close association with Fe, has been shown to have an important function in the formation of hemoglobin in red blood corpuscles (16,29,90).

Cu has also been found to be a normal constituent of hemocyanin, the respiratory pigment in the blood of certain marine invertebrates, which serves the same function as hemoglobin in mammals (16,20,38,64).

Magnesium

Mg has been known to be a component in the porphyrin moiety of chlorophyll (16) and therefore would be important in the process of photosynthesis in green plants. Since the discovery that Mg was responsible for activating the enzyme, alkaline phosphatase, other researchers have shown that dozens of other enzymes require Mg, at least in vitro (16). These included phosphatases, oxidation-reduction enzymes, kinases, synthesizing enzymes, phosphorylating enzymes, and dehydration enzymes. There is also the probability that Mg is bound to the phosphate groups of many of these enzymes (16).

Mg, as well as K, was shown to be concentrated in the intracellular spaces of soft tissue and has been found in cell mitochondria where many enzymes occur (16,90).

Sodium

Na is considered to be the major cation of extracellular fluids, its intracellular concentration being very low (16,20,90). No one specific function has been attributed to Na (16). It has been known to be essential for the proper functioning of mammalian tissue (16, 20,90). The different concentration between the extra- and intracellular fluids has been shown to be important in maintaining the electrical potential of muscles (16,20,90). Na is also a primary

determinant of body fluid osmolarity and is therefore involved in the regulation of fluid volume (16,20,90). The presence of Na in cell nuclei and in mitochondria has indicated that many enzyme systems function in a Na environment (16,20).

Na has also been found in plants and has been shown to be important for proper tissue functioning (16,20).

Potassium

K has been known to play an essential role in animal and plant metabolism (16,20). K is classified as the major intracellular base ion and has been shown to be an activator of specific enzymes (16,20,90). It therefore plays a predominantly intracellular role which contrasts with the distribution of Na.

K has been shown to have an important function in the enzymatic breakdown of glucose (16,20). It was shown to activate the enzymatic catalysis of the phosphorylation of sugar and the enzyme, pyruvic phosphoferase, which is important in the formation of ATP (16,20). Enzymes of the Krebs cycle are known to exist as complexes associated with the mitochondria (16,20). K^+ , which is required by the cell mitochondria for the production of ATP in the Krebs cycle, has been shown to be bound to these cellular structures (16). Na has been shown to play an antagonistic role to K and inhibits the enzymes activated by K (16,20), that is, there is a balance between the two ions.

Various pathological processes have been known to involve the loss of cellular K (16,20,90). The accompanying transfer of Na from

the extracellular fluid in the cell could lead to disruption or inhibition of the enzymatic machinery.

The rate of amino acid utilization for protein regeneration has also been shown to depend on cellular stores of K (16,20,90). K has also been demonstrated as an important mineral for proper muscle functioning. Energy production of muscle cells and the mechanism of contraction were functions shown to be related to the intracellular K concentration in the extracellular fluids bathing the muscle cells (16,20,90).

There has been no evidence to prove that K is stored (16.) In addition, the ion has been shown to be involved in the acid-base equilibria in the body (16,20,90).

Although the function of K in plants is not known, it has been shown to be absolutely essential for proper growth, and appears to be involved in the synthesis of proteins and carbohydrates (16,20).

Insect Minerals

A number of different minerals, some only as traces, are consumed and are present in the tissues of an insect. A dietary source of macro (Mg, Na, K, etc.) and trace or micro (Fe, Cu, etc.) inorganic ions has been shown to be essential for insects, but relatively little work on salt requirements has been carried out because traces of elements are often present as impurities in other dietary factors (2,39). The balance between the respective components of a diet is of primary importance since it relates to metabolic processes (52).

There is general agreement among researchers that the ash of insects contains K, Na, Mg, Ca, Zn, Cu, Al, Si, P, As, S, Cl, Mn, Fe, (1) and Mo (J. L. Nation, personal communication, 1971). All or some of these minerals may influence the chemical reactions taking place in the tissues, but it has been difficult to determine whether some of the major and/or trace elements are utilized specifically, as well as their active concentrations (39). Chapman (13) has stated that minerals are important in the insect for the maintenance of an ionic balance suitable for the activity of living cells, as co-factors of some enzyme systems, and as integral parts of others.

Quantitative and qualitative mineral cation studies on insects have, for the most part, been limited to the hemolymph, and the rectal and malpighian tubule fluids (13,38,39,117). These studies have mainly been concerned with Mg, Na, and K due to their known importance in maintaining a normal internal homeostasis. The wide variations in hemolymph mineral concentration among the insect orders have also been of interest to many researchers. Very little quantitative data is available on whole body or specific tissue-organ mineral content for insects.

The following discussion on insect minerals will be limited to the 5 cations studied in this dissertation, although several others have been shown to be important in insect metabolism (39,41,42).

Iron

Quantitative data on Fe content in insect tissues is scarce. The metabolism of Fe has been described by Bowen (9,10), Waterhouse (108,109), and Waterhouse and Day (110) for several species of insects. Their extensive

research on Lucilia cuprina larvae indicated that there was a small zone in the center of the midgut where Fe was absorbed and that specific cells in this area were responsible for the Fe uptake. The accumulation of what may be thought of as reserve stores of heavy metals in the insect midgut has been known for many other insect species (13).

In describing the Ba and Mn metabolism of social wasps, Bowen (9) postulated that Fe follows the same metabolic route common to all divalent cations.

Fe has been shown to be present in the hemolymph of some insects, as a structural component of hemoglobin (13) in the digestive secretions of Popillia japonica larvae (51), and in the cytochromes of insects as an Fe-porphyrin ring complex (13,41).

Audioradiographic Fe-59 studies by Poulson and Bowen (82) with four species of Drosophila larvae have shown that some tissues contain higher Fe concentrations in the nucleus than others. Fe-59 research on salivary glands, midgut, malpighian tubules, and rectum have indicated that Fe-59 molecules were not bound to the chromosomes, but were localized in the nucleoplasm (77).

All insects are capable of synthesizing cytochromes which are known to be essential in respiration (13). The various cytochromes differ in the forms of their heme grouping (13,41). They are usually present in small amounts, but in the flight muscles, the concentration is high enough to produce a reddish-brown color (13).

Only a few insects living in conditions of low oxygen tension have been shown to contain hemoglobin, and these were colored red by the pigment showing through the integument (13). Hemoglobin has also

been shown to serve a respiratory function (13,41). In Chironomus larvae the hemoglobin was found to be in solution in the blood, while in the larvae of Gasterophilis it was concentrated in the fat body (13).

Bilins, a linear Fe-porphyrin arrangement, are formed by the opening out of ring porphyrins as a results of oxidation and are typically blue or green (13). In Chironomus the bilins from the hemoglobin of the larvae were shown to accumulate in the fat of the adult and impart a green color to the newly emerged adult fly (13). The pericardial cells of Rhodnius also became green when bilins derived from injected hemoglobin were accumulated (13). Rhodnius has also been shown to store Fe from the hemoglobin in the gut epithelium and bilins in the nephrocytes (13), which may represent a type of storage excretion.

Copper

Comar and Bronner (20) have presented quantitative Cu analysis of whole adult, larval, and pupal insects, as well as the hemolymph of the Southern armyworm and the horse bottle fly. An excellent literature review on invertebrate Cu metabolism was presented by Dethier (32). Many of the following references were extracted from his extensive report.

Analyses of Cu by Melvin (65), Okamoto et al. (75,76), Waterhouse (109), and Clare (17) have shown the Cu distribution in insect tissues. They found that the alimentary canal and malphigian tubules contained the largest concentration of Cu while the muscles, cuticle, blood, gonads, and foregut were less concentrated.

Histochemical studies of Cu in the sheep blowfly, Lucilia cuprina by Waterhouse (109) have shown that in a region of the larval midgut there were two small bands of cuprophilic or Cu-absorbing cells. He also showed that the malpighian tubules contained Cu and that no Cu was present in the pericardial and ventral nephrocytes, muscles, foregut, and food. The cuprophilic cells were later shown to contain esterases and cytochrome oxidase, with mitochondria scattered throughout the area (13). Results of Waterhouse's experiments (109) showed that certain cells of the midgut were selectively concerned with absorption of Cu and that the malpighian tubules were concerned with its elimination. Poulson (80) and Poulson and Bowen's (81) study of 40 species of Drosophila has confirmed the presence of special Cu-absorbing cells in the midgut of insects.

In another study, Poulson, et al. (83) fed a Cu-64 solution to fruit flies. The Cu was shown to accumulate in the intestinal tract and malpighian tubules mainly in a form that could not be demonstrated by histochemical technique. He concluded that Cu-uptake followed two distinct pathways, one for ionic forms, and the other for bound forms.

Melvin (65) and Clare (17) have analyzed exuviae, and egg cases of several species of insects and found appreciable concentrations of Cu. From the results, they concluded that the insects were exhibiting a type of storage excretion.

In another experiment, Waterhouse (109) determined the Cu content and distribution in the developmental stages of Lucilia cuprina. He found variations in the egg, larva, and adult stages. The insect egg shell was found to contain the greatest concentration of Cu, with the whole egg, newly hatched larva, and the adult with approximately the same Cu content.

Clare (17) has found an increase in the Cu concentration in the cuticle, muscles, and fat body when a Cu-enriched diet was fed to Periplaneta americana and Blatella germanica. This indicated that more Cu was absorbed into the hemolymph and distributed to these tissues. Waterhouse (109) indicated that the Cu content was regulated when increased levels were administered to various insects. Analysis of Cu in insects has suggested that a large percentage of it is firmly bound in organic combination (32). Dethier (32) has stated that at least one-third of the total Cu concentration was in this form, specifically, as a structural component of the enzyme, tyrosinase.

Sussman (98) has extensively reviewed the functions of tyrosinase and found that four separate enzymes, dopaoxidase, catechol oxidase, diphenolase, and polyphenolase, actually make up a "tyrosinase complex." He has also postulated the occurrence of other copper-protein oxidases in insects. Bodine and co-workers (6) have suggested that protyrosinase, an inactive form of tyrosinase which was found in grasshopper eggs and mealworm larvae, actually belonged to the "tyrosinase complex." Danneel (30) has also described a dopa-phenolase-catecholase system in Drosophila.

The freshly moulted insect cuticle is known to be initially soft and colorless, but then rapidly darkens and hardens. Pryor (84) was the first to prove that these changes in the ootheca of a cockroach and the cuticle of a blowfly larva resulted from the quinone tanning of a protein. Researchers have demonstrated that these Cu-phenols arise from the oxidation of blood tyrosine by tyrosinase (31,40,106).

It has also been suggested that as in vertebrates there may be some relationship between Cu and Fe metabolism, but nothing is known

of this relationship in insects (36,78). Attempts have been made to show that tyrosinase is of major importance in insect metamorphosis and also as a terminal oxidase in the respiration of intact insects (32). Although Cu has been speculated to be part of a respiratory pigment, no such pigment has been isolated from insects (78) even though experiments have indicated that tyrosinase did increase the rate of oxygen uptake in damaged insect tissues (98). Heller (48) has suggested that tyrosinase oxidizes certain substrates which are then reduced by cozymase as part of the respiratory system.

Several authors (5,21,49) have also proposed that tyrosinase in the hemolymph and corpuscles of various insects, by acting upon the phenolic products of protein breakdown, assist in the removal of these toxic materials. Cu in the hemolymph, mostly complexed to tyrosinase, has been quantitatively determined (32).

Excessive but sublethal amounts of Cu in the diet were shown to induce marked morphological abnormalities in Periplaneta americana and Blatella germanica. Clare (17) demonstrated that roach wings became curled and more brittle than normal. In addition, the roaches, as well as blowflies, exhibited a marked increase in water content.

Magnesium

Quantitative estimates of Mg have mainly been limited to the insect hemolymph, where the Mg concentration has usually been found to be relatively high (13,38). In phytophagous insects this was shown to reflect the high level of Mg obtained in the diet, since it is a constituent of chlorophyll (13). In many Lepidoptera larvae the Mg

concentration has been found to fall when they stop feeding, although a fairly large concentration still occurred (13). It is a general consensus among researchers that all insects tend to concentrate this metal (13).

The concentration of Mg has been found to vary greatly, depending on the insect species, and stage of development. In Phasmida, Mg has been found to almost replace Na (13).

Adult Lepidoptera have been generally found to contain less Mg in the hemolymph than the larvae (13). Other orders have been shown to be roughly similar in Mg levels in both larval and adult stages, while others have been found to resemble the Lepidoptera (13).

Several insect enzymes have been shown in vitro to require the Mg $2+$ as a co-factor for enzyme activation. The following enzymes have been found: Trehalose-6-phosphatase, L-ketoglutarate dehydrogenase, acetate-activating enzyme, succinic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, hexokinase, isocitric dehydrogenase, phosphofructokinase, malic enzyme, inorganic phosphatase, oxalocetic carboxylase, phosphohydrolase, L-glycero-phosphate oxidase, luciferase, apyrase, adenosine triphosphatase, adenylate kinase and nucleotide dephosphorylating enzymes (39,41). Mg has also been proposed as being a structural component of adenosine triphosphatase, making up a Mg-ATP coenzyme complex (39,41).

The products of the various metabolic reactions have been isolated and identified by in vitro experimentation (41). Although the specific enzymes have not been isolated, they are assumed to be present to catalyze the formation of the products and are thought to resemble the vertebrate enzymes in structure and function (41). There is presently

no data of value to suggest Mg^{2+} activation in any other insect enzyme system (41), although the enolase, pyruvic kinase, and 3-phosphoglyceric-1-kinase enzymes are assumed to be the same in all animal tissues, and, therefore, would require Mg for activation (39,41).

A model to explain the mechanism by which Mg^{2+} differentially influences chromosomal RNA synthesis has been proposed by Lezzi (62). He has found that Mg^{2+} induced, in isolated salivary gland nuclei of two species of Chironomids, a specific enlargement of one Balbiani ring, while Na^{+} or K^{+} did not. This specific enlargement was presumed to indicate an increase in RNA synthesis in this chromosome region.

The digestive secretions of Popillia japonica were analyzed and were found to contain several cations including Mg, K, Na, and Fe (51). It was postulated that these basic cations acted as a buffer system to neutralize the large concentration of acidic anions.

It has also been proposed that Mg was involved in the ionic mechanism of nerve transmission (13). It has been suggested that Mg can substitute for the extremely low concentrations of Na in many herbivorous insects and, therefore, maintain the proper ionic balance for neural conduction. In other insects high concentrations of Mg were found to block neural conduction.

Mg concentration has also been shown, in vitro and in vivo, to be important in maintaining and regulating the insect heartbeat (58). Alone, and in combination with other ions, it was shown to cause stimulation or paralysis of the heart muscle in several insect species.

Sodium and Potassium

Na and K will be discussed together since the effect of one is usually dependent on the concentration of the other. Like Mg, quantitative determinations of Na and K have mainly been limited to the insect body fluids where they have been shown to function in regulating internal osmolarity and acid-base equilibria (39,41). Nervous system analysis has also favored these 2 minerals (100-105).

Na has been found to be the most abundant cation in the insect hemolymph, but in Lepidoptera, Hymenoptera, and some Coleoptera there were relatively small amounts and it contributed only 10% of the total osmolar concentration (13). The absolute concentration of K was found to be usually lower than that of Na, ranging from 2-100% of the total osmolar concentration (13).

The Na/K ratio has been shown to vary considerably among the insect orders. High values for the Na/K ratio ($\text{Na/K} \approx 40$) have been found to occur in Odonata, Orthoptera, Diptera, and some Coleoptera, while in the phytophagous Coleoptera, Hymenoptera, and Lepidoptera the Na/K ratio was considerably lower, even less than one (13). It has been suggested that these last orders evolved with the angiosperms, which contained relatively little Na, and the adaptation of the insects to a lower Na/K ratio reduced the amount of regulation which was necessary to maintain a fairly constant Mg concentration in the hemolymph (13).

It was shown the Na and K, as well as Mg, were absorbed from the midgut and rectum into the hemolymph by a process of active and/or passive absorption (13,100). The rate of absorption and final concentrations was found to be dependent on the concentration of anions and the insect environment-aquatic or terrestrial. No storage of these ions was shown to take place in the epithelium or midgut tissues.

The importance of Na and K concentration in the normal conduction of a nerve impulse in an insect has been shown to be dependent on the nerve cell's ability to transport these ions across its plasma membrane (50,53,56,104).

Na and K levels have been determined in the abdominal and thoracic ganglia of the ventral nerve chord in several Orthopterans (mainly cockroaches and locusts) to determine the cation fluxes occurring between the hemolymph and nervous system (100-105). These ionic changes, as observed by changes in the Na to K ratio, were shown to be related to the permeability of the continuous cellular and fibrous membrane which enveloped the nervous system (73,107). This membrane, the perilemma, was found to serve a protective function by limiting the entry of Na^+ and K^+ as well as acetylcholine molecules into the underlying nervous tissue (104,107).

Na and K have been shown to be involved in the normal functioning of the dorsal vessel in various insect species (58). It was shown that regulation of the heart beat, that is, paralysis or stimulation, could be affected by changes in the Na and K concentration on the heart muscle.

K levels in the hemolymph were also shown to affect the general activity of locusts (13). A low concentration of K in the hemolymph was shown to raise the muscle resting potential. As a result, the action potential arising from the stimulation was higher and the insect would jump farther and more often than when the K level was high. The K concentration in the hemolymph was also found to increase substantially before moulting and was presumed to be responsible for much of the inactivity occurring at this time (13,55). Various concentrations of

Na and K were demonstrated to stimulate or inhibit the opening and closing of the respiratory spiracles in several insects (54).

Several enzymes have been shown to require Na^+ and/or K^+ as a co-factor for activation. In vitro experimentation of insect tissues have shown that pyruvic kinase, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase required K^+ while glycogen phosphorylase was found to require both Na^+ and K^+ (39,41).

It has been postulated that Na and K may be involved in the mechanism(s) of nuclear activation (39,41). Experimental evidence has suggested that the ratio of the concentration of Na^+ and K^+ may determine whether genes were active or quiet in Chironomid salivary gland chromosomes. By stepwise alteration of this ratio it was possible to induce a series of puffs in gene loci in a sequence which paralleled the natural sequence in the period from late larval to middle pupal life. This has suggested that ecdysone and juvenile hormone may not act directly on the chromosomes, but may exert their effects in altering the Na/K ratio of the nucleus.

The in vivo and in vitro uptake of Na-22 from the hemolymph into cell nuclei of larval salivary glands was measured and compared. The uptake curves were discussed in relation to recent findings on the induction of puffs in polytene chromosomes by inorganic ions (63).

CHAPTER III

METHODS AND MATERIALS

Atomic Absorption Analysis

Insect Preparation

Twenty-two species of insects representing 8 orders were obtained from colonies at the USDA Laboratories,¹ Department of Entomology and Nematology, and the J. Hillis Miller Medical Center at the University of Florida, Gainesville, Florida. Insects in the larval, pupal, nymphal, and adult stages were analyzed for mineral content (see Appendix Table 1), although only adults were incorporated into the statistical models. Adult male and female insects were pooled together from several independent samples to obtain a sufficient tissue weight for a mineral analysis. In several instances, it was necessary to combine different age groups to obtain the necessary weight.

The insects were maintained in holding cages or jars from 12-24 hours without food to assure that their guts would be voided and that the elemental analysis would be based entirely upon tissue-organ

¹Insects Affecting Man and Animals Research Laboratory; Insect Attractants, Behavior, and Basic Biology Research Laboratory.

mineral concentrations and not undigested food in the gastrointestinal system.

All the insects with the exception of Musca domestica and one sample of Attagenus megatoma were heat-killed in Blue-M oven maintained at 80-85° C. The other two species were freeze-killed at -18° C and stored until analysis.

Dehydration

The atomic absorption mineral analysis was based on milligrams of element per kilogram of dry body tissue or parts per million (71). A dry weight analysis was used to reduce the individual weight variations caused by moisture content, and therefore assure a greater degree of quantitative accuracy and experimental reproducibility (J. L. Nation, personal communication, 1971).

The dehydration time varied with the insect group, size and quantity usually being the determining factors. Time ranges were determined for the different insect orders by removing several samples from the oven and weighing then at 12, 24, 36, 48, 60, and 72 hour intervals. When the weight loss reached an equilibrium point (indicating complete dehydration) the insects were removed from the oven and placed in a drying desiccator containing silica gel and Drierite^R (CaSO₄). The samples were then weighed on a Mettler type H15 balance, placed in labelled crucibles, and transferred to a Thermolyne type 1500 furnace to be ashed.

Ashing

The furnace temperature was slowly raised to 400-500° C over a 4 hour period and maintained there for 12-18 hours. When the furnace had cooled, the samples were removed and 0.5 ml 2N HNO₃ was added to each crucible. The samples were placed back into the furnace and slowly dried at under 100° C for one hour. The temperature was again raised to 400-500° C and held there for 4-5 hours to complete the ashing procedure. When the oven had cooled to 70° C the samples were removed. The appearance of a uniformly white or grey colored ash was used as the deciding criterion as to whether the insect tissue was sufficiently ashed. If the ash appeared blotched and/or lumpy, 0.5 ml 2N HNO₃ was added and the sample(s) re-ashed for 3-4 hours at 400° C.

Solution of the ash was effected by adding 1.0 ml 1N HCl to each crucible. Solutions of the ash for samples of greater than 0.5 g dry body weight were made up to a volume of 10.0 ml with deionized water. A final ash volume of 5.0 ml was used when the dry sample material weighed less than 0.5 g. Volumetric flasks and pipettes were used in preparing the standards and samples for a mineral analysis.

In a few instances the ash did not completely go into solution with the addition of the 1.0 ml 1N HCl even though the external appearance seemed uniformly grey or white. When this occurred, the HCl was evaporated in an oven at 80° C, 0.5 ml 2N HNO₃ added, and the samples re-ashed for 2-3 hours at 300-400° C.

Mineral Determination

A Techtron model AA-100 Atomic Absorption Spectrophotometer (see Figure 4) was used in all mineral determinations with air-acetylene gas mixtures. The standard machine settings for the wavelength, gain, lamp position, lamp current, and air and acetylene flow rate (see Figures 5 and 6) varied for each mineral to be determined. The internal arrangement of the machine is also shown in Figure 6. The ideal ranges of concentrations for determining the various elements in parts per million were as follows: K, 1-8; Na, .2-.8; Mg, .2-.8; Cu, 1-8; and Fe, 1-8 (71).

A series of three to four working standards per mineral were prepared each day prior to an analysis from Fisher Chemical Company 1000 ppm shelf standards. The stock solutions were prepared August, 1970, and refrigerated until use (J. L. Nation, personal communication, 1971). Except for Cu, the standard stock solutions were prepared in deionized water (71). Because Cu was determined in undiluted samples, standards were prepared in 0.1 N HCl (71). The standards were graded to correspond to the ideal absorption range for each mineral. A dilution range for sample determination was 1:200 - 1:500 for Mg, K, and Na, and 1:5 - 1:10 for Fe. No dilution was used for Cu. Three determinations were made for each mineral in the sequence: standards-samples-standards-samples-standards-samples (71). After each determination of a standard or sample, deionized water was aspirated through the sample intake valve to verify a return of the meter reading to 100% transmission, and adjustment made when necessary (71).

Figure 4. Techtron Type AA-100 Atomic Absorption Spectrophotometer (57).

TYPE AA-100 ATOMIC ABSORPTION SPECTROPHOTOMETER

Figure 5. Instrument settings of Techtron Type AA-100
Atomic Absorption Spectrophotometer (57).

Figure 6. Instrument settings and internal components of Techtron Type AA-100 Atomic Absorption Spectrophotometer (57).

MONOCHROMATOR, PHOTOMULTIPLIER
HOUSING AND BURNER

POWER SUPPLY UNIT. HOLLOW CATHODE
LAMPS, BURNER AND SPRAY CHAMBER.

READOUT UNIT, GAS INLETS AND WAVE LENGTH ADJUST CONTROL

Results were either recorded on a Varicord model 43 chart recorder or read directly from the meter. After completing a series of five mineral determinations, the mean absorbance for each standard was graphically plotted against its corresponding concentration in parts per million (71). The parts per million of the specific mineral in the sample were then read directly off the corresponding graph. Graphic plots of absorbances over the standard ranges were linear for Fe, Cu, and K, and slightly curvilinear for the Mg and Na. All glassware was cleaned in a dichromate-sulfuric acid solution and rinsed in deionized water (71).

The total body mineral concentration for each insect species was calculated with the following formula:

$$\begin{array}{l} \text{Insect total body} \\ \text{mineral content in} \\ \text{parts per million} \end{array} = \frac{\begin{array}{l} \text{sample} \\ \text{dilution} \end{array} \times \frac{\begin{array}{l} \text{parts per million of} \\ \text{specific mineral} \\ \text{in solution} \end{array}}{\begin{array}{l} \text{milligram dry body} \\ \text{weight of insect tissue} \end{array}} \times 1000$$

These values were then used in the final correlation analysis.

Radiation and LD₅₀ Procedure

Mixed groups of adult male and female insects were irradiated at the Insects Affecting Man and Animals USDA Laboratory with a cobalt-60 source (see Figure 7) at a dose rate of 1200 R/min, or with a 300 KVP Maxitron x-ray therapy unit with 1 mm of aluminum filtration at a dose rate of 2.16 kR/min, to obtain an LD₅₀/24 hour dose for a species population (25-29). Three replications per point were obtained for

Figure 7. Cobalt-60 insect irradiator.

verification of the LD₅₀ dose. Insects were maintained under maximal survival conditions after irradiation and mortality counts made at 24 hours.

The LD₅₀ data for bedbugs, bodylice, houseflies, and silver fish was taken from Cole, et al. (19), who used a cobalt-60 source with a dose rate of 1000 R/min. The LD₅₀/28 day and mean mortality doses were extrapolated from the radiation literature (19,22,23,67,68,72,99).

Data Analysis

An IBM 360 computer was used to devise simple and multiple regression statistical models using the method of least squares for analysis. The computer programs were formulated using APL language by the Department of Statistics, University of Florida, Gainesville, Florida. By manipulating the mineral data, it was possible to obtain several linear models that showed an excellent correlation between insect mineral content and radiation sensitivity as defined by our LD₅₀/24 hour, LD₅₀/28 hour day, and LD₅₀/mean mortality dose parameters.

To test the strength of the relationships, R and r-coefficients, as well as "t" and F-statistics were calculated (66). The standard error for each predicted or expected LD₅₀ was also determined (66). Curvilinearity in the models was also tested (J. A. Cornell, personal communication, 1971). Due to the small samples and great variations across insect orders in the models, a significance level of at least .10 was used to indicate an adequate correlation between mineral content and insect radiation sensitivity.

CHAPTER IV

RESULTS AND DISCUSSION

Statistical Methodology

Several statistical models were formulated which showed a direct and/or indirect relationship between insect mineral cation concentration and radiation sensitivity as defined by one of the parameters selected.

The insect total body Fe, Cu, Mg, Na, and K were obtained by atomic absorption spectrophotometric analysis of the insect tissues. Linear models were formulated by computer manipulation of the five minerals in the form of ratios and/or whole numbers. No model was observed to be curvilinear in response. The statistical methodology is presented initially so that the results will be meaningful to the reader.

The data was analyzed by the method of least squares and resulted in the formulation of simple and multiple regression equations or models which would serve to predict a species-specific radiation dose when the mineral content of that species was known. Mineral cation content would then serve as an effective biological indicator of insect radiation sensitivity.

Simple regression analysis of the data was based on the slope equation for a straight line, $y = a(x) + b$ (26), where y = expected or predicted LD_{50} radiation dose in rads or Roentgens, a = slope, x = mineral content in parts per million (ppm), and b = intercept.

The coefficient of correlation, r , was calculated with each simple regression model to indicate the strength of the linear relationship between the two variables, y and x (66). When the coefficient of correlation was used, a value of zero would indicate no correlation at all, while the limiting values $+1$ or -1 would indicate either a perfect positive or negative correlation between y and x .

A positive correlation indicated by a $+r$ would show that the higher the concentration of a specific mineral or ratio of minerals as defined by x in the simple regression equation, the greater the radiation tolerance of an insect species. A negative correlation, $-r$, for a simple regression model would indicate that the greater the concentration of minerals in the insect tissues, the more sensitive the insect would be to ionizing radiation, hence, an inverse relationship.

A significance level of .05 or .10 was chosen for all regression analyses so that comparisons could be made as to the validity of the different models. Since the statistical models were based on small samples, the "t"-statistic was chosen as the final criterion as to the usefulness of a simple regression model (66). The "t"-statistic was calculated with each simple regression formula to indicate the probability of obtaining a valid LD_{50} response from a given set of mineral data.

Multiple regression analysis represented a multi-dimensional figure based on the linear equation, $y = b_0 + b_1(x_1) + b_2(x_2) + b_3(x_3) + \dots + b_n(x_n)$ (66), where y = the expected or predicted LD_{50} in rads

or Roentgens, $b_0 \dots b_n$ = the estimated parameters (LD_{50} and minerals) of the model, and $x_1 \dots x_n$ = total body insect mineral content in parts per million (ppm).

The multiple regression prediction equation was based upon a number of variables, $x_1, x_2, x_3, \dots x_n$ and was obtained by the method of least squares in exactly the same manner as that employed for the simple linear models (66).

Multiple regression analysis was used to explain a "combined effect" resulting from the interaction of several independent mineral variables represented by $x_1, x_2, x_3, \dots x_n$ in the form of ratios and or whole numbers. These values, when analyzed together in a single equation, could then be used to predict a species-specific radiation lethality dose in a given time period.

The coefficient of multiple determination, $\underline{R}$, was used to test the slope or linear strength of each multiple regression model and was represented as being equivalent to $\underline{r}$. Therefore $\pm \underline{r} = \pm \underline{R}$ for means of comparison (J. A. Cornell, personal communication, 1971).

The F-statistic at a .05 or .10 significance level was used to analyze the validity of a multiple regression model and to determine the limits of its prediction capability. In some special cases there was a degree of ambiguity between the relationship of a high linear coefficient value, R , for a model that produced excellent LD_{50} predictions, and a poor F-statistic (see Tables 15, 19, 20). When this occurred all models significant at the .05 and .10 levels as well as models which were not significant at the .10 level were shown for reasons of comparison.

The standard error (SE) was calculated for each predicted or estimated LD_{50} in both the simple and multiple regression models to place bounds for error, plus or minus, around each predicted radiation dose (J. A. Cornell, personal communication, 1971).

A statistical range of error, ± 10 -15%, should be expected in the LD_{50} values experimentally obtained by x and/or gamma irradiation of the insects, since there is an error in measuring actual radiation exposure inherent in the ion chambers currently available (H. L. Cromroy, personal communication, 1971).

The major problem encountered was the use of models which consisted of non-representative numbers of insect species. The results could be misleading or misinterpreted if one did not consider the following relationships between the test statistics (J. A. Cornell, personal communication, 1971):

$$(1) \quad t = \sqrt{F}$$

$$(2) \quad F = t^2$$

$$(3) \quad t = \frac{[\sqrt{n-2}] \quad r}{\sqrt{1-r^2}},$$

where t = "t"-statistic for a simple regression model, N = number of species in a model, $n-2$ = degrees of freedom, and r = coefficient of correlation;

$$(4) \quad F = \frac{[N - (K+1)] \quad R^2}{K (1-R^2)},$$

where F = F-statistic for a multiple regression model, N = number of species in a model, K = number of parameters (mineral variables) in a model, $N - (K+1)$ and K = degrees of freedom.

- (5) Null hypothesis (H_0): $a = 0$ (slope = 0)
 Alternative hypothesis (H_a): $a \neq 0$

for simple regression analysis by
 t-test at the .05 or .10 level

- (6) Null hypothesis (H_0): $b_0 \dots b_n = 0$
 Alternative hypothesis (H_a): $b_0 \dots b_n \neq 0$

for multiple regression analysis
 by F-test at the .05 or .10 level

Acceptance of H_0 is in effect the rejection of the predictor equation based on the test statistic. Rejection of H_0 is acceptance of H_a and therefore is a measure of the validity of the predictor equation.

By studying these formulae, one can better understand the relationship between the test statistics and therefore understand the validity or inadequacy of a model (see Tables 15, 19, 20). In most cases this was seen to be related to the number of species and/or number of mineral parameters in a model.

LD₅₀/24 Hour Predictor Models

Nineteen species representing Anoplura, Diptera, Coleoptera, Hemiptera, Homoptera, Hymenoptera, and Orthoptera were available for deriving a model based on a 24 hour radiation exposure. The 24 hour x and/or gamma radiation doses, as well as the total body mineral content, were calculated for each species and a correlation analysis was performed to test the relationship between insect mineral content and radiation sensitivity.

The primary objective was to derive a single equation that could be used as an accurate predictor or biological indicator of radiation sensitivity across the insect orders, once the mineral content for a particular species was known.

The predicted $LD_{50}/24$ hour radiation doses were based on the calculated slope or linear regression formula, $y = 2.2147E12 (x) + 112.0065$, where $x = Cu/(Na \times Mg)^2$ in parts per million (see Table 1). Although the "t"-statistic for the 19 insect species was significant, the slope equation did not account for as much variation between species as expected.

Additional analyses were performed on the 19 species in an attempt to improve on the prediction capability of the simple regression model. Multiple regression analysis of the data revealed the predictor equation, $y = b_0 + b_1 (Na) + b_2 (Cu/(Na \times Mg)^2) + b_3 (K/Cu) + b_4 (Cu/Fe)$, where $b_0 = 68.4260$, $b_1 = 0.0061$, $b_2 = 3.4360E12$, $b_3 = 0.0689$, and $b_4 = -109.9400$ (see Table 2).

The results of the simple and multiple regression models failed to account for the large species variation between the observed and expected LD_{50} radiation doses. In an attempt to reduce the variation, the 19 species were sub-divided into categories based on feeding preference, since it is known that minerals are ingested and accumulated in the insect tissues in relation to their diet (39,52).

The following sub-divisions were chosen: (a) stored-product feeders, (b) organic-plant feeders, and (c) blood-feeders. The individual insects in the 19 species model that pertained to a specific feeding group were then separated and analyzed under one of the above sub-divisions.

Table 1. Analysis of the simple regression model, $y = 2.2147E12 (Cu/(Na \times Mg)^2) + 112.0665$ representing the $LD_{50}/24$ hr radiation response for nineteen species of insects.

Species	Cu/(Na x Mg) ² in ppm	Observed LD_{50} in KR	Expected LD_{50} in KR $\pm$ SE	r	t
<u>Sitophilus oryzae</u>	16/(993x609) ²	203	206 $\pm$ 18	+0.66	+3.63*
<u>Oryzaephilus surinamensis</u>	22/(912x724) ²	210	221 $\pm$ 21		
<u>Tribolium castaneum</u>	17/(989x629) ²	310	207 $\pm$ 18		
<u>Lasioderma serricorne</u>	14/(3636x723) ²	195	117 $\pm$ 18		
<u>Trogoderma inclusum</u>	16/(1221x488) ²	245	214 $\pm$ 20		
<u>Attagenus megatoma</u>	51/(1876x428) ²	345	288 $\pm$ 37		
<u>Periplaneta americana</u>	23/(1569x462) ²	52	211 $\pm$ 19		
<u>Blatella germanica</u>	26/(2438x852) ²	91	125 $\pm$ 17		
<u>Nauphoeta cinerea</u>	20/(6174x626) ²	146	115 $\pm$ 19		
<u>Leucophaea madorae</u>	18/(2473x700) ²	118	125 $\pm$ 17		
<u>Cimex lectularius</u>	16/(2108x368) ²	155	171 $\pm$ 14		
<u>Pediculus humanus</u>	21/(3280x487) ²	175	130 $\pm$ 16		
<u>Culex pipens quinquefasciatus</u>	37/(1810x1885) ²	140	119 $\pm$ 18		
<u>Stomoxys calcitrans</u>	33/(2160x3987) ²	145	113 $\pm$ 19		
<u>Periplaneta fuliginosa</u>	27/(2309x678) ²	96	136 $\pm$ 15		
<u>Musca domestica</u>	19/(2593x983) ²	93	118 $\pm$ 18		
<u>Acheta domestica</u>	16/(4149x766) ²	125	116 $\pm$ 19		
<u>Myzus persicae</u> ^b	89/(1442x1296) ²	95	168 $\pm$ 14		
<u>Salenopsis saevissima richteri</u> ^c	9/(1172x459) ²	175	183 $\pm$ 15		

a,b Analysis based on a mixed sample of adults and nymphs -- all other species analyzed in the adult stage

c Analysis based on a sample of mixed castes

* Significant at the .05 level

Table 2. Analysis of the multiple regression model, $y = 68.4260 + 0.0061 (\text{Na}) + 3.4360\text{E}12 (\text{Cu}/(\text{Na} \times \text{Mg})^2) + 0.06893 (\text{K}/\text{Cu}) + (-109.9400 (\text{Cu}/\text{Fe}))$ representing the $\text{LD}_{50}/24$ hr radiation response for nineteen species of insects.

Species	Na in ppm	Cu/(Na x Mg) ² in ppm	K/Cu in ppm	Cu/Fe in ppm	Observed LD_{50} in kR	Expected LD_{50} in kR±SE	R	F
<u>Sitophilus oryzae</u>	993	16/(993x609) ²	3181/16	16/45	203	196 ± 20	+0.75	+4.46*
<u>Oryzaephilus surinamensis</u>	912	22/(912x724) ²	8053/22	22/77	210	239 ± 26		
<u>Tribolium castaneum</u>	989	17/(989x629) ²	6516/17	17/48	310	211 ± 22		
<u>Lasioderma serricorne</u>	3636	14/(3636x723) ²	13295/14	14/207	195	154 ± 27		
<u>Trogoderma inclusum</u>	1221	16/(1221x483) ²	5311/16	16/73	245	231 ± 23		
<u>Attageus megatoma</u>	1876	51/(1876x428) ²	6788/51	51/75	345	286 ± 45		
<u>Periplaneta americana</u>	1569	23/(1569x462) ²	6194/23	23/50	52	198 ± 20		
<u>Blattella germanica</u>	2438	26/(2438x852) ²	10956/26	26/85	91	100 ± 22		
<u>Nauphoeta cinerea</u>	6174	20/(6174x626) ²	20293/20	20/89	146	155 ± 45		
<u>Leucophaea maderae</u>	2473	18/(2473x700) ²	18904/18	18/38	118	124 ± 41		
<u>Cimex lectularius</u>	2108	16/(2108x368) ²	5833/16	16/942	155	195 ± 27		
<u>Pediculus humanus humanus</u> ^a	3280	21/(3280x487) ²	5911/21	21/449	175	131 ± 35		
<u>Culex pipiens quinquefasciatus</u>	1810	37/(1810x1885) ²	21190/37	37/110	140	93 ± 25		
<u>Stomoxys calcitrans</u>	2160	33/(2160x3987) ²	13050/33	33/189	115	91 ± 26		
<u>Periplaneta fuliginosa</u>	2309	27/(2309x678) ²	16225/27	27/59	96	112 ± 23		
<u>Musca domestica</u>	2593	19/(2593x983) ²	10507/19	19/121	93	116 ± 19		
<u>Acheta domesticus</u>	4149	16/(4149x766) ²	15642/16	16/100	125	147 ± 26		
<u>Myzus persicae</u> ^b	1442	89/(1442x1296) ²	8244/89	89/139	95	101 ± 38		
<u>Solenopsis saevissima richteri</u> ^c	1172	9/(1172x459) ²	3908/9	9/93	175	204 ± 27		

a, b Analysis based on mixed samples of adults and nymphs -- all other species analyzed in the adult stage

c Analysis based on a sample with mixed castes

* Significant at the .05 level

Stored-Product Feeders

Analysis of six species of Coleoptera, which were reared on grain and/or flour, revealed a great improvement in the predicted LD₅₀ doses when compared to the values obtained in the 19 species model.

A simple linear model, $y = 244.5774 \text{ (Cu/Fe)} + 172.0181$, was derived (see Table 3). Multiple regression analysis of the six stored-product insects produced two models for predicting a 24 hour radiation lethality response based on mineral content. The following two linear equations were derived:

$$(1) \quad y = b_0 + b_1 \text{ (Mg)} + b_2 \text{ (Cu/K)} + b_3 \text{ (Cu/Fe)}, \quad \text{where} \\ b_0 = 424.5906, \quad b_1 = -0.3263, \quad b_2 = -46506.5039, \quad \text{and } b_3 = 589.8412 \\ \text{(Table 4);}$$

$$(2) \quad y = b_0 + b_1 \text{ (Cu)} + b_2 \text{ (Cu/Fe)} + b_3 \text{ (Mg/K)}, \quad \text{where} \\ b_0 = 308.3822, \quad b_1 = -7.0798, \quad b_2 = 687.3172, \quad \text{and } b_3 = -1229.1410 \\ \text{(Table 5).}$$

Organic-Plant Feeders

Eight species of laboratory-reared insects, which fed on organic or decayed plant matter, were analyzed to test the validity of the diet-based sub-division. Only one true plant-feeder was presented in the models due to a lack of LD₅₀ data as well as a lack of sufficient tissue weight to represent this group. The simple and multiple regression models were based on Orthoptera, mainly cockroaches, although Diptera and Thysanura were also represented.

Table 3. Analysis of the simple regression model, $y = 244.5774 (\text{Cu/Fe}) + 172.0181$ representing the $\text{LD}_{50}/24$ hr radiation response for six species of adult stored-product insects.

<u>Species</u>	<u>Cu/Fe in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Sitophilus oryzae</u>	16/45	203	256 $\pm$ 17	+0.80	+2.68*
<u>Oryzaephilus surinamensis</u>	22/77	210	240 $\pm$ 18		
<u>Tribolium castaneum</u>	17/48	310	256 $\pm$ 17		
<u>Lasioderma serricorne</u>	14/207	195	189 $\pm$ 29		
<u>Trogoderma inclusum</u>	16/73	245	227 $\pm$ 19		
<u>Attagenus megatoma</u>	51/75	345	340 $\pm$ 37		

* Significant at the .05 level

Table 4. Analysis of the multiple regression model, $y = 424.5906 + (-0.3263 \text{ (Mg)}) + (-46506.5039 \text{ (Cu/K)}) + 589.8412 \text{ (Cu/Fe)}$ representing the $LD_{50}/24 \text{ hr}$ radiation response for six species of adult stored-product insects.

<u>Species</u>	<u>Mg in ppm</u>	<u>Cu/K in ppm</u>	<u>Cu/Fe in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>R</u>	<u>F</u>
<u>Sitophilus oryzae</u>	609	16/3181	16/45	203	202 $\pm$ 15	+0.98	+17.14*
<u>Oryzaephilus surinamensis</u>	724	22/8053	22/77	210	228 $\pm$ 13		
<u>Tribolium castaneum</u>	629	17/6516	17/48	310	304 $\pm$ 16		
<u>Lasioderma serricorne</u>	723	14/13295	14/207	195	179 $\pm$ 13		
<u>Trogoderma inclusum</u>	488	16/5311	16/73	245	255 $\pm$ 18		
<u>Attagenus megatoma</u>	428	51/6788	51/75	345	339 $\pm$ 17		

* $F_{3,2}$ -- Significant at the .10 level

Table 5. Analysis of the multiple regression model, $y = 308.3822 + (-7.0798 \text{ (Cu)} + 687.3172 \text{ (Cu/Fe)} + (-1229.1410 \text{ (Mg/K)})$ representing the $LD_{50/24}$ hr radiation response for six species of stored-product insects.

Species	Cu in ppm	Cu/Fe in ppm	Mg/K in ppm	Observed LD_{50} in kR	Expected LD_{50} in kR $\pm$ SE	R	F
<u>Sitophilus oryzae</u>	16	16/45	609/5181	203	200 $\pm$ 21	+0.98	+13.62*
<u>Oryzaephilus surinamensis</u>	22	22/77	724/8053	210	237 $\pm$ 9		
<u>Tribolium castaneum</u>	17	17/48	629/6516	310	309 $\pm$ 20		
<u>Lasioderma serricorne</u>	14	14/207	723/13295	195	188 $\pm$ 18		
<u>Trogoderma inclusum</u>	16	16/73	488/5311	245	234 $\pm$ 10		
<u>Attagenus megatoma</u>	51	51/75	428/6788	345	341 $\pm$ 21		

* $F_{3,2}$ -- Significant at the .10 level

The method of least squares produced four simple regression equations which showed a high degree of linear correlation between insect mineral content and insect radiation sensitivity. The following four formulae and associated statistics were derived:

$$(1) \quad y = -27456.8938 \text{ (Cu/K)} + 152.671 \text{ (Table 6),}$$

$$(2) \quad y = -6509.5691 \text{ (Cu/Na)} + 158.6133 \text{ (Table 7),}$$

$$(3) \quad y = 0.0054 \text{ (K)} + 25.8061 \text{ (Table 8),}$$

$$(4) \quad y = 0.0166 \text{ (Na)} + 51.3646 \text{ (Table 9).}$$

Results obtained by multiple regression analysis showed a marked improvement over the prediction capability of the simple regression models. Two predictor equations were derived which indicated a strong relationship between mineral content and insect radiation sensitivity. The following are the equations that were formulated:

$$(1) \quad y = b_0 + b_1 \text{ (Na)} + b_2 \text{ (Cu/K)}, \quad \text{where}$$

$$b_0 = 110.2092, \quad b_1 = 0.0087, \quad \text{and} \quad b_2 = -18940.1062 \quad \text{(Table 10);}$$

$$(2) \quad y = b_0 + b_1 \text{ (K)} + b_2 \text{ (Cu/Na)}, \quad \text{where}$$

$$b_0 = 92.6478, \quad b_1 = 0.0031, \quad \text{and} \quad b_2 = -3925.0502 \quad \text{(Table 11).}$$

Blood-Feeders

The final sub-division based on feeding preference was the blood-feeders. Since the mineral content of one species of Anoplura was obtained from a mixed sample of nymphs and adults, it was decided that separation of these two stages would be advisable in the final correlation analysis. The result was a model based on five species which represented

Table 6. Analysis of the simple regression model, $y = -27456.8938 \text{ (Cu/K)} + 152.6571$ representing the LD_{50} / 24 hr radiation response for eight species of adult organic-plant feeding insects.

<u>Species</u>	<u>Cu/K</u> <u>in ppm</u>	<u>Observed LD_{50}</u> <u>in kR</u>	<u>Expected LD_{50}</u> <u>in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Periplaneta americana</u>	23/6194	52	49 $\pm$ 10	-0.93	-6.10*
<u>Blatella germanica</u>	26/10956	91	87 $\pm$ 5		
<u>Nauphoeta cinerea</u>	20/20293	146	125 $\pm$ 5		
<u>Leucophaea maderae</u>	18/18904	118	126 $\pm$ 6		
<u>Periplaneta fuliginosa</u>	27/16225	96	107 $\pm$ 4		
<u>Musca domestica</u>	19/10507	93	104 $\pm$ 4		
<u>Acheta domesticus</u>	16/15642	125	124 $\pm$ 5		
<u>Thermobia domestica</u>	29/14041	98	96 $\pm$ 4		

* Significant at the .05 level

Table 7. Analysis of the simple regression model, $y = -6509.5691 (\text{Cu/Na}) + 158.6133$ representing the $\text{LD}_{50}/24$ hr radiation response for eight species of adult organic-plant feeding insects.

<u>Species</u>	<u>Cu/Na in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Periplaneta americana</u>	23/1569	52	62 $\pm$ 8	-0.92	-5.78*
<u>Blattella germanica</u>	26/2438	91	89 $\pm$ 5		
<u>Nauphoeta cinerea</u>	20/6174	146	137 $\pm$ 7		
<u>Leucophaea maderae</u>	18/2473	118	111 $\pm$ 4		
<u>Periplaneta fuliginosa</u>	27/2309	96	83 $\pm$ 5		
<u>Musca domestica</u>	19/2593	93	112 $\pm$ 4		
<u>Acheta domesticus</u>	16/4149	125	133 $\pm$ 7		
<u>Thermobia domestica</u>	29/2858	98	93 $\pm$ 4		

* Significant at the .05 level

Table 8. Analysis of the simple regression model, $y = .0054 (K) + 25.8061$ representing the $LD_{50}/24$ hr radiation response for eight species of adult organic-plant feeding insects.

Species	K in ppm	Observed LD_{50} in KR	Expected LD_{50} in KR $\pm$ SE	r	t
<u>Periplaneta americana</u>	6194	52	59 $\pm$ 9	+0.91	+5.34*
<u>Blatella germanica</u>	10956	91	85 $\pm$ 5		
<u>Nauphoeta cinerea</u>	20293	146	136 $\pm$ 8		
<u>Leucophaea maderae</u>	18904	118	128 $\pm$ 7		
<u>Periplaneta fuliginosa</u>	16225	96	114 $\pm$ 5		
<u>Musca domestica</u>	10507	93	83 $\pm$ 6		
<u>Acheta domesticus</u>	15642	125	111 $\pm$ 5		
<u>Thermobia domestica</u>	14041	98	102 $\pm$ 4		

* Significant at the .05 level

Table 9. Analysis of the simple regression model, $y = 0.0166 (\text{Na}) + 51.3646$ representing the $\text{LD}_{50}/24$ hr radiation response for eight species of adult organic-plant feeding insects.

<u>Species</u>	<u>Na</u> <u>in ppm</u>	<u>Observed LD_{50}</u> <u>in kR</u>	<u>Expected LD_{50}</u> <u>in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Periplaneta americana</u>	1569	52	77 ± 8	+0.86	+4.12*
<u>Blatella germanica</u>	2438	91	92 ± 6		
<u>Nauphoeta cinerea</u>	6174	146	154 ± 14		
<u>Leucophaea maderae</u>	2473	118	92 ± 6		
<u>Periplaneta fuliginosa</u>	2309	96	90 ± 6		
<u>Musca domestica</u>	2593	93	94 ± 6		
<u>Acheta domesticus</u>	4149	125	120 ± 7		
<u>Thermobia domestica</u>	2858	98	99 ± 6		

* Significant at the .05 level

Table 10. Analysis of the multiple regression model, $y = 110.2092 + 0.0087 (\text{Na}) + (-18940.1062 (\text{Cu/K}))$ representing the $\text{LD}_{50}/24$ hr radiation response for eight species of adult organic-plant feeding insects.

Species	Na in ppm	Cu/K in ppm	Observed LD_{50} in kR	Expected LD_{50} in kR $\pm$ SE	R	F
<u>Periplaneta americana</u>	1569	23/6194	52	52 $\pm$ 4	+0.99	+146.35*
<u>Blattella germanica</u>	2438	26/10956	91	87 $\pm$ 2		
<u>Nauphoeta cinerea</u>	6174	20/20293	146	145 $\pm$ 4		
<u>Leucophaea maderae</u>	2473	18/18904	118	114 $\pm$ 3		
<u>Periplaneta fuliginosa</u>	2309	27/16225	96	99 $\pm$ 2		
<u>Musca domestica</u>	2593	19/10507	93	99 $\pm$ 2		
<u>Acheta domesticus</u>	4149	16/15642	125	127 $\pm$ 2		
<u>Thermobia domestica</u>	2858	29/14041	98	96 $\pm$ 2		

* $F_{2,5}$ -- Significant at the .05 level

Table 11. Analysis of the multiple regression model, $y = 92.6478 + 0.0031 (K) + (-3925.0502 (Cu/Na))$ representing the $LD_{50}/24$ hr radiation response for eight species of adult organic-plant feeding insects.

<u>Species</u>	<u>K</u> <u>in ppm</u>	<u>Cu/Na</u> <u>in ppm</u>	<u>Observed LD₅₀</u> <u>in kR</u>	<u>Expected LD₅₀</u> <u>in kR $\pm$ SE</u>	<u>R</u>	<u>F</u>
<u>Periplaneta americana</u>	6194	23/1569	52	53 $\pm$ 3	+0.99	+147.1100*
<u>Blatella germanica</u>	10956	26/2438	91	85 $\pm$ 2		
<u>Nauphoeta cinerea</u>	20293	20/6174	146	143 $\pm$ 3		
<u>Leucophaea maderae</u>	18904	18/2473	118	122 $\pm$ 2		
<u>Periplaneta fuliginosa</u>	16225	27/2309	96	97 $\pm$ 3		
<u>Musca domestica</u>	10507	19/2593	93	97 $\pm$ 3		
<u>Acheta domesticus</u>	15642	16/4149	125	126 $\pm$ 3		
<u>Thermobia domestica</u>	14041	29/2858	98	96 $\pm$ 2		

* $F_{2,5}$ -- Significant at the .05 level

three orders: Anoplura, Diptera, and Hemiptera. The following two models were obtained by simple regression analysis:

$$(1) \quad y = -30.6599 \text{ (Mg/Na)} + 171.5961 \text{ (Table 12);}$$

$$(2) \quad y = -0.0147 \text{ (Mg)} + 172.2073 \text{ (Table 13).}$$

Analysis based on a combination of minerals in the form of fractions and whole numbers produced two multiple regression formulas that could be used to predict a species-specific $LD_{50}/24$ hour radiation dose. The following multiple regression equations or models were formulated:

$$(1) \quad y = b_0 + b_1 \text{ (Cu)} + b_2 \text{ (Fe)} + b_3 \text{ (Mg/Na)}, \text{ where}$$

$$b_0 = 224.1558, b_1 = -1.2260, b_2 = -0.0467, \text{ and } b_3 = -32.4359$$

(Table 14);

$$(2) \quad y = b_0 + b_1 \text{ (Fe)} + b_2 \text{ (Mg/Na)}, \text{ where}$$

$$b_0 = 187.2418, b_1 = -0.0251, b_2 = -37.9907 \text{ (Table 15).}$$

$LD_{50}/28$ Day Predictor Models

Seven species of adult insects representing two orders, Coleoptera and Orthoptera, were analyzed by simple and multiple regression analysis to determine if the total body mineral content for an insect species was related to an $LD_{50}/28$ day radiation mortality dose.

Correlation analysis of the seven species resulted in the following two simple regression predictor equations:

Table 12. Analysis of the simple regression model, $y = -30.6599 \text{ (Mg/Na)} + 171.5961$ representing the $LD_{50}/24$ hr radiation response for five species of blood-feeding insects.

<u>Species</u>	<u>Mg/Na in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Cimex lectularius</u>	368/2108	155	166 $\pm$ 5	-0.96	-5.73*
<u>Pediculus humanus humanus</u>	487/3280	175	167 $\pm$ 5		
<u>Pediculus humanus humanus</u> ^a	487/3280 ^b	170	167 $\pm$ 4		
<u>Culex pipiens quinquefasciatus</u>	1885/1810	140	140 $\pm$ 7		
<u>Stomoxys calcitrans</u>	3987/2160	115	115 $\pm$ 5		

^a Nymphs -- other species analyzed in the adult stage

^b Based on same mineral content as adults due to analysis of the two stages in a mixed sample

* Significant at the .05 level

Table 13. Analysis of the simple regression model, $y = -0.0147 (\text{Mg}) + 172.2073$ representing the $\text{LD}_{50}/24$ hr radiation response for five species of blood-feeding insects.

<u>Species</u>	<u>Mg in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Cimex lectularius</u>	368	155	167 ± 6	-0.94	-4.69*
<u>Pediculus humanus humanus</u>	487	175	165 ± 5		
<u>Pediculus humanus humanus^a</u>	487 ^b	170	165 ± 5		
<u>Culex pipens quinquefasciatus</u>	1885	140	145 ± 5		
<u>Stomoxys calcitrans</u>	3987	115	114 ± 9		

^a Nymphs -- all other species analyzed in the adult stage

^b Based on the same mineral content as adults due to analysis of the 2 stages in a mixed sample

* Significant at the .05 level

Table 14. Analysis of the multiple regression model, $y = 224.1587 + (-1.2260 \text{ (Cu)}) + (-0.0467 \text{ (Fe)}) + (-32.4359 \text{ (Mg/Na)})$ representing the $LD_{50}/24 \text{ hr}$ radiation response for five species of blood-feeding insects.

<u>Species</u>	<u>Cu</u> <u>in ppm</u>	<u>Fe</u> <u>in ppm</u>	<u>Mg/Na</u> <u>in ppm</u>	<u>Observed LD_{50}</u> <u>in kR</u>	<u>Expected LD_{50}</u> <u>in kR $\pm$ SE50</u>	<u>R</u>	<u>F</u>
<u>Cimex lectularius</u>	16	942	368/2108	155	155 $\pm$ 4	+0.99	+62.87*
<u>Pediculus humanus humanus</u>	21	449	487/3280	175	173 $\pm$ 3		
<u>Pediculus humanus humanus</u> ^a	21 ^b	449 ^c	487/3280 ^d	170	173 $\pm$ 3		
<u>Culex pipens quinquefasciatus</u>	37	110	1885/1810	140	140 $\pm$ 4		
<u>Stomoxys calcitrans</u>	33	189	9987/2160	115	115 $\pm$ 4		

^a Nymphs -- all other species analyzed in the adult stage

^{b,c,d} Based on identical mineral content as adults due to analysis of the 2 stages in a mixed sample

* $F_{3,1}$ -- Significant at the .10 level

Table 15. Analysis of the multiple regression model, $y = 187.2418 + (-0.0251 (\text{Fe})) + (-37.9907 (\text{Mg/Na}))$ representing the $\text{LD}_{50}/24$ hr radiation response for five species of blood-feeding insects.

<u>Species</u>	<u>Fe in ppm</u>	<u>Mg/Na in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>R</u>	<u>F</u>
<u>Cimex lectularius</u>	942	368/2108	155	157 ± 5	+0.99	+40.70*
<u>Pediculus humanus humanus</u>	449	487/3280	175	170 ± 3		
<u>Pediculus humanus humanus</u> ^a	449 ^b	487/3280 ^c	170	170 ± 3		
<u>Culex pipiens quinquefasciatus</u>	110	1885/1810	140	145 ± 4		
<u>Stomoxys calcitrans</u>	189	3987/2160	115	112 ± 5		

^a Nymphs -- all other species analyzed in the adult stage

^{b, c} Based on identical mineral content as adults due to analysis of the 2 stages in a mixed sample

* $F_{3,1}$ -- Not significant at the .10 level

$$(1) \quad y = -0.0024 (Mg + Na) + 11.4310 \quad (\text{Table } 16);$$

$$(2) \quad y = -0.3593 ((Fe \times Na)/K) + 10.1094 \quad (\text{Table } 17).$$

Multiple regression analysis of the $LD_{50}/28$ day data resulted in the formulation of three models. The prediction capability of these models for determining an $LD_{50}/28$ day radiation dose was shown to be superior to those obtained from the simple regression models. The following models were derived:

$$(1) \quad y = b_0 + b_1 (Cu/K) + b_2 (Mg + Na) + b_3 (Fe \times Na), \quad \text{where} \\ b_0 = 39.5350, \quad b_1 = -3009.0000, \quad b_2 = -0.0161, \quad \text{and } b_3 = 0.0001 \\ (\text{Table } 18);$$

$$(2) \quad y = b_0 + b_1 (Cu/K) + b_2 (Mg + Na) + b_3 (Fe \times Na) + \\ b_4 ((Fe \times Na)/K), \quad \text{where} \\ b_0 = 47.182, \quad b_1 = -7523.900, \quad b_2 = -0.0173, \quad b_3 = 2.2863 \text{ E-5}, \\ \text{and } b_4 = 1.3975 \quad (\text{Table } 19);$$

$$(3) \quad y = b_0 + b_1 (Cu/K) + b_2 (Mg + Na) + b_3 (Fe \times Na) + \\ b_4 ((Na + Mg)/K), \quad \text{where} \\ b_0 = 43.6770, \quad b_1 = -5403.3000, \quad b_2 = -0.0180, \quad b_3 = 0.0001, \\ \text{and } b_4 = 22.6400 \quad (\text{Table } 20).$$

$LD_{50}/\text{Mean-Mortality Predictor Models}$

Simple and multiple regression models were derived for determining an LD_{50} radiation dose based on the mean-mortality (mean-life expectancy) for eight species of laboratory-reared insects. Insects representing the

Table 16. Analysis of the simple regression model, $y = -0.0024 (Mg + Na) + 11.4310$ representing the $LD_{50}/28$ day radiation response for seven species of adult insects.

<u>Species</u>	<u>Mg + Na in ppm</u>	<u>Observed LD_{50} in krad</u>	<u>Expected LD_{50} in krad $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Sitophilus oryzae</u>	609+ 993	4.08	7.55 $\pm$ 1.99	-0.62	-1.78*
<u>Blatella germanica</u>	852+2438	3.10	3.45 $\pm$ 1.86		
<u>Periplaneta americana</u>	462+1569	2.35	6.51 $\pm$ 1.69		
<u>Oryzaephilus surinamensis</u>	724+ 912	11.00	7.46 $\pm$ 1.96		
<u>Tribolium castaneum</u>	629+ 989	13.19	7.51 $\pm$ 1.98		
<u>Acheta domesticus</u>	766+4149	0.97	-0.49 ^a $\pm$ 3.60		
<u>Epilachna varivestis</u>	1605+ 983	2.45	5.15 $\pm$ 1.55		

a The negative value represents an $LD_{50} \approx 0$

* Significant at the .10 level

Table 17. Analysis of the simple regression model, $y = -0.3593 ((\text{Fe} \times \text{Na})/\text{K}) + 10.1094$ representing the $\text{LD}_{50}/28$ day radiation response for seven species of adult insects.

Species	(Fe x Na)/K in ppm	Observed LD ₅₀ in krad	Expected LD ₅₀ in krads $\pm$ SE	r	t
<u>Sitophilus oryzae</u>	(45 x 993)/3181	4.08	5.05 $\pm$ 1.65	-0.56	-1.51*
<u>Blatella germanica</u>	(85 x 2438)/10956	3.10	3.28 $\pm$ 2.12		
<u>Periplaneta americana</u>	(50 x 1569)/6194	2.35	5.52 $\pm$ 1.64		
<u>Oryzaephilus surinamensis</u>	(77 x 912)/8053	11.00	6.96 $\pm$ 1.97		
<u>Tribolium castaneum</u>	(48 x 989)/6516	13.19	7.49 $\pm$ 2.18		
<u>Acheta domesticus</u>	(100x4149)/15642	0.97	0.58 $\pm$ 3.53		
<u>Epilachna varivestis</u>	(85 x 983)/16341	2.45	8.26 $\pm$ 2.55		

* Significant at the .10 level

Table 18. Analysis of the multiple regression model, $y = 39.5350 + (-3009.0000 \text{ (Cu/K)}) + (-0.0161 \text{ (Mg + Na)}) + 0.0001 \text{ (Fe x Na)}$ representing the $LD_{50}/28$ day radiation response for seven species of adult insects.

Species	Cu/K in ppm	Mg + Na in ppm	Fe x Na in ppm	Observed LD_{50} in krad	Expected LD_{50} in krad $\pm$ SE	R	F
<u>Sitophilus oryzae</u>	16/3181	609+ 993	45x 993	4.08	3.79 $\pm$ 1.72	+0.95	+8.98*
<u>Blatella germanica</u>	26/10956	852+2438	85x2438	3.10	1.52 $\pm$ 1.09		
<u>Periplaneta americana</u>	23/6194	462+1569	50x1569	2.35	3.82 $\pm$ 1.17		
<u>Oryzaephilus surinamensis</u>	22/8053	724+ 912	77x 912	11.00	12.63 $\pm$ 1.68		
<u>Tribolium castaneum</u>	17/6516	629+ 989	48x 989	13.19	10.83 $\pm$ 1.36		
<u>Acheta domesticus</u>	16/15642	766+4149	100x4149	0.97	1.36 $\pm$ 2.00		
<u>Epilachna varivestis</u>	19/16341	1605+ 983	85x 983	2.45	3.20 $\pm$ 2.01		

* $F_{3,3}$ -- Significant at the .10 level

Table 19. Analysis of the multiple regression model, $y = 47.1820 + (-7523.9000 \text{ (Cu/K)}) + (-0.0173 \text{ (Mg + Na)}) + 2.2863E-5 \text{ (Fe x Na)} + 1.3975 \text{ (Fe x Na)/K}$ representing the LD₅₀/28 day radiation response for seven species of adult insects.

Species	Cu/K in ppm	Mg + Na in ppm	Fe x Na in ppm	(Fe x Na)/K in ppm	Observed LD ₅₀ in krad	Expected LD ₅₀ in krad ± SE	R	F
<u>Sitophilus oryzae</u>	16/3181	609+ 993	45x 993	(45 x 993)/3181	4.08	3.43±1.62	+0.97	+8.26*
<u>Blatella germanica</u>	26/10956	852+2438	85x2438	(85 x2438)/10956	3.10	3.57±1.95		
<u>Periplaneta americana</u>	23/6194	462+1569	50x1569	(50 x1569)/6194	2.35	3.17±1.21		
<u>Oryzaephilus surinamensis</u>	22/8053	724+ 912	77x 912	(77 x 912)/8053	11.00	12.58±1.56		
<u>Tribolium castaneum</u>	17/6516	629+ 989	48x 989	(48 x 989)/6516	13.19	11.23±1.30		
<u>Acheta domesticus</u>	16/15642	766+4149	100x4149	(100x4149)/15642	0.97	0.58±1.95		
<u>Epilachna varivestis</u>	19/16341	1605+ 983	85x 983	(85 x 983)/16341	2.45	2.59±1.93		

* F_{4,2} -- Not significant at the .10 level

Table 20. Analysis of the multiple regression model, $y = 43.6770 + (-5403.3000 (\text{Cu/K})) + (-0.0180 (\text{Mg} + \text{Na})) + 0.0001 (\text{Fe} \times \text{Na}) + 22.6400 ((\text{Na} + \text{Mg})/\text{K})$ representing the $\text{LD}_{50}/28$ day radiation response for seven species of adult insects.

Species	Cu/K in ppm	Mg + Na in ppm	Fe x Na in ppm	(Na + Mg)/K in ppm	Observed		Expected		R	F
					LD_{50} in krad	LD_{50} in krad	LD_{50} in krad $\pm$ SE	LD_{50} in krad		
<u>Sitophilus oryzae</u>	16/3181	609+ 993	45x 993	(993 + 609)/3181	4.08	4.74 $\pm$ 2.15			+0.96	+6.20*
<u>Blatella germanica</u>	26/10956	852+2438	85x2438	(2438+ 852)/10956	3.10	1.01 $\pm$ 1.31				
<u>Periplaneta americana</u>	23/6194	462+1569	50x1569	(1569+ 462)/6194	2.35	2.68 $\pm$ 1.85				
<u>Oryzaephilus</u> <u>surinamensis</u>	22/8053	724+ 912	77x 912	(912 + 724)/8053	11.00	12.04 $\pm$ 1.92				
<u>Tribolium castaneum</u>	17/6516	629+ 989	48x 989	(989 + 629)/6516	13.19	11.54 $\pm$ 1.68				
<u>Acheta domesticus</u>	16/15642	766+4149	100x4149	(4149+ 766)/15642	0.97	1.70 $\pm$ 2.15				
<u>Epilachna varivestis</u>	19/16341	1605+983	85x 983	(983 +1605)/16341	2.45	3.43 $\pm$ 2.15				

* $F_{4,2}$ -- Not significant at the .10 level

orders Coleoptera, Diptera, Lepidoptera, and Orthoptera were incorporated into the regression models.

Analysis of the data revealed several equations which indicated a direct relationship between insect mineral content and the LD_{50} /mean-mortality dose parameter. Simple regression analysis resulted in the formulation of the predictor equation:

$$y = 499.7257 \text{ (Mg/K)} + 27.9200 \text{ (Table 21)}.$$

Multiple regression analysis resulted in the formation of three mineral-based models. The following models were derived:

$$(1) \quad y = b_0 + b_1 \text{ (Mg/K)} + b_2 \text{ (Cu/Mg)} + b_3 \text{ (Fe/K)} + b_4 \text{ (Na/Mg)}, \text{ where} \\ b_0 = -110.1670, \quad b_1 = 887.5769, \quad b_2 = -631.3156, \quad b_3 = 5554.3929, \\ b_4 = 8.8102 \quad \text{(Table 22)};$$

$$(2) \quad y = b_0 + b_1 \text{ (Mg/K)} + b_2 \text{ (Cu/Mg)} + b_3 \text{ (Fe/K)} + b_4 \text{ (Mg/Na)}, \text{ where} \\ b_0 = -36.7632, \quad b_1 = 704.4908, \quad b_2 = -833.3842, \quad b_3 = 3454.4009, \\ \text{and } b_4 = -21.1603 \quad \text{(Table 23)};$$

$$(3) \quad y = b_0 + b_1 \text{ (Mg/K)} + b_2 \text{ (Cu/Mg)} + b_3 \text{ (Fe/K)}, \text{ where} \\ b_0 = -51.5398, \quad b_1 = 457.4505, \quad b_2 = -597.3484, \quad b_3 = 5524.6261 \\ \text{(Table 24)}.$$

Mineral-Based LD_{50} Predictions

Atomic absorption spectroscopy was also used to determine the total body Cu, Fe, Mg, Na, and K concentration for adult insects whose LD_{50} radiation doses were not available (see Appendix Table 1). Those species

Table 21. Analysis of the simple regression model, $y = 499.7257 \text{ (Mg/K)} + (-27.9200) \text{ representing the } LD_{50}/\text{mean-mortality radiation response for eight species of adult insects.}$

<u>Species</u>	<u>Mg/K in ppm</u>	<u>Observed LD_{50} in kR</u>	<u>Expected LD_{50} in kR $\pm$ SE</u>	<u>r</u>	<u>t</u>
<u>Tribolium castaneum</u>	629/6516	13.19	20.32 $\pm$ 5.62	+0.69	+2.32*
<u>Oryzaephilus surinamensis</u>	724/8053	12.19	17.01 $\pm$ 5.47		
<u>Periplaneta americana</u>	462/6194	3.00	9.35 $\pm$ 6.45		
<u>Blatella germanica</u>	852/10956	3.00	10.94 $\pm$ 6.12		
<u>Acheta domesticus</u>	766/15642	2.50	-3.45 ^a $\pm$ 10.48		
<u>Epilachna varivestis</u>	1605/16341	7.60	21.16 $\pm$ 5.71		
<u>Musca domestica</u>	983/10507	51.00	18.83 $\pm$ 5.51		
<u>Trichoplusia ni</u>	1018/7043	46.00	44.31 $\pm$ 12.84		

^a Negative value represents an $LD_{50} \approx 0$

* Significant at the .05 level

Table 22. Analysis of the multiple regression model, $y = -110.1670 + 887.5769 (\text{Mg/K}) + (-631.3156 (\text{Cu/Mg})) + 5554.3929 (\text{Fe/K}) + 8.8102 (\text{Na/Mg})$ representing the LD_{50} /mean-mortality radiation response for eight species of adult insects.

Species	Mg/K in ppm	Cu/Mg in ppm	Fe/K in ppm	Na/Mg in ppm	Observed		
					LD_{50} in kR	Expected LD_{50} in kR $\pm$ SE	R F
<u>Tribolium castaneum</u>	629/6516	17/629	48/6516	989/629	13.19	13.71 $\pm$ 2.09	+0.99 +26.63*
<u>Oryzaephilus</u> <u>surinamensis</u>	724/8053	22/724	77/8053	912/724	12.19	15.37 $\pm$ 3.37	
<u>Periplaneta americana</u>	462/6194	23/462	50/6194	1569/462	3.00	-0.82 ^a $\pm$ 4.42	
<u>Blattella germanica</u>	852/10956	26/852	85/10956	2438/852	3.00	8.10 $\pm$ 2.04	
<u>Acheta domesticus</u>	766/15642	16/766	100/15642	4149/766	2.50	3.01 $\pm$ 4.71	
<u>Epilachna varivestis</u>	1605/16341	18/1605	85/16341	983/1605	7.60	3.92 $\pm$ 4.19	
<u>Musca domestica</u>	983/10507	19/983	121/10507	2593/983	51.00	48.07 $\pm$ 4.57	
<u>Trichoplusia ni</u>	1018/7043	17/1018	42/7043	727/1018	46.00	47.14 $\pm$ 4.85	

^a Negative value represents an $\text{LD}_{50} \approx 0$

* $F_{4,3}$ -- Significant at the .05 level

Table 23. Analysis of the multiple regression model, $y = -36.7632 + 704.4908 (\text{Mg/K}) + (-833.3842 (\text{Cu/Mg})) + 3454.4009 (\text{Fe/K}) + (-21.1603 (\text{Mg/Na}))$ representing the LD_{50} /mean-mortality radiation response for eight species of adult insects.

Species	Mg/K in ppm	Cu/Mg in ppm	Fe/K in ppm	Mg/Na in ppm	Observed LD_{50} in kR	Expected LD_{50} in kR $\pm$ SE	R	F
<u>Tribolium castaneum</u>	629/6516	17/629	48/6516	629/989	13.19	21.29 $\pm$ 4.66	+0.94	+5.76*
<u>Oryzaephilus</u> <u>surinamensis</u>	724/8053	22/724	77/8053	724/912	12.19	18.23 $\pm$ 7.02		
<u>Periplaneta americana</u>	462/6194	23/462	50/6194	462/1569	3.00	-4.55 ^a $\pm$ 9.12		
<u>Blatella germanica</u>	852/10956	26/852	85/10956	852/2438	3.00	12.12 $\pm$ 4.78		
<u>Acheta domesticus</u>	766/15642	16/766	100/15642	766/4149	2.50	-1.93 ^b $\pm$ 9.09		
<u>Epilachna varivestis</u>	1605/16341	19/1605	85/16341	1605/983	7.60	6.02 $\pm$ 9.53		
<u>Musca domestica</u>	983/10507	19/983	121/10507	983/2593	51.00	45.05 $\pm$ 9.17		
<u>Trichoplusia ni</u>	1018/7043	17/1018	42/7043	1018/727	46.00	42.25 $\pm$ 9.41		

a,^bNegative values represent an $\text{LD}_{50} \cong 0$

* $F_{4,3}$ -- Significant at the .10 level

Table 24. Analysis of the multiple regression model, $y = -51.5398 + 457.4504 (\text{Mg/K}) + (-597.3484 (\text{Cu/Mg})) + 5524.6261 (\text{Fe/K})$ representing the LD_{50} /mean-mortality radiation response for eight species of adult insects.

Species	Mg/K in ppm	Cu/Mg in ppm	Fe/K in ppm	Observed LD_{50} in kR	Expected LD_{50} in kR $\pm$ SE $_{50}$	R	F
<u>Tribolium castaneum</u>	629/6516	17/629	48/6516	13.19	17.64 $\pm$ 4.26	+0.90	+5.72*
<u>Oryzaephilus surinamensis</u>	724/8053	22/724	77/8053	12.19	24.95 $\pm$ 5.64		
<u>Periplaneta americana</u>	462/6194	23/462	50/6194	3.00	-2.72 ^a $\pm$ 10.01		
<u>Blatella germanica</u>	852/10956	26/852	85/10956	3.00	8.87 $\pm$ 4.62		
<u>Acheta domesticus</u>	766/15642	16/766	100/15642	2.50	-6.61 ^b $\pm$ 9.36		
<u>Epilachna varivestis</u>	1605/16341	19/1605	85/16341	7.60	15.15 $\pm$ 7.63		
<u>Musca domestica</u>	983/10507	19/983	121/10507	51.00	43.52 $\pm$ 10.10		
<u>Trichoplusia ni</u>	1018/7043	17/1018	42/7043	46.00	37.69 $\pm$ 9.78		

a,b,Negative values represent an $\text{LD}_{50} \cong 0$

* F_{3,4} -- Significant at the .10 level

that were applicable to a specific regression model were then analyzed by computer to determine the predicted or estimated LD_{50} radiation dose. The standard error (SE) for each predicted LD_{50} was also calculated (see Appendix Tables 2-21). The data is presented so that future comparisons between the predicted and observed LD_{50} radiation doses could be made, therefore testing the validity of a mineral-based predictor model.

Mechanisms of Death

$LD_{50}/24$ Hour Models

Analyses of the simple and multiple regression models have indicated the major importance of metallic cations in the determination of radiation sensitivity for insects in the adult stage.

The results of the simple and multiple regression models were significant for 19 species. However, they have indicated that the physiological, biochemical, and/or nutritional variations encountered between species, as well as within species representing unrelated orders, were related to the variation in the predicted $LD_{50}/24$ hour radiation doses on a species level. Therefore, the models could not be adequately explained by ratios and/or concentrations of single mineral cations.

The variation in the observed and expected $LD_{50}/24$ hour doses was shown to be significantly reduced when the above models were subdivided into the following categories based on feeding preferences: stored-product feeders, organic-plant feeders, and blood-feeders.

Regression analysis of the diet-based sub-divisions indicated that the mechanisms of insect death from an exposure to large doses of ionizing radiation were determined in part by levels or concentrations of specific cations obtained by feeding. These minerals would be metabolized and distributed to the various insect tissues and organs where they would interact in the numerous physiological and biochemical reactions involved in the insect metabolism. One might consider that these minerals affect a mechanism for inducing radiation protection or sensitization, therefore retarding or increasing the lethal response to ionizing radiation.

Several of the models based on diet used a single cation more than once in a predictor equation. This would indicate a "weighted" mineral variable which could be thought of as being more important in retarding or activating the mechanism of death due to a massive exposure of ionizing radiation.

The primary effect from ionizing radiation on cations distributed in the insects' water-rich O_2 -containing, cellular environment would be oxidation of the cations via an HO_2 -free radical mechanism (12). The extent of the reaction would be dependent upon the concentrations of O_2 specific cations, as well as other substances in the cell (12). Since most inorganic cations are very small, this indirect effect from ionizing radiation would predominate (12).

The HO_2 -free radical might also interact with organic molecules in the insect cells and tissues and drastically alter them. The following reactions are possible (79): (1) $HO_2 \cdot + RH \longrightarrow R \cdot + H_2O_2$; and (2) $RH + HO_2 \cdot \longrightarrow RO \cdot$ (organic free radical) + H_2O .

A lethal effect on insect cellular metabolism would be expected if RH was an essential metabolic component, possibly complexed to an inorganic cation. H_2O_2 could also act as a metabolic inhibitor if present in large concentrations (79).

It was presumed that a massive exposure to ionizing radiation would initiate certain reactions causing an almost immediate inducement of the mechanism(s) of death in 24 hours or less in 50% of an insect population.

Since death of the insect was rapid, it was hypothesized that an exposure to the $LD_{50}/24$ hour dose would produce a mineral-related death by either or both of the following mechanisms: (1) inhibition of the CNS; and (2) inhibition of respiratory metabolism. Because the insects received a total body exposure, a combination of these effects could be expected.

It must be presumed that minerals alone would not account for the death of an organism as biologically complex as an insect. The radiation response would probably involve an inter-relationship between mineral and non-mineral related systems.

The diet-based sub-divisions indicated that the mechanism(s) for radiation death in a relatively short time period would vary according to the insect group by virtue of the concentration of specific cations obtained by feeding. This might help to explain why species' sensitivity to ionizing radiation (LD_{50} doses) has been shown to vary according to geographical location (H. L. Cromroy, personal communication, 1971).

The mechanism for CNS death in an insect from an $LD_{50}/24$ hour radiation dose could be shown to be directly and/or indirectly related to total body mineral content. The following two minerals would be of major importance in the activation of a CNS death: Na and K.

The mechanism of death would involve the direct interaction of free radicals ($\text{HO}_2 \cdot$, etc.) with Na and K and cause a binding of these cations to other inorganic or organic molecules, therefore altering or shifting the normal Na/K ratio responsible for proper neural conductance. The same effect could be expected in insects where Na was almost completely substituted by Mg.

An indirect CNS effect produced by radiation destruction of membrane permeability surrounding the perilemma, blood cells, etc., could also be expected to drastically upset normal ionic fluxes due to the release of abnormally large concentrations of minerals. In effect, changes in cell permeability due to the polar binding of minerals would result. This radiation-induced destruction of membrane integrity has been shown to occur with exposures to large doses of ionizing radiation (12).

The destruction of the membranes could alter the normal K, Na, and Mg fluxes required by the insect heart and, therefore, produce cardiac failure by the inhibition or over-stimulation of the cardiac muscles (58).

Respiratory death in insects (74) could be produced by the inhibition of the pathways of respiratory metabolism: glycolysis, citric acid cycle, pentose-phosphate shunt, and/or the electron transport system.

Free radical oxidation of cytochrome Fe ($\text{Fe}^{2+} \longrightarrow \text{Fe}^{3+}$) as well as oxidation of the Cu component of the cytochrome oxidase system ($\text{Cu}^{1+} \longrightarrow \text{Cu}^{2+}$) of the insect could result in respiratory death by inhibition of terminal oxidation (86,87). High doses of ionizing radiation were shown to inactivate several enzyme systems in biological organisms (12,74). Na^+ and K^+ have also been shown to act as co-factors

for the activation of several insect enzymes (41). Binding of these cations by the action of free radicals could result in the destruction of several reactions important in respiratory metabolism.

The divalent ion of Mg, Mg^{2+} , has been shown to be an essential co-factor in many of the enzyme reactions comprising respiratory metabolism (41). Free radical oxidation of Mg ($Mg^{2+} \longrightarrow Mg^{3+}$) would upset the normal catalytic activities initiated only by Mg^{2+} ion and therefore would inhibit the normal pathways of many essential energy or ATP-releasing reactions. The destruction or inhibition of any one reaction link in the total chain of events comprising respiratory metabolism could produce death of the insect.

Phosphorylation or ATP production has been shown to be reduced in cells following a moderate to high dose of ionizing radiation (12). Casarett (12) has stated that "this decrease appears to result from an uncoupling of the phosphorylation mechanism from those of oxidation, since oxidation proceeds without phosphorylation following radiation exposure. Some effects occur in either the electron transport system of the mitochondria or more likely in the associated mechanisms of ATP formation"(p. 96).

Studies with the electron microscope have revealed massive disruption in mitochondria when various tissues were exposed to ionizing radiation (H. L. Cromroy, personal communication, 1971).

The inhibition or uncoupling of phosphorylation from oxidation has been considered by many researchers to be a primary effect of radiation which can result in the death of the cell (12). Other researchers have suggested that its relationship to cell death was secondary to other cellular changes (12). Therefore, $LD_{50}/24$ hour

simple and multiple regression models containing a species-specific concentration of Cu, Fe, and Mg could be related directly and/or indirectly to radiation-induced respiratory death.

Much of the observed $LD_{50}/24$ hour radiation response could be related to CNS death and/or death by inhibition of respiratory metabolism. The following acute radiation syndrome was observed in many insects (59): the initial effect from the radiation exposure was a cessation of feeding, accompanied by a period of inactivity or sluggishness. This stage was followed by rapid flying or walking movements and/or twitching which terminated in a period of quiescence characterized by loss of balance, sluggishness, and death. Although many of the insects were immobile, a rapid twitching of the legs and wings was observed. Death was usually observed as occurring in a period between 6-24 hours after radiation exposure.

$LD_{50}/28$ Day and $LD_{50}/\text{Mean-Mortality Models}$

The $LD_{50}/28$ day and $LD_{50}/\text{mean-mortality}$ (longevity) dose models could not be separated into feeding groups based on diet, due to an insufficient quantity of insect species to obtain LD_{50} data. The $LD_{50}/28$ day and $LD_{50}/\text{mean-mortality}$ models also represented the use of "weighted" mineral values in order to derive the estimated LD_{50} .

Although mineral content was shown to be related to radiation sensitivity, the results of the $LD_{50}/28$ day and $LD_{50}/\text{mean-mortality}$ dose simple and multiple regression models were not as significant as the models obtained from the $LD_{50}/24$ hour analyses. This was partly

the result of species variation between and within orders (i.e., diet) in a regression model and partly the substantially lower whole body radiation doses received by the insects.

The insect response to low doses of ionizing radiation would involve mechanisms affecting a delayed mortality. This delayed response was observed when insects were irradiated with an $LD_{50}/28$ day and LD_{50} mean-mortality radiation dose. The observed radiation syndrome was not as pronounced, but did contain many of the responses observed from the $LD_{50}/24$ hour exposure. Since the test period was so long, 28 days or longer, only periodic observations were recorded.

The drastic effects produced from a massive radiation exposure would be attenuated and therefore result in a more subtle or prolonged CNS and/or respiratory response via the same type of free radical mechanism as was previously discussed in the $LD_{50}/24$ hour models. This also would be affected by repair mechanisms within the insect. Damage from a radiation insult depends at a cellular level to a large extent on the ability of repair mechanisms (H. L. Cromroy, personal communication, 1971). Presently, there is no available information on the role of minerals in repair mechanisms.

Several authors (5,21,49) have proposed that tyrosinase in the hemolymph and corpuscles of various insects, by acting upon the phenolic products of protein breakdown, assists in the removal of these toxic materials. Low doses of ionizing radiation could also interfere with the Cu component of tyrosinase and, in effect, lead to a buildup of the toxic substances, which would result in the poisoning of the insect.

In addition to the attenuated CNS and/or respiratory effects, a radiation-induced inactivation of the tyrosinase enzyme system might occur due to the binding from oxidation of the Cu component by free radicals. Radiation interaction with this Cu-enzyme complex could upset the normal processes of moulting and metamorphosis (32,74). Therefore, an alteration in the normal formation of the cuticle, as well as the life cycle of the insect would result. This could very well be a major mineral-related effect causing radiation death in insects over a long period of time, particularly in immature insects.

Tyrosinase has been shown to be important in controlling the formation of the cuticle (84). Sussman (98) has found that four separate enzymes, dopa oxidase, catechol oxidase, diphenolase, and polyphenolase, make up a "tyrosinase enzyme complex." Other authors (6) have described a similar enzyme system.

Gastrointestinal effects could be a major factor leading to death of the insect from low doses of ionizing radiation. Radiation interaction with Cu and Fe, etc., causing a binding by oxidation via free radicals could upset proper mineral absorption in the midgut of the insect and therefore disrupt the normal metabolism of foods. General active and/or passive transport of ions would be affected (74), and there is a strong probability that the production of the peritrophic membrane would be interrupted (H. L. Cromroy, personal communication, 1971). Minerals have been shown to affect a buffer system in the gastrointestinal tract of insects (51).

Researchers (108-111) have shown the presence of bands or zones of Cu and Fe absorbing cells in specific areas of the midgut. Radiation disruption of proper mineral excretion could also result (74).

The malpighian tubules were shown to concentrate Cu for elimination. If ionizing radiation tied up Cu so that abnormally high concentrations accumulated in the insect tissues, the malpighian tubules could not handle the unusual concentrations, and death by Cu-poisoning would result (17).

The possibility of long-term radiation effects on mineral permeability in the insect hemocytes could also be postulated. In this case, ionizing radiation would interfere with the mechanisms of clotting, as well as the ability of the insect to ward off infection and fight disease. Radiation shortens life-span in vertebrates by increasing susceptibility to disease and rate of mutation (74). Large concentrations of Na, K, and Mg, have been found to occur in the hemolymph of insects (13,38). This fact, in combination with the experimentally demonstrated radiation destruction of insect hemocytes (43), would help substantiate the postulates for a long-term radiation death based on mineral content.

It has been postulated that concentrations of Na and K were involved in the mechanism(s) of nuclear activation in insects and, therefore, determined whether genes were active or quiet (39,41). It was suggested that ecdysone and juvenile hormone did not act directly on the chromosomes, but exerted their effects by altering the Na/K ratio of the nucleus. Low doses of ionizing radiation could initiate a free-radical mechanism which would upset the critical levels of Na and K, and thereby inhibit molting and metamorphosis, as well as other hormonal-controlled reactions.

A delayed death due to inhibition of chromosomal RNA synthesis could also be postulated if the radiation-induced production of free

radicals were to oxidize Mg ($\text{Mg}^{2+} \longrightarrow \text{Mg}^{3+}$). Lezzi (62) has explained a model by which Mg^{2+} differentially influenced chromosomal RNA concentrations in the salivary gland nuclei of chironomids. Mg^{2+} has also been shown to be a co-factor in several nucleotide dephosphorylating enzyme systems (39,41).

The $\text{LD}_{50}/28$ day and $\text{LD}_{50}/\text{mean-mortality}$ simple and multiple regression models would, therefore, represent the use of various mineral concentrations (Cu, Fe, Mg, Na, and K) to indicate a delayed radiation response affecting insect death by the interaction of several hypothetical mechanisms.

CHAPTER V

CONCLUSION

Results from simple and multiple regression analysis have indicated that mineral content can be used as an effective biological indicator of insect radiation sensitivity on a species level. A high correlation between insect total body Cu, Fe, Mg, Na, and K concentration and an LD₅₀/24 hour x or gamma radiation exposure was observed when species were separated into categories based on their general diet preference.

Several dose-related mechanisms for indicating death in a short period of time (≤ 24 hours) were postulated. Insect death resulting from high doses of ionizing radiation was tentatively explained as being related directly and/or indirectly to inhibition of the CNS and/or inhibition of respiratory metabolism via a free radical mechanism for inducing cation oxidation at the molecular level. This was partly based on the observed radiation syndrome.

Regression analysis of the LD₅₀/28 day and LD₅₀/mean-mortality dose models also demonstrated a correlation between insect mineral content and long-term death resulting from low doses of ionizing radiation. LD₅₀ predictions based on the LD₅₀/28 day and LD₅₀/mean-mortality models were not as significant as the LD₅₀/24 hour models.

This was the result of both a variation in diet and the inherent variability within insect orders representing the models.

A tentative hypothesis was given for the mechanisms affecting a delayed radiation death by free-radical oxidation of specific cations induced by low doses of ionizing radiation. The fatal effects were postulated as involving a combined inhibitory response of several of the following biologically active cation sites: CNS, respiratory system, tyrosinase enzyme system, buffer system, gastro-intestinal and excretory systems, active-passive transport system, RNA synthesizing system, etc.

Analysis of additional biologically important cations (i.e., Zn, Mn, Ca, etc.) could increase the significance of the relationship between mineral content and radiation sensitivity.

APPENDIX

Appendix Table 1. Total body insect^a mineral content determined by atomic absorption spectrophotometry.

<u>Species</u>	<u>Concentration of Minerals in ppm</u>				
	<u>Cu</u>	<u>Fe</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>
<u>Coleoptera</u>					
<u>Lasioderma</u> <u>serricorne</u> (a)	14	207	723	3636	13295
<u>Tribolium</u> <u>castaneum</u> (a)	17	48	629	989	6516
<u>Attagenus</u> <u>megatoma</u> (a)	51	75	428	1876	6788
<u>Sitophilus</u> <u>oryzae</u> (a)	16	45	609	993	3181
<u>Trogoderma</u> <u>inaculum</u> (a)	16	73	488	1221	5311
<u>Oryzaephilus</u> <u>surinamensis</u> (a)	22	77	724	912	8053
<u>Epilachna</u> <u>varivestis</u> (a)	19	85	1605	983	16341
<u>Epilachna</u> <u>varivestis</u> (l)	22	309	2215	397	18584
<u>Diabrotica</u> <u>undecimpunctata</u> (a)	111	48	441	2697	4162
<u>Diabrotica</u> <u>balteata</u> (a)	102	56	549	2137	7509
<u>Orthoptera</u>					
<u>Nauphoeta</u> <u>cinerea</u> (a)	20	89	626	6174	20293
<u>Nauphoeta</u> <u>cinerea</u> (n)	15	34	355	4982	9707
<u>Periplaneta</u> <u>americana</u> (a)	23	50	462	1569	6194
<u>Blatella</u> <u>germanica</u> (a)	26	85	852	2438	10956
<u>Periplaneta</u> <u>fuliginosa</u> (a)	27	59	678	2309	16225
<u>Leucophaea</u> <u>maderae</u> (a)	18	38	700	2473	18904
<u>Periplaneta</u> <u>australasiae</u> (a)	25	60	529	2880	14902
<u>Blaberus</u> <u>discoidalis</u> (a)	12	37	545	2655	8234
<u>Periplaneta</u> <u>brunnea</u> (a)	29	62	561	4653	15263
<u>Acheta</u> <u>domesticus</u> (a)	16	100	766	4149	15642

^a
 (a) = adult
 (n) = nymph
 (l) = larva
 (p) = pupa

Appendix Table 1. (Continued)

<u>Species</u>	Concentration of Minerals in ppm				
	<u>Cu</u>	<u>Fe</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>
<u>Lepidoptera</u>					
<u>Trichoplusia ni</u> (a)	17	42	1018	727	7043
<u>Trichoplusia ni</u> (l)	21	85	1202	1908	24362
<u>Trichoplusia ni</u> (p)	19	50	2865	744	10002
<u>Pseudoplusia includens</u> (a)	22	48	1464	765	9744
<u>Pseudoplusia includens</u> (l)	20	33	1260	894	19683
<u>Pseudoplusia includens</u> (p)	19	30	1820	732	8568
<u>Plodia interpunctella</u> (a)	10	30	652	629	5416
<u>Plodia interpunctella</u> (l)	16	30	501	685	8757
<u>Gnorimoschema operculella</u> (a)	23	49	498	1041	9535
<u>Cadra cautella</u> (a)	9	36	300	590	8500
<u>Cadra cautella</u> (l)	11	24	289	618	8286
<u>Decidamea inscriptum</u> (l)	17	15	954	480	15893
<u>Hyphantria cunea</u> (l)	48	38	666	3878	24160
<u>Hemerocampa leucostigma</u> (l)	13	36	567	1800	19690
<u>Diptera</u>					
<u>Musca domestica</u> (a)	19	121	983	2593	10507
<u>Musca domestica</u> (l)	11	75	1572	2091	11111
<u>Musca domestica</u> (p)	23	107	1980	3706	11664
<u>Stomoxys calcitrans</u> (a)	33	189	3987	2160	13050
<u>Stomoxys calcitrans</u> (l)	9	70	904	1899	11691
<u>Stomoxys calcitrans</u> (p)	14	27	2761	2117	14672
<u>Culex pipens quinquefasciatus</u> (a)	37	110	1885	1810	21190
<u>Culex pipens quinquefasciatus</u> (l)	121	340	732	8360	31709
<u>Culex pipens quinquefasciatus</u> (p)	43	92	1105	3530	6446
<u>Anastrepha suspensa</u> (a)	25	231	660	1989	12143
<u>Aedes aegypti</u> (l)	30	139	365	7536	12203
<u>Haemotobia irritans</u> (a)	18	658	597	2439	7621
<u>Drosophila melanogaster</u> ^b (a)	23	388	301	2777	9155
<u>Hippelates pusio</u> (a)	17	75	1320	1650	8470
<u>Hymenoptera</u>					
<u>Apis mellifera</u> ^c (a)	12	58	1770	184	7440
<u>Solenopsis saevissima richteri</u> (a)	9	93	459	1172	3908

^bvestigial wing^cNation and Robinson (71)

Appendix Table 1. (Continued)

<u>Species</u>	Concentration of Minerals in ppm				
	<u>Cu</u>	<u>Fe</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>
<u>Hemiptera</u>					
<u>Cimex lectularius</u> (a)	16	942	368	2108	5833
<u>Homoptera</u>					
<u>Myzus persicae</u> (a,n)	89	139	1296	1442	8244
<u>Anoplura</u>					
<u>Pediculus humanus humanus</u> (a,n)	21	449	487	3280	5911
<u>Thysanura</u>					
<u>Thermobia domesticus</u> (a)	29	--	--	2858	14041
<u>Siphonaptera</u>					
<u>Xenopsylla cheopis</u> (a)	33	112	107	3393	4378
<u>Others</u>					
<u>Rhipicephalus sanguineus</u> ^d (a)	20	905	65	3422	464
<u>Armadillidium vulgare</u> ^e (a)	trace	trace	184	4136	12135

^dArachnida
^eCrustacea

Appendix Table 2. Predicted LD₅₀/24 hr radiation doses for 16 species of insects based on the simple regression model, $y = Cu/(Na \times Mg)^2$ representing 19 species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀</u> <u>in kR ± SE</u>
<u>Hippelates pusio</u>	120 ± 18
<u>Epilachna varivestis</u>	129 ± 16
<u>Anastrepha suspensa</u>	144 ± 14
<u>Pseudoplusia includens</u>	151 ± 14
<u>Periplaneta australasiae</u>	136 ± 15
<u>Blaberus discoidalis</u>	125 ± 17
<u>Periplaneta brunnea</u>	121 ± 18
<u>Trichoplusia ni</u>	180 ± 14
<u>Gnorimoschema operculella</u>	297 ± 40
<u>Haematobia irritans</u>	131 ± 16
<u>Nauphoeta cinerea</u> ^b	123 ± 17
<u>Plodia interpunctella</u>	238 ± 25
<u>Drosophila melanogaster</u> ^c	185 ± 15
<u>Diabrotica undecimpunctata</u>	286 ± 37
<u>Diabrotica balteata</u>	277 ± 34
<u>Apis mellifera</u>	365 ± 57

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 3. Predicted LD₅₀/24 hr radiation doses for 16 species of insects based on the multiple regression model, $y = 68.4260 + 0.0061 (\text{Na}) + 3.4360\text{E}12 (\text{Cu}/(\text{Na} \times \text{Mg}))^2 + 0.0689 (\text{K}/\text{Cu}) + (-109.9400 (\text{Cu}/\text{Fe}))$ representing 19 species of insects.

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Hippelates pusio</u>	101 ± 24
<u>Epilachna varivestis</u>	135 ± 43
<u>Anastrepha suspensa</u>	152 ± 20
<u>Pseudoplusia includens</u>	113 ± 30
<u>Periplaneta australasiae</u>	118 ± 21
<u>Blaberus discoidalis</u>	114 ± 19
<u>Periplaneta brunnea</u>	96 ± 42
<u>Trichoplusia ni</u>	163 ± 24
<u>Gnorimoschema operculella</u>	341 ± 48
<u>Haematobia irritans</u>	138 ± 26
<u>Nauphoeta cinerea</u> ^b	111 ± 41
<u>Plodia interpunctella</u>	272 ± 40
<u>Drosophila melanogaster</u> ^c	220 ± 29
<u>Diabrotica undecimpunctata</u>	84 ± 168
<u>Diabrotica balteata</u>	139 ± 117
<u>Apis mellifera</u>	481 ± 88

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 4. Predicted LD₅₀/24 hr radiation doses for four species of insects based on the simple regression model, $y = 244.5774 (Cu/Fe) + 172.0181$ representing six species of stored-product insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Solenopsis saevissima richteri</u>	196 ^b ± 27
<u>Cadra cautella</u>	234 ± 18
<u>Gnorimoschema operculella</u>	285 ± 21
<u>Plodia interpunctella</u>	251 ± 17

^aRefer to Appendix Table 1 for mineral content of species

^bExperimentally determined LD₅₀/24 hr = 175 kR

Appendix Table 5. Predicted LD₅₀/24 hr radiation doses for four species of insects based on the multiple regression model, $y = 424.5906 + (-0.3263 \text{ (Mg)}) + (-46506.5039 \text{ (Cu/K)}) + 589.8412 \text{ (Cu/Fe)}$ representing six species of stored-product insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Solenopsis caevissima richteri</u>	223 ^b ± 26
<u>Cadra cautella</u>	426 ± 51
<u>Gnorimoschema operculella</u>	424 ± 36
<u>Plodia interpunctella</u>	319 ± 21

^aRefer to Appendix Table 1 for mineral content of species

^bExperimentally determined LD₅₀/24 hr = 175 kR

Appendix Table 6. Predicted LD₅₀/24 hr radiation doses for four species of insects based on the multiple regression model, $y = 308.3822 + (-7.0798 \text{ (Cu)}) + 687.3172 \text{ (Cu/Fe)} + (-1229.1410 \text{ (Mg/K)})$ representing six species of stored-product insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Solenopsis saevissima richteri</u>	167 ^b ± 16
<u>Cadra cautella</u>	375 ± 42
<u>Gnorimoschema operculella</u>	401 ± 37
<u>Plodia interpunctella</u>	314 ± 28

^aRefer to Appendix Table 1 for mineral content of species

^bExperimentally determined LD₅₀/24 hr = 175 kR

Appendix Table 7. Predicted LD₅₀/24 hr radiation doses for six species of insects based on the simple regression model, $y = -27456.8938 (Cu/K) + 152.6571$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Anastrepha suspensa</u>	97 ± 4
<u>Periplaneta australasiae</u>	106 ± 4
<u>Blaberus discoidalis</u>	111 ± 4
<u>Periplaneta brunnea</u>	101 ± 4
<u>Nauphoeta cinerea</u> ^b	110 ± 4
<u>Drosophila melanogaster</u> ^c	83 ± 5

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 8. Predicted LD₅₀/24 hr radiation doses for six species of insects based on the simple regression model, $y = -6509.5691 (\text{Cu/Na}) + 158.6133$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀</u> <u>in kR ± SE</u>
<u>Anastrepha suspensa</u>	78 ± 6
<u>Periplaneta australasiae</u>	102 ± 4
<u>Blaberus discoidalis</u>	128 ± 6
<u>Periplaneta brunnea</u>	118 ± 5
<u>Nauphoeta cinerea</u> ^b	138 ± 8
<u>Drosophila melanogaster</u> ^c	104 ± 4

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 9. Predicted LD₅₀/24 hr radiation doses for six species of insects based on the simple regression model, $y = .0054 (K) + 25.8061$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE⁵⁰</u>
<u>Anastrepha suspensa</u>	92 ± 5
<u>Periplaneta australasiae</u>	107 ± 5
<u>Blaberus discoidalis</u>	71 ± 7
<u>Periplaneta brunnea</u>	109 ± 5
<u>Nauphoeta cinerea</u> ^b	79 ± 6
<u>Drosophila melanogaster</u> ^c	76 ± 7

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 10. Predicted LD₅₀/24 hr radiation doses for six species of insects based on the simple regression model, $y = 0.0166 (Na) + 51.3646$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Anastrepha suspensa</u>	84 ± 7
<u>Periplaneta australasiae</u>	99 ± 6
<u>Blaberus discoidalis</u>	95 ± 6
<u>Periplaneta brunnea</u>	129 ± 8
<u>Nauphoeta cinerea</u> ^b	134 ± 9
<u>Drosophila melanogaster</u> ^c	98 ± 6

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 11. Predicted LD₅₀/24 hr radiation doses for six species of insect based on the multiple regression model, $y = 110.2092 + 0.0087 (\text{Na}) + (-18940.1062 (\text{Cu/K}))$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Anastrepha suspensa</u>	89 ± 2
<u>Periplaneta australasiae</u>	104 ± 2
<u>Blaberus discoidalis</u>	105 ± 2
<u>Periplaneta brunnea</u>	115 ± 3
<u>Nauphoeta cinerea</u> ^b	124 ± 3
<u>Drosophila melanogaster</u> ^c	87 ± 2

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 12. Predicted LD₅₀/24 hr radiation doses for six species of insects based on the multiple regression model, $y = 92.6478 + 0.0031 (K) + (-3925.0502 (Cu/Na))$ representing eight species of organic-plant feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Anastrepha suspensa</u>	82 ± 2
<u>Periplaneta australasiae</u>	105 ± 2
<u>Blaberus discoidalis</u>	100 ± 5
<u>Periplaneta brunnea</u>	116 ± 2
<u>Nauphoeta cinerea</u> ^b	111 ± 5
<u>Drosophila melanogaster</u> ^c	88 ± 3

^aRefer to Appendix Table 1 for mineral content of species

^bNymphs -- all other species analyzed in the adult stage

^cvestigial wing

Appendix Table 13. Predicted LD₅₀/24 hr radiation doses for four species of arthropods based on the simple regression model, $y = -30.6599 \text{ (Mg/Na)} + 171.5961$ representing five species of blood-feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Hippelates pusio</u> ^b	147 ± 4
<u>Haematobia irritans</u>	164 ± 4
<u>Rhipicephalus sanguineus</u> ^c	171 ± 5
<u>Xenopsylla cheopis</u>	171 ± 5

^aRefer to Appendix Table 1 for mineral content of species

^bMay feed on oozing wounds, etc.

^cArachnida -- other species represent Insecta

Appendix Table 14. Predicted LD₅₀/24 hr radiation doses for four species of arthropods based on the single regression model, $y = -0.0147 (\text{Mg}) + 172.2073$ representing five species of blood-feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Hippelates pusio</u> ^b	153 ± 4
<u>Haematobia irritans</u>	163 ± 5
<u>Rhipicephalus sanguineus</u> ^c	171 ± 6
<u>Xenopsylla cheopis</u>	171 ± 6

^aRefer to Appendix Table 1 for mineral content of species

^bMay feed on oozing wounds, etc.

^cArachnida -- other species represent Insecta

Appendix Table 15. Predicted LD₅₀/24 hr radiation doses for four species of arthropods based on the multiple regression model, $y = 224.1587 + (-1.2260 \text{ (Cu)}) + (-0.0467 \text{ (Fe)}) + (-32.4359 \text{ (Mg/Na)})$ representing five species of blood-feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR $\pm$ SE</u>
<u>Hippelates p. sio</u> ^b	174 $\pm$ 10
<u>Haematobia irritans</u>	163 $\pm$ 2
<u>Rhipicephalus sanguineus</u> ^c	157 $\pm$ 5
<u>Xenopsylla cheopis</u>	177 $\pm$ 11

^aRefer to Appendix Table 1 for mineral content of species

^bMay feed on oozing wounds, etc.

^cArachnida -- other species represent Insecta

Appendix Table 16. Predicted LD₅₀/24 hr radiation doses for four species of arthropods based on the multiple regression model, $y = 187.2418 + (-0.0251 (\text{Fe})) + (-37.9907 (\text{Mg/Na}))$ representing five species of blood-feeding insects.^a

<u>Species</u>	<u>Expected LD₅₀ in kR ± SE</u>
<u>Hippelates pusio</u> ^b	155 ± 4
<u>Haematobia irritans</u>	161 ± 3
<u>Rhipicephalus sanguineus</u> ^c	164 ± 5
<u>Xenopsylla cheopis</u>	183 ± 8

^aRefer to Appendix Table 1 for mineral content of species

^bMay feed on oozing wounds, etc.

^cArachnida -- other species represent Insecta

Appendix Table 17. Predicted LD₅₀/28 day radiation doses for 13 species of insects based on the simple regression model, $y = -0.0024 (Mg + Na) + 11.4310$ representing seven species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀ in krad</u>
<u>Lasioderma serricorne</u>	0.86 ± 2.94
<u>Trogoderma inclusum</u>	7.29 ± 1.90
<u>Periplaneta fuliginosa</u>	4.19 ± 1.67
<u>Musca domestica</u>	2.76 ± 2.11
<u>Solenopsis saevissima richteri</u>	7.48 ± 1.97
<u>Hippelates pusio</u>	4.43 ± 1.66
<u>Cadra cautella</u>	9.27 ± 2.71
<u>Anastrepha suspensa</u>	5.01 ± 1.56
<u>Pseudoplusia includens</u>	6.03 ± 1.60
<u>Trichoplusia ni</u>	7.20 ± 1.88
<u>Gnorimoschema operculella</u>	7.70 ± 2.05
<u>Plodia interpunctella</u>	8.32 ± 2.29
<u>Apis mellifera</u>	6.69 ± 1.73

^aRefer to Appendix Table 1 for mineral content of species

Appendix Table 18. Predicted LD₅₀/28 day radiation doses for 13 species of insects based on the multiple regression model, $y = 39.5350 + (-3009.0000 \text{ (Cu/K)} + (-0.0161 \text{ (Mg + Na)}) + 0.0001 \text{ (Fe x Na)}$ representing seven species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀ in krad ± SE</u>
<u>Lasioderma serricorne</u>	46.35 ± 13.76
<u>Trogoderma inclusum</u>	12.19 ± 1.72
<u>Periplaneta fuliginosa</u>	0.85 ± 1.71
<u>Musca domestica</u>	9.97 ± 2.41
<u>Solenopsis saevissima richteri</u>	17.69 ± 2.90
<u>Hippelates pusio</u>	-1.17 ^b ± 2.02
<u>Cadra cautella</u>	24.19 ± 4.09
<u>Anastrepha suspensa</u>	39.79 ± 10.29
<u>Pseudoplusia includens</u>	0.69 ± 2.19
<u>Trichoplusia ni</u>	7.41 ± 1.27
<u>Gnorimoschema operculella</u>	13.02 ± 1.74
<u>Plodia interpunctella</u>	15.52 ± 2.29
<u>Apis mellifera</u>	4.22 ± 2.71

^aRefer to Appendix Table 1 for mineral content of species

^bNegative value represents an LD₅₀ ≈ 0

Appendix Table 19. Predicted LD₅₀/28 day radiation doses for 13 species of insects based on the multiple regression model, $y = 47.1820 + (-7523.9000 \text{ (Cu/K)}) + (-0.0171 \text{ (Mg } \pm \text{ Na)}) + 2.2863\text{E-5 (Fe x Na)} + 1.3975 \text{ (Fe x Na)/K}$ representing seven species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀ in krad $\pm$ SE</u>
<u>Lasioderma serricorne</u>	59.79 $\pm$ 16.77
<u>Trogoderma inclusum</u>	19.83 $\pm$ 6.41
<u>Periplaneta fuliginosa</u>	-2.29 ^b $\pm$ 3.00
<u>Musca domestica</u>	20.66 $\pm$ 8.97
<u>Solenopsis saevissima richteri</u>	42.45 $\pm$ 20.32
<u>Hippelates pusio</u>	3.95 $\pm$ 4.57
<u>Cadra cautella</u>	27.60 $\pm$ 4.68
<u>Anastrepha suspensa</u>	49.43 $\pm$ 12.33
<u>Pseudoplusia includens</u>	-2.32 ^c $\pm$ 3.17
<u>Trichoplusia ni</u>	5.63 $\pm$ 1.86
<u>Gnorimoschema operculella</u>	11.35 $\pm$ 2.10
<u>Plodia interpunctella</u>	16.89 $\pm$ 2.39
<u>Apis mellifera</u>	3.28 $\pm$ 2.15

^aRefer to Appendix Table 1 for mineral content of species

^{b,c}Negative values represent an LD₅₀ $\approx$ 0

Appendix Table 20. Predicted LD₅₀/mean-mortality radiation doses for 13 species of insects based on the simple regression model, $y = (499.7257 \text{ (Mg/K)} + (-27.9200))$ representing eight species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀</u> <u>in kR ± SE</u>
<u>Lasioderma serricorne</u>	-0.74 ^b ± 9.50
<u>Trogoderma inclusum</u>	18.00 ± 5.47
<u>Attagenus megatoma</u>	3.59 ± 8.05
<u>Leucophaea maderae</u>	-9.42 ^c ± 12.74
<u>Periplaneta fuliginosa</u>	49.96 ± 15.08
<u>Hippelates pusio</u>	-10.28 ^d ± 13.08
<u>Cadra cautella</u>	-0.75 ^e ± 9.51
<u>Anastrepha suspensa</u>	47.16 ± 13.96
<u>Pseudoplusia includens</u>	5.16 ± 7.57
<u>Blaberus discoidalis</u>	-9.55 ^f ± 12.79
<u>Periplaneta brunnea</u>	-1.82 ^g ± 9.88
<u>Gnorimoschema operculella</u>	11.23 ± 6.06
<u>Plodia interpunctella</u>	32.23 ± 8.44

^aRefer to Appendix Table 1 for mineral
b,c,d,e,f,g content of species
Negative values represent LD₅₀ ≈ 0

Appendix Table 21. Predicted LD₅₀/mean-mortality radiation doses for 13 species of insects based on the multiple regression model, $y = -110.1670 + 887.5769 (\text{Mg/K}) + (-631.3156 (\text{Cu/Mg})) + 5554.3929 (\text{Fe/K}) + 8.8102 (\text{Na/Mg})$ representing eight species of adult insects.^a

<u>Species</u>	<u>Expected LD₅₀</u> <u>in kR ± SE</u>
<u>Lasioderma serricorne</u>	5.64 ± 8.79
<u>Trogoderma inclusum</u>	4.84 ± 5.84
<u>Attagenus megatoma</u>	-3.00 ^b ± 15.55
<u>Leucophaea maderae</u>	-5.13 ^c ± 7.77
<u>Periplaneta fuliginosa</u>	8.04 ± 6.94
<u>Hippelates pusio</u>	-5.72 ^d ± 8.59
<u>Cadra cautella</u>	4.69 ± 10.95
<u>Anastrepha suspensa</u>	4.56 ± 5.53
<u>Pseudoplusia includens</u>	2.16 ± 5.02
<u>Blaberus discoidalis</u>	-1.46 ^e ± 9.75
<u>Periplaneta brunnea</u>	-4.54 ^f ± 6.92
<u>Gnorimoschema operculella</u>	4.56 ± 75.45
<u>Plodia interpunctella</u>	2.64 ± 3.32

^aRefer to Appendix Table 1 for mineral content of species
^{b,c,d,e,f} Negative values represent an LD₅₀ < 0

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Richard Levy was born June 29, 1944, in Brooklyn, New York. He attended the University of Florida where he graduated with the degrees Bachelor of Science in Zoology and Bachelor of Science in Entomology in 1967 and 1968, respectively. He was admitted to graduate school in the College of Forestry in 1968 where he worked as a graduate research assistant in wildlife ecology.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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