BROMIDE RESIDUES IN TISSUES FROM RATS MAINTAINED ON DIETS FUMIGATED WITH METHYL BROMIDE

by

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INTRODUCTION

The use of pesticides, insecticides, and fumigants in the United States and the world during the nineteenth century was considered to be the best method for the control of agricultural insect pests. The concern for chemical residue concentrations in foods does not arise from the food products normal content of this chemical or from apprehension of introducing a foreign substance into the diet, but from the toxic levels of chemical residues which can occur in foods exposed to chemical compounds. Such compounds could be fumigants, pesticides, and insecticides used in the prevention of disease, spoilage, and insect damage to food products, Kraybill (1966). In 1966 Kraybill said, "The uncontrolled application of insecticides with resultant contamination of feeds is a grave economic and health problem since this leads to accumulation of persistent type pesticides or their metabolites in the tissues of meat animals."

Thompson (1966) stated that fumigation is one method available for controlling insect infestation in stored products, and, of the fumigants available, one of outstanding importance is methyl bromide. Under normal fumigation conditions methyl bromide is a lethal, odorless, colorless gas whose b.p. = 4.5°C at 760 mm Hg. Shrader et al. (1942) noted that this compound diffuses rapidly from foods after fumigation. However, when a fumigant is used on any substance, and is sorbed, it may leave a residue. The resulting residue is thought to be inorganic bromide.

Elimination of pests is especially important in the food industries from both an economic and a public health standpoint. Successful fumigation practice involves the effective destruction of insects without leaving toxic or undesirable residues in the fumigated product. Methyl bromide is a fumigant which has proven to be valuable in protecting stored commodities from pest and rodent damage. This gas is unusually effective, fast acting, highly penetrating, and safe to use if proper precautions are utilized. Le Goupil (1932) was the first to report on the insecticidal value of methyl bromide. Thompson (1966) reported that the value of methyl bromide as a fumigant stems only in part from its high toxicity to most pests: its most important attribute is that it is not highly sorbed by most commodities and can therefore penetrate easily into them and air off quickly upon ventilation at the end of the treatment. Getzendaner et al. (1968) noted the use of methyl bromide for commodity fumigation has greatly increased in recent years.

However, there is a paucity of knowledge and data on the metabolic fate of methyl bromide in man and animals. The small amount of data available was obtained from experiments where information was required to guide the Food and Drug Administration in setting tolerance limits for bromides in food products. In 1965, the World Health Organization stated that very little information was available on the residues left in various items of food treated with methyl bromide. They also noted that chronic toxic levels had not been determined, and long-term studies on the effect of inorganic bromide should be conducted on rats.

The data available at present is insufficient for the estimation of acceptable daily intakes of inorganic bromide for man.

Getzendaner et al. (1968) stated that there is a lack of published information useful for establishing tolerances of bromide residues on processed foods resulting from direct fumigation in chambers or from fumigation of warehouses or other buildings which might contain foods. The use of methyl bromide can lead to higher bromide residues in tissues of animals or humans which consume such a treated food. Assessing the hazards of bromide residues in foods or air on the basis of total daily intake of this element has been considered. However, there is insufficient data available to evaluate the significance of residues from this fumigant. Little is known concerning the tissue wherein the bromide resides, much less its metabolic fate in the body.

There are many methods for determining bromide in biological tissues. The AOAC method (Mapes and Shrader 1957) is time consuming and difficult to replicate. An alternative method is the use of neutron activation analysis (NAA) which requires no chemical preparations. The NAA method is faster than any other commonly used procedure (chemical, ion-exchange chromatography, potentiometric titration, spectroscopic); has equal or better accuracy, repeatability, and sensitivity; and is relatively free of serious interference.

Inorganic bromide occurs naturally in all plants and animals yet its role in body metabolism, if any, has yet to be determined. Studies by many workers have verified that the bromide concentration in MeBr fumigated foods does increase. Bromides incorporated in body tissues from breathing polluted air may also be a contributing factor. However

the response of various body organs to bromide have not been investigated thoroughly enough to determine the effect of bromide residues in foods on human and animal behavior, growth and function.

Many researchers have reported that more work is needed to provide information on the effect(s) of bromide residues in foods consumed by animals; and that a more reliable method of analysis of bromide in tissues is needed. Therefore, a study of the effect(s) of feeding rats diets fumigated with various levels of methyl bromide, and analyzing the tissues for residues by neutron activation analysis was undertaken.

OBJECTIVES

The objectives of this study were:

1. To study the effect(s) of diets fumigated with methyl bromide on organ size, growth rate and physical activity of rats
2. To identify the site of tissue deposition of bromide residues
3. To apply a computer program to the analysis of data obtained from a lithium-germanium detector used in neutron activation analysis

REVIEW OF LITERATURE

F.D.A. Approved Levels of Inorganic Bromide in Foods

Bromide residues may occur in food commodities from many sources. Table 1 lists some of the more common brominated fumigants in use today, Bohannon et al. (1969). Exposure of foods to these chemicals is one of the major causes of bromide contamination. These residues may also accrue from adding potassium bromate to malts in distilled spirits, or occur in foods grown on soil treated with the nematocide 1,2-dibromo-3-chloropropane.

A list of the tolerance limits for residual bromide set by the F.D.A. for various food commodities was published in the Federal Register (1967). They are as follows:

1. 400 parts per million in or on dried eggs and processed herbs and spices
2. 325 ppm in or on parmesan cheese and roquefort cheese
3. 250 ppm in or on concentrated tomato products and dried figs
4. 125 ppm in or on cereal flours; macaroni and noodle products; pie, cake, biscuit, cookie and bread mixes; breading; dried vegetables; soya flour; and flours of barley, milo (sorghum), oats, rice and rye

According to this same article, the following foodstuffs should not be exposed to methyl bromide or should be exposed only with extreme care:

1. Iodized salt stabilized with sodium hyposulphite
2. Full fat soya flour
3. Baking sodas and salt blocks used for cattle licks

Table 1. Brominated Fumigants

Fumigant	Formula
Bromoform	$CHBr_3$
Ethyl Bromide	C_2H_5Br
Methyl Bromide	CH_3Br
Bromobenzen	C_6H_5Br
1-Bromopropene	C_3H_7Br
3-Bromopropene	$CH_2{:}CH{\cdot}CH_2Br$
2-Bromopropane	$CH_3\ CHBr{\cdot}CH_3$
1,1,1-Tribromoethane	$CBr_3{\cdot}CH_3$
Ethylene Dibromide	$CH_2Br{\cdot}CH_2Br$
2-Bromobutane	$C_2H_5{\cdot}CHBr{\cdot}CH_3$
1,3-Dibromopropane	$CH_2Br{\cdot}CH_2{\cdot}CH_2Br$

4. Fresh fruits and vegetables

The exposure of iodized salt, soya flour and baking sodas to methyl bromide may cause unpleasant odors, while the exposure of fresh fruits or vegetables to this fumigant can lead to their rapid deterioration if improperly handled.

The Effectiveness of Methyl Bromide Against Insects

Fumigation is one of the most effective methods of controlling stored-product pests. According to Shepard and Buzicky (1939) the use of methyl bromide as an insect fumigant increased enormously from 1938 to 1939 in the control of insect infestation of stored foods and green vegetables. These workers reported that the larvae of the black carpet beetle, Attagenus piceus (Oliv.), were the most resistant to methyl bromide, and that low temperature increased the lethal rate among insects tested. In a separate experiment, they found that five times the recommended dosage of fumigant had no effect on the baking qualities of flour. Mackie and Carter (1937) presented some of the first evidence in favor of using methyl bromide to fumigate infested fresh vegetables. They claimed that using normal dosages did not damage the vegetables. Phillips et al. (1938) found that methyl bromide fumigation was practical in the destruction of insects in harvested apples, but extreme care had to be utilized in applying the pesticide or both external and internal injury to the fruit would result. Dustan (1937) was successful in controlling cheese mites with methyl bromide, while Piper and Davidson (1938) found the fumigant excellent in the control of stored-product insects. Gerhardt et al. (1951) found methyl bromide to be one

of the most effective and practical fumigants for use against two common pests of walnuts: the navel orangeworm, Myelois venipars Dyer, and the Mediterranean flour moth, Ephestia kuhniella Zeller. They also found that the bromide residues in the walnut meat became constant after the first 24 hours of fumigation and this level was retained for as much as nine weeks. Lindgren and Vincent (1959) determined that methyl bromide was the most effective fumigant tested in killing adult Tribolium confusum Duv., and Sitophilus oryza (L.).

Bromide Residues in Foodstuffs

Bromide residues can occur in foods either naturally from soil, water, and air or from the application of a brominated chemical substance by man. Automobile exhaust emissions of bromide, from the combustion of ethylene bromide, may easily increase the natural environment's content of this element; thus increasing the bromide content of plant and animal tissues. Many of the foodstuffs produced in the United States may be fumigated with methyl bromide for insect control. The official tolerance levels for methyl bromide, calculated as inorganic bromide, on raw agricultural commodities varies from 5 to 240 parts per million (ppm), depending on the commodity fumigated. Such foods as animal products and fats, herbs, spices, confections, beverages and cereal products have been fumigated with methyl bromide. Dudley (1939) analyzed dried fruits, fresh fruits and vegetables for total bromide following fumigation (at atmospheric pressure) in the laboratory with concentrations of methyl bromide approaching those used in commercial practice (2 lb of CH_3Br/1000 cu ft/24 hrs). He found

that a large part of the absorbed gas is volatilized within 48 hours and escapes into the surrounding air. The residue levels detected were minor in the fruits and vegetables; but milled grains, cheese, nuts and nut meats absorbed greater amounts. Lang (1941) reported that within one hour after exposure, approximately 85 to 95 percent of the volatile methyl bromide residue disappears from a foodstuff. Although the volatile residue is dispersed, significantly high non-volatile bromide residues may occur under certain conditions. The finding of higher bromide residues in milled grains on a laboratory scale (Stenger et al., 1939; Dudley et al., 1940) raised a question as to whether such residues might be objectionable in the fumigated products. These workers feared that the residual bromide might consist in part of free methyl bromide. This fear was well founded because prior to the work by Shrader et al. (1942) no analytical method had been available for distinguishing between the various forms of bromide present in small amounts. The method developed by Shrader et al. (1942) determined the bromide remaining in fumigated flour to be largely inorganic immediately after fumigation and entirely inorganic within several days. Roehm et al. (1942) showed that not only were the residues inorganic bromides, but the levels of these residues were lower under the recommended commercial fumigation conditions than had previously been expected from experiments conducted on a laboratory scale. The amounts of bromide retained by white flour and whole wheat flour were reported to vary from 0.0042% to 0.0091% (Dudley et al., 1940; Stenger et al., 1939; Cotton, 1941). Lubatti and Harrison (1944) determined that the amount of sorbed bromide left in grain products

increases as moisture content of the product and/or temperature increases. However, Winteringham and Harrison (1946) found that the sorption is inhibited by the presence of water. Shrader et al. (1942) summarized the various ways in which methyl bromide may be retained in fumigated products as follows:

1. Inorganic bromides are formed by hydrolysis of the methyl bromide or by its action on sulfur or nitrogen containing constituents of the food product.
2. Additional compounds are formed with unsaturated constituents.
3. There is physical retention by either solution or adsorption.

Methyl bromide is a well-known methylating agent, and Winteringham and Harrison (1946) and Lewis and Eccleston (1945) hypothesized that the residual inorganic bromides are not formed from hydrolysis but from methylation of acid groups (carboxyl, phenol, and thiol) present in the proteins. Lewis and Eccleston also pointed out that the protein fraction of the wheat contained higher residual levels than the starch fractions, and the bromide liberated was proportional to the protein content of the product. Blackburn et al. (1941) and Blackburn and Phillips (1944) showed that methylating agents such as methyl bromide react readily with such proteins as collagen, gelatin and keratin.

Hermitte and Shellenberger (1947) found that when high concentrations of methyl bromide are used to fumigate flour; unpleasant odors occur, mixing tolerances are lessened and loaf volume is reduced. At low fumigation levels no lasting detrimental effects were produced. They also found that the changes in the flour were associated with the gluten protein fractions of flour. Olomucki and Bondi (1955) studied

the problems connected with the sorption of ethylene dibromide (EDB) by grain and concurred with previous workers that the protein fraction was reacting with the fumigant. However, they found the chemical reaction of EDB is less intense than that of methyl bromide as indicated by the residual bromine. Winteringham et al. (1955) postulated that the objectionable odor from wheat flour fumigated with methyl bromide was caused by the reaction of the fumigant with the amino-acid methionine. This reaction would produce a sulphonium salt which would cause the adverse odor. These workers also showed that a large part of the reaction occuring between methyl bromide and gluten was due to methylation of the nitrogen-containing groups of the protein. Bridges (1955) stated the possible sites of N-methylation in the protein were:

1. The nitrogen of the peptide links
2. The terminal amino groups of the protein chains
3. The nitrogen containing groups of the three basic amino acids; histidine, lysine, and arginine
4. The nitrogen of the indole group of tryptophan

It was also pointed out that in N-methylation of proteins the basic amino-acid residues are the most susceptible to attack. He concluded that this N-methylation caused no appreciable loss of any essential amino-acids in wheat protein when fumigated with methyl bromide under normal conditions. Lindgren et al. (1962) determined that fumigation at lower temperatures and higher dosages resulted in lower bromine residues than fumigation at higher temperatures and lower dosages. They also found that the higher the moisture content, temperature and exposure periods, the higher the bromine residue. The parts of the wheat with

the highest bromine residue levels were: bran > shorts > flour > middlings. These results indicated that the wheat fractions highest in fat content (bran and shorts) had the greatest bromine residue levels. In general, the attempts by various researchers to relate bromide residues in fumigated foodstuffs to protein, fat and moisture content have resulted in varied interpretations. However, there is a general consensus among many of these workers that the relationship between bromide residues and both percent fat and percent protein is very close. Getzendaner (1965) reported that methyl bromide was a safe fumigant for the use of spot fumigation of flour mill machinery.

There appears to be some discrepancy among researchers as to the exact chemical reactions which take place between methyl bromide and a product. Most of the research has been centered primarily around the grain industry, leaving gaping holes in research data useful for establishing tolerances in processed foods resulting from direct fumigation in chambers or from fumigations of buildings containing foodstuffs. A general survey study was made by Duggan et al. (1966), and Duggan et al. (1967) on pesticide residue levels in ready-to-eat foods. In a two-year study they found that the frequency of bromide residues had not changed significantly as a whole within food classes.

Beckman et al. (1967) studied the inorganic bromide content of foodstuffs due to soil treatment with fumigants and found that the organically bound bromide ion is not taken up by plants from the soil, but is degraded, liberating inorganic bromide ions into the soil which the plant absorbs and stores in the intercellular fluids. These workers also noted that leafy vegetables contained high levels of inorganic

bromide on a weight basis. However, in all products studied, the bromide concentrations were within FDA limits. Gutenmann et al. (1961) studied bromide residues in cherries resulting from soil treated with Nemagon and found 5 - 10 ppm residue.

The Effect of Methyl Bromide Fumigated Feeds on Animals

Dudley and coworkers (1940, 1942) reported feeding experiments in which rats were maintained for 20 weeks on a diet fumigated to 30 milligrams of bromide per 100 grams with no detrimental effects. Another study was also conducted by these same workers in which the diets were fumigated to 5760 parts per million. This diet was fed to an unspecified number of rats for eight weeks, and the animals developed diarrhea, showed little weight gain, and generally poor condition. Harned et al. (1944) noted that pregnant rats which were fed bromide throughout gestation gave birth to offspring which had a reduced maze-learning capacity. Dudley et al. (1940) reported feeding rabbits diets with bromide levels of 3254 ppm. The animals developed paralysis within two weeks and bronchiopneumonia. Spencer et al. (1944) raised 196 rats on diets fumigated to 26.2 and 63.7 milligrams per 100 grams of residual bromide for one year. They found no discernible ill effects due to bromide ingestion. However, they did show greatly elevated levels of bromide in the blood. In 1960, Rosenblum et al. raised twenty-two beagle dogs for 365 days on food fumigated from 35 mg/kg body weight. They found no evidence of bromide intoxication in any group with the exception of one animal in the highest treatment (150 mg/kg/day) group. The cause of death was related to bromide ingestion,

but the relationship was not clear. These workers noted no significant effects in the surviving dogs hemoglobin, hematocrit, serum proteins or blood urea nitrogen. Getzendaner (1965) fed chickens diets ranging from 5 to 410 ppm of bromide residue. He determined that the bromide content of eggs and chicken tissues reaches a maximum in 30 to 40 days when the dietary intake is maintained at a fixed level. The residue levels ranged from 14 in skin and light meat to 301 in the kidneys.

Bondi et al. (1955) and Olomucki and Bondi (1955) made studies on the effect of grain fumigated with EDB on laying hens. These researchers found that in several cases, decreased egg size occurred and in some cases complete cessation of egg production was observed. The free, unreacted EDB remaining in the feed due to insufficient airing was determined as the toxic element and not the residual inorganic bromide.

Work by Lynn et al. (1963) showed that the milk of lactating dairy cows contained inorganic bromide when the element was in their diet. However, the levels of bromide determined were insignificant in regard to FDA tolerance levels.

Kelly et al. (1968) measured the bromide levels of fresh pork from the coastal area (Smithfield, Virginia) and from the interior midwest (Greensburg, Indiana). The bromide content in pork from the midwest (0-109.5 ppm) was higher than pork from coastal areas (0-78.1 ppm). These workers also discovered that fumigation of cured hams with methyl bromide to control pest infestation resulted in bromide residue levels far above FDA tolerance levels of 125 ppm (173.16 ppm).

Leonard et al. (1970), in an unpublished work, fed four wethers diets ad libitum containing 0, 487, 1,020 and 1,542 ppm bromide as NaBr

for twenty-eight days. They found that lambs other than the control exhibited labored breathing and became lethargic after one week. The lamb on the 1,542 ppm bromide diet became very weak and after 17 days had stopped eating completely. These workers also noted that a pronounced increase in bromide levels was produced in all tissues, with the more metabolically active tissues having the highest bromide residue.

Methods of Detecting Methyl Bromide in Foodstuffs and Animal Tissues

There are several different methods for detecting qualitative and quantitative amounts of methyl bromide in foodstuffs and biological tissues. These methods differ considerably in sensitivity, repeatability and time required to analyze a sample. Kolthoff and Yutzy (1937) used a chemical method which would detect bromide in the presence of considerable chloride. The bromide was oxidized to bromate and determined iodometrically. Geddes and Fehberg (1938) published a method applicable to water soluble bromine in brominated flour. In 1942, Shrader et al. reported on a method for the determination of bromide residues which has been widely used to the present day. They determined the bromide residue attributable to methyl bromide fumigation was the difference between total bromide and inorganic bromide. This conclusion was based on the fact that methyl bromide may escape upon airing or may decompose into inorganic bromide. Since the latter cannot escape, the total bromide should decrease toward a limit while the inorganic bromide should increase toward the same limit. This type of analysis is accurate to less than 10 ppm. Depending upon the amount of equipment and help

available, the time required to run a small number of samples is approximately 48 hours. Results vary with different experienced groups of people using the same method. Mapes and Shrader (1957) modified this method to give better accuracy and repeatability but did little to increase the speed of analysis.

In 1952, Rieman and Lindenbaum used ion-exchange chromatography to detect bromide in the presence of much chloride with good repeatable results. Charin (1954), Helmkamp et al. (1954), and Stern and Shurachman (1958) reported on the use of potentiometric titration for the measurement of chloride ions. However, researchers have had difficulty applying this analytical tool to another member of the halogen family, bromine. Pflaum et al. (1962) reported making an electrode for detecting bromide residues via potentiometric titration. He detected levels as low as 10 ppm; however, more work is needed to prove the reliability of this method. Prokopov (1970) proposed a potentiometric titration method for the detection of bromide. This method looks promising but lacks the speed of other analytical methods. Gutenmann et al. (1961) reported using the modified Schoniger combustion method for determining bromide residues. They detected less than 10 ppm but repeatability was inconsistent. Beckman and Bevenue (1963) and Berck (1965) reported on the use of gas-liquid chromatography for the detection of microamounts of bromide residues in crops fumigated with bromide compounds. This method showed good accuracy and repeatability but was also time consuming. Beckman et al. (1967) reported a polarographic analytical procedure which gives specific and quantitative responses based on the bromide content of food crops. Improved extraction, ashing and oxidizing

systems were used to improve recovery and reliability. However, to analyze a large number of samples with this method would be too time consuming.

Chasson (1968) reported using routine serum profiling to detect bromides in the blood. A quick visual glance at the serum electrolytes is the only work required to detect brominism. Although this is strictly a qualitative and not a quantitative test, it is excellent for fast detection of brominism and the appropriate therapy can be undertaken rapidly.

Dunton (1968) reported using X-ray fluorescence for detecting total bromide in brines. This method determines bromide concentrations from 1 to 1000 ppm in about one minute per sample with an accuracy comparable to that of usual chemical methods.

Neutron Activation Analysis for Detecting Bromide Residues

A Brief History of Neutron Activation Analysis. In 1896, Antoine Henri Becquerel discovered the existence of elements which spontaneously emit invisible, penetrating rays (radioactive elements), Fermi (1952). In 1898, Pierre and Marie Curie discovered radium, and Ernest Rutherford discovered the minute but heavy nucleus which forms the core of the atom, Fermi (1952). This core has been determined to be stable in ordinary elements but unstable in radioactive elements. The radioactivity emitted by these unstable elements was considered to be a type of energy manifestation entirely beyond control. However, in 1933, while bombarding aluminum with α-particles, Joliot and Curie (1934) succeeded in producing radioactivity via artificial means. In their experiment, a nuclear

transformation of aluminum occurred with an accompanying emission of neutrons. The work of Joliot and Curie, along with discoveries by other scientists in the early thirties, prepared the foundation for James Chadwick, while studying the disintegration of light atomic nuclei by α-particles in 1934, to discover the neutron, Tolansky (1956).

Neutrons and protons are the building blocks of nuclei of atoms. Protons carry a positive charge, while neutrons have no charge. The neutrality of neutrons enables them to penetrate matter very easily.

Shortly after the discovery of the neutron, Bohr (1936) postulated a theory on the mechanism of disintegration. He proposed that a nuclear interaction depicts a nuclear particle coming close enough to a nucleus to unite with it to form a new compound nucleus. In the transformation, the new nucleus is unstable and must break up and change to a more stable state. As a result, energy is released by the reaction and radioactive atoms are produced.

Activation analysis is a technique by which a stable isotope of an element is made radioactive through reaction with a nuclear particle such as a neutron, Lyon (1964). The resulting radionuclides are measured by various methods for the induced radioactivity. This measurement enables a researcher to determine the amount of the stable isotope originally in the sample.

Kamen (1951) defined activation analysis as a nuclear reaction analogous to an ordinary chemical reaction. It exhibits a mass change (heat or reaction), an energy of activation, and it proceeds at various reaction rates which are dependent upon the experimental conditions. The primary advantage of neutron activation analysis is its extreme

sensitivity for detecting and measuring certain elements, and its insensitivity for others.

In most cases, light elements (C,N,O) in biological tissues do not become irradiated when bombarded by neutrons. Thus, these substances do not interfere with the measurement of the heavier (Na, K, etc.) elements that are activated.

Neutron activation allows the measurement of most elements heavier than neon (At.Wt. 20.183) with sensitivities up to 10^{-15}g. The actual limits of detection depend upon the neutron flux and the measuring equipment available.

A second advantage of NAA is the possibility of measuring several elements simultaneously. This characteristic enabled Battye et al. (1967) to study the interrelation between trace elements, and their change in concentration with age, sex, etc.

Modern instrumental techniques have made many activation analyses completely nondestructive. This characteristic is important in the "in vivo" studies in man, Taylor et al. (1967). The measurement of the sample's radioactivity can be determined by either beta radiation or gamma radiation detection. The beta radiations emitted by the isolated radioisotope can be measured either by Geiger-Mueller tubes, or liquid-scintillation-counting techniques, Price (1964). The gamma-ray emission is usually measured by gamma-ray scintillation spectrometry, Price (1964).

Problems and Restrictions Encountered When Using Neutron Activation Analysis. There are major interference problems concerning activation analysis. The first concerns the poor resolution of most gamma-ray

detectors due to overlapping of photopeaks of various elements from competing reactions in the same sample.

The second problem occurs when one or more isotopes of a substance have a yield of gamma rays much higher than the trace element being measured. The most notorious element causing this problem in biological tissue studies is ^{24}Na.

A third problem concerns the huge capital outlay in the range of several millions of dollars, necessary to purchase an activation source and a measuring device.

Some restrictions which must be considered before an activation analysis can be undertaken are:

1. The natural abundance of the stable isotope of the element being irradiated
2. The intensity (flux) of the nuclear-particle source
3. The magnitude of competitive nuclear reactions interference

The procedure for activation analysis involves:

1. The selection of the nuclear reaction
2. Preparation of the sample
3. Irradiation of the sample
4. Measurement of the sample's radioactivity
5. Interpretation of data

Step one is usually determined by the nuclear source that is available. Most activation analyses use neutrons to bombard the stable isotope.

Step two can be the most difficult in the whole analysis, especially when studying biological materials, due to interference problems. This

step is usually handled by chemically removing the substance causing the interference, such as ^{24}Na in biological tissues.

There are various nuclear particles which can be used in step three to activate a stable isotope. These are neutrons, protons, photons, deuterons, and alpha particles. The half-life of the induced radionuclide can range from a few microseconds to millions of years. The most widely used nuclear-particle to initiate an activation analysis is the neutron, Leddicotte (1968).

Application of Neutron Activation Analysis. The concentration of many of the various elements in animal and plant tissues is often minute. The phenomenal growth of scientific technology during the past thirty years has led to the development of many new analytical tools. Many of these new methods (mass spectroscopy, potentiometric titrations, gas chromatography, etc.) have been used to gather data which are more accurate and reproducible than data from older techniques. Elementary NAA was used by Hevesy and Levi (1938). This new type of analysis was very similar to current types of neutron activation analyses. King et al. (1939) reported the detection of several hundredths of 1% copper in pure silver. Atchison and Beamer (1956) used a van de Graaff Accelerator to detect known bromide concentrations with less than 3% error. They noted that the presence of any halogen interferes with the detection of another member of the family and that sodium interferes with bromide detection. However, these interferences can be corrected accurately. Bowen (1959) used activation analysis for the determination of chloride, iodide, and bromide concentrations in biological materials. These analyses involved chemical separation and

gave excellent accuracy, repeatability and recovery limits. Guinn and Wagner (1960) introduced a system of purely instrumental neutron activation analysis in which no chemical operations are employed. Bromide concentrations as low as 1.4 ppm were detected. They eliminated interferences by using brief irradiation periods, followed by rapid counting. This technique produced a spectrum in which the short-lived activities developed in the samples were accentuated. Guinn and Potter (1962) used NAA to determine total bromide residues in agricultural crops sprayed with various organic bromo-compounds. They noted that bromide levels as low as 1 ppm were detectable in crop samples and 0.01 ppm in extracts. The method was rapid, non-destructive and accurate. Lediocotte (1968) stated that 90 of the elements can be detected and measured accurately in concentrations as low as 10^{-6} to 10^{-15} g in biological materials. Further work concerning the use of neutron activation analysis to measure bromide residues in tissues and foods has been done by Castro and Schmitt (1962), Anders (1962), Ross (1964), Yule (1966), Samsahl (1967), Shenberg et al. (1967) and others. All of these workers agreed that NAA allowed greater sensitivities in the measurement of residues, and showed unusually low contamination of the element being studied by foreign elements. Therefore, it would seem that such an analytical tool would be invaluable in studying trace elements in animal and plant tissues.

The doors have not been opened fully in many areas of study for the use of neutron activation analysis. This is probably because potential users of this analytical tool are not aware of the possibilities available to them.

EXPERIMENTAL PROCEDURE

Preparation of Fumigation Chamber

Methyl bromide is noninjurious to most materials requiring fumigation; however, utmost care should be utilized with this highly toxic gas fumigant and any type of fumigation chamber used must be airtight. In this experiment, a 55 gallon metal drum was used as the fumigation chamber. Nine inch pieces of pipe were placed through the drum 6 inches from the top and bottom. The piece of pipe at the top extended 3 inches into the drum. This enabled a polyethylene bag, in which the feed to be fumigated was placed, to be secured to the pipe stem inside the barrel and thereby receive the fumigant. The lower pipe extended 2 inches into the barrel. The polyethylene bag was secured around this stem to allow the gas to escape after fumigation. A valve was placed on the outside of the drum on the lower piece of pipe to bleed off the methyl bromide at the end of the fumigation process. A second polyethylene bag was placed over the sealed drum just prior to fumigation and taped down with 3 inch masking tape to further guard against gas leakage.

Fumigation of the Rat Diets

Two-hundred and fifty pounds of a high quality, 4% fat, rat diet were purchased from the Dublin Animal Laboratories in Dublin, Virginia. Fifty pounds of this diet were placed in a polyethylene bag and set in the fumigation chamber. The plastic bag was sealed to the two pipe

stems with masking tape, and the drum was shut and sealed. One pound of DOW methyl bromide gas was admitted into the chamber via plastic tubing run through the upper pipe stem. The feed was fumigated for 24 hours. The barrel was rotated for 10 minutes every six hours to provide equal exposure of the diet to the fumigant. The diet was then aerated for 72 hours. After aeration, the fumigated diet contained 3807 ppm residual bromide as measured by neutron activation analysis.

Mixing of the Experimental Diets

Neutron activation analysis of the fumigated and nonfumigated diets yielded 3807 ppm and 36 ppm, respectively. To obtain treatment levels of 290 ppm, 600 ppm, and 1150 ppm, the fumigated feed was mixed with the nonfumigated feed as shown in Table 2.

The mixed diets were sampled and irradiated to determine the final levels in the diets. The bromide residues found in the three treatment feeds were 290.10 ppm for the lower treatment, 601.00 for the intermediate level and 1177.1 for the highest treatment level. Eighteen thousand one hundred sixty gram lots were chosen because this is sufficient feed to sustain 12 rats ad libidum for 56 days.

The Cage Distribution of the Experimental Rats

The randomization of the placement of the rats in a battery to study growth rate, habits, etc., is important because it minimizes the effect of light, temperature and position from interfering with statistical analyses. Sixty-eight male rats were divided into two experimental groups. Experiment 1 contained 48 rats which were divided

Table 2. Mixing of the Treatment Diets

Type of Feed	Treatment A	Treatment B	Treatment C
Residual Bromine (ppm)	290	600	1,177
Control Feed (gms)	16,920.5	15,436.0	13,120.6
Fumigated Feed (gms)	1,239.5	2,724.0	5,493.4
	18,160.0	18,160.0	18,160.0

into 12 output groups of four animals each. All animals in each output group were from the same litter; therefore, twelve litters were represented. Caution was taken to assure that no rat from the same litter was placed on the same treatment in a different output group. This step eliminated variation among output groups due to litter effect.

Each output group in Experiment 1 contained four rats from the same litter. A control diet and three treatment diets were designated as : Control = 1, 290 ppm = 2, 601 ppm = 3, 1177 ppm = 4. Table 3 shows the result of this distribution. The numbers in the treatment diets represent the residual bromide levels in ppm. The position of each animal in each output group of cages was selected at random from the number 1 - 4.

A 60-cage stainless steel battery was used to house the rats. The battery was divided into 2 sides, which contained 5 tiers of 6 cages each. Experiment 1 utilized the inner 20 cages on each side, leaving the 5 cages running down each end of each side for Experiment 2. The remaining eight rats in Experiment 1 were placed in a separate battery following the same design.

The test rats in Experiment 1 were a disease resistant strain that ranged from 27.5 to 45.0 gms in weight, avg. 32.18 gms. These rats were placed in each output group by litters and their weights were recorded. All rats in both Experiments 1 and 2 were fed ad libidum, and daily feed intake was measured. Their water was changed every two days and their body weights were recorded every three days. The cages and glassware were cleaned weekly and

Table 3. Distribution of Animals in Each Output Group

Output Group Sequence	1	6	2	5	4	8	10	9	7	11	3	12
Cage Sequence	3	1	2	1	1	1	2	4	3	4	2	4
In Each	1	4	4	2	4	3	1	2	1	3	1	3
Output Group	2	2	3	4	3	4	4	1	4	2	4	2

litter paper was changed every two days. The animals were sacrificed at the end of eight weeks.

Experiment 2 was designed to study the effect of the diet with the highest level of residual bromide (1177 ppm) on rats for a period of eight weeks. There were 20 rats in this study divided into four output groups (A-D) of five each. No output group contained more than one rat from the same litter. The same method of care was devoted to the animals in this study as Experiment 1. However, these animals were sacrificed by groups at 14 day intervals; A - 14 days, B - 28 days, C - 42 days, D - 56 days.

Tissue Sample Preparation for Analysis of Bromide Residues

Animals used for Experiment 1 were sacrificed at the end of 56 days and the following samples were removed for study; blood, liver, kidney, testes, spleen, triceps muscle, gastrocnemius muscle, eye ball and stem of optic nerve, lung and bronchii, abdominal fat and heart muscle. The whole organs of the heart, liver, kidneys, testes and lung were weighed immediately upon removal. All samples were wrapped in aluminum foil and frozen at 0°C immediately after dissection.

The frozen tissues from one animal at a time were thawed and representative samples of each type of tissue were taken. Visible fat was removed from all tissues, and the connective tissue surrounding the testes was discarded. After weighing, all samples were placed in polyethylene vials in which the sample would be irradiated. Samples were run in duplicate except for the liver, which was run in triplicate. The testes, kidneys and eyes were placed intact in the vials (one/vial).

All other tissues were dissected to obtain duplicate samples. The liver and blood were the only samples which were not totally analyzed. This was due to the limited size of the vials. Whole blood samples were used instead of dividing the liquid into plasma and serum fractions. The liver was dissected into its four lobes, and representative slices were removed from each lobe and placed in the same vial for analysis. This step was to insure as much representation of the different metabolically active sections of the liver as possible. The lung tissue was not separated from the bronchii. The bone sample was the tibia bone. It was removed from each animal just below the caudal extremity of the tibia and slightly above the distal end. All tissues were removed from the bone and it was split just below the nutrient foramen. The caudal extremity and distal end were placed in separate vials. A sample of fat from both sides of the abdominal cavity was represented in each vial. The heart muscle was divided into the right and left halves. Fat was removed from the coronary sulcus and discarded. All tissues were thawed and prepared the night before they were analyzed to prevent tissue deterioration in the vials prior to analysis. The effect of tissue breakdown in the vials would not alter the residual bromide content, but gases formed in the breakdown processes could cause the sealed vials to rupture around the top. This would allow seepage of tissue fluids from the vial and thereby alter the sample weight, which must be accurate for neutron activation analysis.

Activation of Samples

Samples were prepared approximately 12 hours prior to activation. They were placed in a pneumatic tube and fired from an analysis lab, approximately 107 feet from the reactor, into the core of the reactor. It took the samples four seconds to travel this distance. A set of standards was prepared which contained 671.45 ppm bromide. A standard was irradiated prior to each group of animal tissues. The rat tissue samples were divided into two irradiation groups, 30 and 60 seconds. The blood, liver, testes, kidneys and bone were irradiated for 30 seconds. The remaining tissues (spleen, triceps muscle, gastrocnemius muscle, eyes, lungs, fat and heart muscle) were activated for 60 seconds. Thirty and sixty second irradiation times were found to be sufficient to yield good gamma-ray emissions for detection and resolution in the lithium-germanium detector used.

Sample number 1 of the fumigated feed was irradiated for ten minutes and was too hot (2R) to handle safely and analyze immediately. The time required for this sample to cool to be placed in a detection apparatus was critical because the short-lived bromine-80 isotope was chosen for detection. This isotope has a half-life of 18 minutes. The sample irradiated for 10 minutes had to cool for a few days before it could be analyzed. By this time, the bromine-80 had decayed and was not measurable. For this reason, 30 and 60 second irradiation times were tested and found to yield excellent results.

The natural abundance of bromine is divided into the following masses; bromine - 79 (50.52%) and bromine - 81 (49.48%). Bromine 79

was picked to measure the residual bromide because a shorter activation time was required to yield good resolution and detection. The decrease in the irradiation time allowed faster processing of the eighteen hundred samples without sacrificing accuracy in results.

Activated samples were removed from the reactor and placed in a lithium-germanium detector to measure the gamma-ray emissions from the activated bromide. This detector stored the information (gamma-ray emissions) and punched the data out automatically onto IBM punch cards. These cards were collected, sorted, labeled and placed in the proper order to be analyzed by an IBM 360 computer.

Computer Program Used to Determine Bromine Residues in Samples

A computer program was designed to place the analyses on the following ratio:

$$\frac{\text{gamma-ray emissions of X}}{\text{gamma-ray emissions of std.}} = \frac{\text{weight of X}}{\text{weight of std.}}$$

The printout gave the results as ppm residual bromine. The computer program used in this work is shown in Appendix A.

RESULTS AND DISCUSSION

A preliminary study was conducted to determine the best available method for detecting bromide residues. A potentiometric titration procedure was studied and proved to be inaccurate. The problem was thought to be the instability of the electrode. The Shrader et al. (1942) technique is one of the oldest and most widely used methods for determining bromide residues in organic materials. An experiment was conducted to test this method against a relatively new analytical tool (NAA) and found the Shrader technique to be more time consuming and to differ with results obtained by the newer method.

Table 4 shows the results of an experiment which determined the residual bromide of the 4 diets used in Experiment 1 by both NAA and the Shrader method. A large difference between the results of the two methods was noted, if one considers that the USDA bromide tolerance in meat products for human consumption is 125 ppm. A comparison of the results obtained for treatment diets B and C by NAA and the Shrader analysis shows a discrepancy of 121 and 3 3 ppm, respectively. If USDA tolerance limits are to be taken seriously, more research must be undertaken to determine which of these methods is the most reliable. The variation in results may be attributed to contamination of the different samples in the muffle furnace or impurities in the chemicals used in the Shrader method. There is less error in the NAA analysis because there is no chemical preparation of the samples and, provided the nuclear reactor and detection equipment function properly, there are fewer steps required to obtain results, which also lower the human

Table 4. Bromide Residues in Diets as Determined By NAA and the Shrader Technique

Type of Feed	Neutron Activation Analysis (ppm)			Shrader Technique (ppm)		
	Range	Average	S.D.	Range	Average	S.D.
Control	35.23-36.95	36'	0.88	107-121	114	9.90
Treatment A	271.41-298.62	290'	12.98	330-376	349	32.53
Treatment B	589.4-612.4	600	16.26	451-504	477	37.48
Treatment C	1165.7-1188.6	1177	16.19	746-831	793	60.10

'Average of triplicate samples, all other samples run in duplicate

error factor. The NAA method is also approximately 30 times faster than the older method. For these reasons, NAA was chosen as the method to be used for the determination of the residual bromide both in the fumigated diets and in the tissues of the rats fed treated feeds.

Growth and Mortality

The growth period was 8 weeks for the rats in Experiment 1. Throughout this period the rats on each diet showed no signs of intoxication, exhibited normal behavior patterns and appeared in excellent condition. Each rat was weighed every three days. Growth rate records show conclusively that the rats on the three fumigated diets performed as well as the rats on the control diets. Table 5 shows the growth data obtained from the rats in Experiment 1. There were no statistically significant differences between the control animals and the three different treatment groups. However, treatment A had a higher feed efficiency than treatment C, and the rats gained an average of 13.85 g more than the rats on treatment C.

Three rats from Experiment 1 died during the test. Two of these rats were on treatment A (290 ppm) for 7 and 10 days, respectively, before death. The other animal was on treatment B (600 ppm) and died after seven days on this diet. The rats were examined post-mortem by a veterinarian and the cause of death was diagnosed as pneumonia. Bacterial cultures yielded a hemolytic Streptococcus from the lung and liver of each rat. The significance of the streptococci is open to conjecture. The incidence of infection was higher in two of the three treatment groups than in the control group (0). However, the good

Table 5. Mean Growth Data From Experiment 1

Group	No. Animals In Group	Feed Eaten (gms)	Gms Gain	Feed Efficiency	Bromine Consumed mg/day
Control	12	928.17	243.5	3.85	0.62
Treatment A	10	953.49	251.39	3.79	4.90
Treatment B	11	946.76	250.14	3.81	10.16
Treatment C	12	938.17	238.54	3.99	19.72

performance of the remainder of the rats on the treatment diets combined with the early deaths of the three rats and the low bromide levels in their diets indicates that the bromide in the diets did not contribute to their demise.

One rat from Experiment 2 died after 13 days on treatment C from a massive bacterial meningitis. Streptococcus sp. and Staphylococcus sp. were isolated from the liver and lungs of this animal. Again, as in Experiment 1, the remainder of the rat colony in Experiment 2 performed normally and appeared healthy for the duration of the experiment. Therefore, the fumigated diet does not seem to have been the cause of death.

Organ Weights

The rats from Experiment 1 were sacrificed after eight weeks and Table 6 shows weights obtained on the liver, kidney, spleen, lungs, heart and testes. A statistical analysis revealed no significant difference in the values obtained from the control animals versus the treatment animals. However, Table 7 shows a statistically significant trend in Experiment 2 for the spleen to enlarge and then decline in weight between 42 to 56 days by 0.2 gms. This can probably be attributed to animal variation rather than the high level of residual bromides in the diet (1177 ppm), since none of the other metabolically active organs from animals in Experiment 2 followed this pattern. The changes in weights between the other treatment groups of tissues were not statistically significant.

Table 6. Organ Weights of Rats in Experiment 1

Diet	No. Samples	Days On Diet	Organ Weights (gms)					
			Heart	Liver	Kidney	Testes	Lung	Spleen
Control	12	56	0.91	10.10	1.79	2.75	1.40	0.57
Treatment A	10	56	0.92	10.41	1.85	2.81	1.33	0.55
Treatment B	11	56	0.97	10.22	1.82	2.75	1.34	0.55
Treatment C	12	56	0.87	9.75	1.79	2.68	1.39	0.58

Table 7. Organ Weights of Rats in Experiment 2

Group	No. Samples	Days On Diet	Organ Weights (gms)				
			Heart	Liver	Spleen	Kidney	Testes
1	4	14	0.54	4.44	0.45	1.20	1.13
2	5	28	0.82	7.73	0.68[a]	1.73	2.20
3	5	42	0.77	10.47	0.69[a]	1.93	2.55
4	5	56	0.85	10.79	0.49	1.92	2.72

[a] $P < .01$ for groups 2 and 3 versus 4

Tissue Analyses

At the end of the growth period for Experiment 1 of this study, the blood, liver, testes, kidney, bone, spleen, triceps and gastrocnemius muscles, eyes, lungs, abdominal fat and heart were analyzed for total bromide by a NAA procedure described by Furr (1970). The results of this work are summarized in Tables 8 and 9. It is evident that there is a significant difference between the control animals and all three treatment levels. A consistent linear relationship exists between the level of bromide contained in the feed and found in the treated tissues. As the residual bromide in the feed increased, the tissue bromide levels also increased. These results agree with previous studies by Spencer et al. (1944) if only the control diets are compared. The results of this study showed bromide levels in much higher concentrations in the tissues of rats which were on diets lower in residual bromide than studies by previous workers. The bromide levels reported by Spencer et al. (1944) were considered low at the time their study was conducted. However, newer restrictions on the levels of bromide acceptable by the USDA have made these findings quite significant and the bromide levels not "quite low" as reported, but rather high. Dudley et al. (1940), Flinn (1941) and Rosenblum et al. (1960) reported that bromide feeding experiments caused little or no deleterious effects on experimental animals. This work supports their findings; however, another question needs to be considered. If lower levels of bromide in the diets in this experiment yielded higher residues in the tissues than previously reported, should more care be taken in adding bromide

Table 8. Bromide Content of 30-Second Irradiated Rat Tissues in Experiment 1

Means[1] of Bromide Levels						
Diet	No. Animals/ Group	ppm Br/gm Blood	ppm Br in desiccated Tissue			
			Liver	Testes	Kidney	Bone
Control	12	11.43	6.17	12.87	10.94	14.29
Treatment A	10	208.77	91.40	176.66	139.31	82.28
Treatment B	11	372.30	175.92	333.04	292.12	164.57
Treatment C	12	631.08	304.45	610.62	527.93	343.83

[1]All means significantly different at $P < .01$ treatment

Table 9. Bromide Content of 60-Second Irradiated Rat Tissues in Experiment 1

Means[1] of Bromide Levels								
Diet	No. Animals/ Group	ppm Br in desiccated tissue Spleen	Triceps	Gastrocnemius	Eyes	Lung	Fat	Heart
Control	12	11.93	8.18	5.84	16.09	15.71	2.50	14.77
Treatment A	10	179.86	59.60	52.02	251.02	218.59	26.79	106.48
Treatment B	11	319.07	108.53	103.07	492.38	416.15	57.16	211.92
Treatment C	12	541.27	178.78	179.53	856.83	648.18	99.30	359.72

[1]All treatment means significantly different at $P < .01$

containing compounds to crops which meat producing animals consume? Possibly there is an ulterior approach to this question in that the USDA tolerance limits should be raised for bromide residues in meats and meat products. Current national consumer sentiments concerning the increased use of pesticides or increased tolerance limits would refute this idea because the users of these man made chemicals have applied them to such an extent that today's generation and generations of the future will feel the repercussions. Bohannon et al. (1969) stated that the most important fact to remember about pesticide pollution is that it is injected into the environment by man in a premeditated manner. The medical implications of pesticide residues in food products have forced the Food and Drug Administration to initiate extensive residue research on the effects of chemical control of pests. The consumer's protest in recent years led by Ralph Nader and Rachel Carson, has also applied pressure on the government to limit the extensive application of pesticides on food commodities. However, with the banning of DDT and other major pesticides, which have been shown to cause ecological damage, a need has arisen for something to fill this void in insect control. One possible approach could be finding new or proving older pesticides to be safe. The results of this work and work by other researchers indicate that the bromide residue levels in foods could be higher than the present limitations without ill effects. In 1955, Winteringham studied the effects of sodium bromide on 70 adult humans. He fed them approximately 4375 mg of NaBr per week and noted the average bromide contents of the blood and urine increased significantly during the test period. However, these levels dropped

rapidly on cessation of the bromide treatments, and no significant physiological or neurological effects were detected. A similar study concerning residents of Africa was also reported in this paper by Winteringham. The same results were reported. These experiments indicate the need for further research to validate his findings. If high levels of sodium bromide can be consumed by humans for the periods of time Winteringham (1958) reports, with no discernible ill effects, surely a closer look should be made at the established bromide levels allowed in foods by the F.D.A.

The same method of analysis on the tissue samples of Experiment 1 for residual bromide was used in Experiment 2. All four groups in Experiment 2 were fed the same diet containing 1177 ppm residual bromide. The groups were slaughtered at 2-week intervals to observe the bromide accumulations in the tissues over an eight-week period. The results of this study are shown in Table 10. An analysis of variance and Duncan's multiple range test revealed significantly higher levels of bromide in the kidneys of group 2 (on the diet for two weeks) than the other three groups. The same analysis detected a significant difference in the residual bromide levels of the gastrocnemius muscle between groups 2 and 4. Although statistical tests revealed no significant difference between treatment means of the other tissues, all tissues except the testes reached their highest concentrations of bromide when the rats were on the diet for two to four weeks. Apparently, the bromide levels in the tissues are building to a maximum tolerance level, and then being either removed from the body or possibly transferred by the

Table 10. Bromide Content of 30 and 60 Second Irradiated Rat Tissues in Experiment 2

Means of Bromide Levels												
				ppm Br in desiccated tissue								
Diet	Group	Time On Diet	ppm Br/gm Blood*	Liver*	Testes*	Kidney*	Spleen	Triceps	Gastroc	Eyes	Lung	Heart
C	1	14	712.83	332.23	501.70	501.98	662.13	318.15	234.90	1531.07	675.90	424.20
C	2	28	671.53	307.46	558.26	556.66[a]	683.44	327.80	318.74[b]	2252.69	748.34	423.14
C	3	42	620.10	299.10	571.24	470.36	662.48	259.68	238.68	2154.42	696.98	356.02
C	4	56	663.66	292.84	496.22	470.06	518.84	216.00	182.34[b]	850.00	664.78	355.72

*30-second irradiated samples, all others irradiated 60 seconds

[a]Group 2 treatment mean significantly different from other 3 treatment means at $P < .05$

[b]Group 2 treatment mean is significantly different from group 4 treatment mean at $P < .05$

blood to another organ. Bromide balance studies using labeled bromide might help to answer this question.

No work was done in an attempt to correlate an increase or bromide concentrations in the tissues with a decrease in concentrations of other members of the halogen family. Spencer et al. (1944) reported a chloride/bromide ratio determined in rats fed bromide fumigated feeds. These researchers noted no difference in this ratio in any tissues from rats ingesting the same quantity of bromide in the food, except the brain. The ratio did decrease significantly between treatment groups. The higher the bromide in the feed, the lower the chloride/bromide ratio.

It is also apparent from the data in Tables 8, 9 and 10 that the more metabolically active the tissue, i.e., eye > blood > lung > spleen, etc., the more bromide tends to accumulate. The most notable data are those reported for the eyes. In Experiment 1 the highest bromide residues detected in the eyes were 856.83 ppm. The figures for Experiment 2 are even higher. Possible explanations for these accumulations are bromination of β-carotenes as they are transported through the blood to the eye; accumulation of bromides in the ocular fluids; or a build up of bromide salts around the tear ducts as moisture evaporates. If the bromide is affecting the animals sight through these residue levels, it may explain the lethargic actions reported by Rosenblum et al. (1958) in his dogs. The fact that the eyes had these high levels of bromide residues may warrant further studies of this tissue in larger experimental animals, i.e., sheep, hogs, etc. This would enable the eye to be dissected into its component parts and analyzed

more thoroughly. The larger eye size from a species other than the rat would also enable the researcher to study this tissue under the electron microscope to observe any histological abnormalities which could possibly be attributed to these high bromide residue levels.

There also is a trend for the bromide to concentrate in lean meat rather than fat. A comparison of the muscle and fat values in Table 9 supports this concept also noted by Kelly and Graham (1968). The data collected on the residue levels in the testes may warrant further studies to determine the bromide effects on reproductive functions. The results of this study are in agreement with findings by Spencer et al. (1944), Rosenblum et al. (1960), Dudley et al. (1940) and others concerning the general health of the animals. As in previous studies, the animals in this experiment showed no signs of bromide intoxication, nutritional deficiencies and made normal weight gains. Dudley et al. (1940) conclude: "It appears that such amounts of bromide as encountered in foods fumigated with methyl bromide, under commercial conditions, would, on the basis of our evidence, probably not contain sufficient quantities of bromide residues to produce deleterious effects. For this reason, milled cereal products fumigated with one pound of methyl bromide per 1000 cubic feet will probably offer no risk when such food is consumed." Fitzhugh (1955) stated the amount of a substance for use in a food should show no chronic toxicity to animals in an amount equivalent to 100 times the amount which is proposed for use in the total human diet. Although this study did not use feed levels of this magnitude, levels approximately 10 times the maximum level of bromides allowed in meat products were fed and showed no detrimental effects.

This study was undertaken to determine where the residual bromides in feeds are deposited when ingested by rats. The study was not designed to determine what compounds or states in which the bromides were bound. However, these data show that when levels much lower than those used by other workers were fed, significantly higher levels than previously reported were detected in the tissues examined. These data also point out the need for further research concerning the tolerance limits that should be allowed in food products, to examine this discrepancy. Work is needed to determine a chronic toxic level for bromides in rats. Although this work concurs with previous researchers on the health of the animals, there appears to be a need for further research to determine if these high levels are detrimental to certain vital functions in food producing species. Does the bromide affect visual acuity, and thereby cause the animal to act lethargic? Would the bromide levels found in the fat and lean affect the fat-to-lean ratio in meat producing animals? Would the high levels of bromides found in the testes affect fertility? Only further research in these areas can answer these questions.

SUMMARY

A preliminary study was undertaken to determine a rapid method of analysis for bromide residues occurring in tissues of animals fed methyl bromide. Neutron activation analysis was chosen because it was faster, more precise and had a lower standard deviation between samples than other methods considered. A computer program was devised to measure the data collected as ppm.

Sixty-eight rats were divided into two experimental groups of 48 and 20 rats, respectively. Experiment 1 was divided into 12 groups of 4 rats each. Each group contained 1 rat on either a non-fumigated control diet or one of each of the three treatment diets. The three treatment diets were fumigated with methyl bromide and contained 290, 601, and 1177 ppm residual bromide, respectively. The rats in Experiment 1 were paired according to weight and litter, and no rats from the same litter were in more than one of the 12 groups. These rats were raised for 56 days and then sacrificed. There were no significant differences in growth rate or organ weights between treatments; however, a significant difference in the bromide levels found in the tissues between treatments was noted. The eye was noted as containing the highest level of residual bromides.

Experiment 2 was designed to study the 20 remaining animals' response to treatment C (1177 ppm Br), and to determine the bromide build-up in the tissues every two weeks. The 20 rats were divided into four groups of five rats each. These groups were sacrificed every two weeks for eight weeks. There was no significant difference

between groups in Experiment 2 concerning growth rate, general health and condition. However, two factors of importance were noted in this trial. When the organ weights were statistically analyzed, a significant loss in weight was detected in the spleens between the groups sacrificed after six weeks (0.69 gm) and eight weeks (0.49 gm) on the treatment diet. Tissue analysis for bromides revealed a statistically significant difference between the levels found in the kidneys of rats on the diet for two weeks when compared to the other three groups. The gastrocnemius muscle was also significantly higher in bromides in the two-week group than the four-week group.

In the tissue analyses for bromide residues, it appears that the higher the metabolic activity of the organ analyzed, the greater the bromide accumulation. The inference of these residues is not discernible at this time. This work has opened new doors for further research in this area, in view of the fact that there is a good possibility such high residues, as were reported, may alter some body metabolic functions. Further research will possibly answer these questions.

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APPENDICES

APPENDIX A

COMPUTER PROGRAM

```
C
      DIMENSION CHAN(210),ID(2),CORRCT(210)
      ITER=1
C     THIS STATEMENT SETS THE UPPER LIMIT ON THE NO. OF CHANNELS READ
   11 READ(5,13)IDECAY,WTSAMP
   13 FORMAT(I3,F8.0)
C     THIS STATEMENT READS THE DECAY TIME AND SAMPLE WEIGHT
      READ(5,1,END=100)(ID(I),I=1,2),(CHAN(I),I=188,209)
    1 FORMAT (A4,A2,52X,2F6.0/10X,10F6.0/10X,10F6.0)
      DECAYT=IDECAY
C     THIS STATEMENT SETS ALL VALUES =0
      II=0
      PEAK=0.0
      ADDCH=0.0
      SUMCH=0.0
      ADDCOR=0.0
C     THIS STATEMENT DETECTS ABNORMAL DRIFT IN THE PEAK
      DO 2 I=191,197
C     THESE STATEMENTS DETERMINE WHERE THE PEAK IS LOCATED AND ITS VALUE
      IF(II.EQ.197)GO TO 6
      IF(CHAN(I).LT.CHAN(I+1))GO TO 2
      IF(CHAN(I).GT.PEAK)GO TO 19
      GO TO 2
   19 PEAK=CHAN(I)
      II=I
    2 CONTINUE
      WRITE(6,4)PEAK,II
C     THESE STATEMENTS SET BOUNDARIES FOR DETERMINING THE PEAK AREA
C     THESE STATEMENTS DETERMINE CORRECTED CHANNEL COUNTS AND THEIR SUM
```

```
C                              APPENDIX A (CONT.)
C
C
     4 FORMAT(1H ,'PEAK=',F8.2,'CHANNEL=',I3)
       IF(II.EQ.193)GO TO 17
       IF(II.EQ.194)GO TO 15
       IF(II.EQ.195)GO TO 15
       IF(II.EQ.196)GO TO 18
C      THESE STATEMENTS DETERMINE PPM RESIDUES IN THE TISSUES
    16 DO 8 J=N,M
       IF(J.GT.NS.AND.J.LT.NM)GO TO 8
       SUMCH=SUMCH+CHAN(J)
     8 CONTINUE
       X=SUMCH/4.0
       DO 9 K=NS,NM
       CORRCT(K)=CHAN(K)-X
       ADDCOR=ADDCOR+CORRCT(K)
       ADDCH=ADDCH+CHAN(K)
     9 SQRTCH=SQRT(ADDCH)
       WRITE(6,10)(ID(I),I=1,2),ADDCOR,ADDCH,SQRTCH
    10 FORMAT(1H ,'IDENTIFICATION=',A4,A2,/
      1' ADDITION OF CORRECTED COUNTS=',F7.0,/
      1' ADDITION OF UNCORRECTED CHANNEL POINTS=',F7.0,/
      1' SQRT OF UNCORRECTED CHANNEL SUM=',F7.0)
       IF(WTSAMP.LT.600.0)GO TO 12
       STDCCS=ADDCOR
       ITER=ITER+1
       STDDT=DECAYT
       GO TO 11
    12 A=ADDCOR/STDCCS
       B=0.0385*((DECAYT-STDDT)/60.0)
       PPM=(A*671.45*EXP(B))/WTSAMP
```

```
C
C
C
      WRITE(6,14)PPM
   14 FORMAT(1H ,'PARTS PER MILLION=',F12.3,////)
      GO TO 11
    6 WRITE(6,7)
    7 FORMAT(1H ,'ABNORMAL DRIFT')
      GO TO 11
   15 N=191
      M=198
      NS=192
      NM=197
      GO TO 16
   17 N=190
      M=198
      NS=191
      NM=196
      GO TO 16
   18 N=192
      M=200
      NS=193
      NM=199
      GO TO 16
  100 STOP
      END
```

APPENDIX B

BROMIDE LEVELS DETECTED IN TISSUES* FROM RATS ON EXPERIMENT 1.

Output Group	Diet[a] Fed	Tissue Analyzed for Bromide Residues (ppm)						
		Blood	Liver	Testes	Kidney	Bone	Spleen	Triceps
1	Control	13.1	2.1	9.5	7.5	5.2	7.7	7.1
1	A	184.2	70.2	166.3	117.4	74.6	140.6	40.8
1	B	365.8	134.1	332.2	317.8	182.7	324.6	77.1
1	C	608.5	257.0	609.3	513.1	336.4	495.4	169.2
2	Control	9.2	4.9	10.8	11.2	22.1	17.4	13.4
2	A	191.6	89.3	171.2	143.0	80.0	160.8	33.4
2	B	371.7	180.9	348.0	358.7	203.0	329.0	94.1
2	C	623.8	433.4	596.9	600.0	308.3	507.8	160.3
3	Control	11.9	13.3	8.5	17.1	7.8	8.0	10.0
3	B	443.3	178.3	394.0	272.2	40.3	393.5	146.3
3	C	685.2	280.3	579.8	357.5	521.7	820.3	174.2
4	Control	12.3	2.0	15.0	8.4	18.5	7.3	5.7
4	A	194.1	67.6	158.9	132.1	68.2	138.0	49.0
4	C	672.8	324.4	675.8	623.5	379.6	499.3	141.3
5	Control	8.9	8.9	16.8	4.3	10.8	8.1	6.5
5	B	372.3	176.1	346.2	260.8	161.3	270.0	90.5
5	C	650.2	371.8	635.1	589.0	325.5	590.3	169.6
6	Control	12.9	4.9	23.3	11.6	9.8	12.7	9.6
6	A	204.6	126.6	175.2	152.0	53.6	128.9	38.9
6	B	382.7	179.0	330.7	283.4	186.6	266.4	128.9
6	C	603.1	269.4	617.1	506.6	367.6	461.6	221.7
7	Control	13.3	8.7	13.3	12.0	20.6	11.9	6.1
7	A	197.6	71.6	179.6	127.2	75.0	164.4	77.7
7	B	346.6	142.3	289.8	244.3	184.0	240.8	100.7
7	C	630.9	294.8	645.1	568.7	325.4	545.2	154.4
8	Control	6.1	3.2	9.4	9.9	12.7	10.8	3.2

continued

Appendix B continued

Output Group	Diet[a] Fed	Tissue Analyzed for Bromide Residues (ppm)						
		Blood	Liver	Testes	Kidney	Bone	Spleen	Triceps
8	A	220.6	98.5	188.1	157.0	99.7	162.0	61.5
8	B	376.2	166.1	215.4	268.3	159.0	393.8	143.8
8	C	611.7	314.7	552.6	577.6	301.6	576.9	196.1
9	Control	10.3	6.4	12.7	11.2	12.6	8.8	2.3
9	A	210.0	75.0	172.0	159.7	79.0	159.7	79.0
9	B	401.1	187.9	344.5	266.1	179.9	344.8	116.1
9	C	593.3	298.2	601.5	510.5	339.1	491.2	212.7
10	Control	13.1	3.2	13.6	9.4	13.8	17.6	12.3
10	A	192.9	63.0	155.6	147.4	78.2	122.2	57.2
10	B	397.1	177.1	327.7	333.7	139.2	283.0	110.8
10	C	603.6	273.2	584.9	500.0	339.4	523.8	174.7
11	Control	13.0	5.7	8.5	15.8	9.6	19.1	10.0
11	A	219.0	82.0	183.4	137.7	108.3	144.1	39.2
11	B	216.5	172.2	321.9	280.1	163.3	324.1	83.2
11	C	634.3	248.3	586.5	490.1	297.0	510.9	194.2
12	Control	13.0	10.7	13.0	12.9	28.0	13.8	12.0
12	A	225.7	151.7	194.0	173.3	98.9	362.7	117.3
12	B	412.7	243.8	396.5	299.3	202.3	369.2	119.2
12	C	655.5	287.9	642.8	498.5	284.4	472.5	176.9

*All tissues samples were run in duplicate and averaged

[a]A = treatment diet with 290 ppm residual bromide
B = treatment diet with 601 ppm residual bromide
C = treatment diet with 1177 ppm residual bromide

APPENDIX C

BROMIDE LEVELS DETECTED IN TISSUES* FROM RATS ON EXPERIMENT 1

Output Group	Diet[a] Fed	Tissue Analyzed For Bromide Residues (ppm)				
		Gastrocnemius	Eyes	Lung	Fat	Heart
1	Control	5.0	22.7	12.6	2.3	6.0
1	A	42.9	205.0	189.2	21.3	89.3
1	B	101.1	439.0	384.6	47.8	221.0
1	C	181.6	703.3	494.0	73.7	349.9
2	Control	1.9	10.3	10.9	3.4	6.1
2	A	48.1	191.1	217.8	21.3	96.1
2	B	83.7	536.7	388.5	68.0	190.5
2	C	170.8	805.6	577.6	81.9	319.8
3	Control	4.2	28.9	15.2	1.0	8.6
3	B	104.0	564.2	439.8	35.9	206.6
3	C	247.3	1096.9	802.2	86.4	511.1
4	Control	5.0	21.6	15.2	1.5	9.9
4	A	44.6	253.4	208.8	25.9	96.8
4	C	151.9	963.0	713.6	58.4	264.6
5	Control	2.6	6.6	17.1	1.7	4.7
5	B	76.0	464.1	366.5	51.1	194.7
5	C	149.3	843.8	668.3	143.2	310.6
6	Control	2.3	4.6	14.8	1.0	8.0
6	A	56.6	200.5	197.7	21.4	88.1
6	B	84.7	133.5	417.7	61.9	185.5
6	C	214.3	918.1	593.9	114.4	378.6
7	Control	8.7	15.6	14.5	2.2	10.3
7	A	42.5	237.2	216.2	20.9	99.9
7	B	91.7	452.0	382.1	39.6	210.1
7	C	204.5	878.3	579.7	99.6	384.9
8	Control	4.1	7.1	19.4	2.5	9.1

continued

APPENDIX C continued

Output Group	Diet[a] Fed	Tissue Analyzed For Bromide Residues (ppm) Gastrocnemius	Eyes	Lung	Fat	Heart
8	A	52.5	253.0	212.0	24.1	101.1
8	B	134.6	667.0	544.1	88.2	265.6
8	C	140.2	763.6	732.1	132.6	342.2
9	Control	3.0	15.1	14.0	2.1	8.2
9	A	57.7	297.5	205.9	27.1	118.5
9	B	124.8	551.6	359.4	55.5	211.7
9	C	178.6	933.0	684.7	91.1	323.4
10	Control	3.9	4.2	17.7	5.4	9.7
10	A	53.9	153.4	187.0	14.4	81.5
10	B	108.6	452.2	344.3	55.7	192.1
10	C	157.9	874.0	542.7	95.8	357.1
11	Control	2.3	26.4	17.2	3.9	11.8
11	A	48.2	206.?	197.9	18.9	102.2
11	B	98.0	526.7	458.7	74.7	170.4
11	C	177.8	918.9	650.6	93.7	380.4
12	Control	6.0	30.0	19.9	3.0	12.5
12	A	71.0	421.6	303.7	72.1	170.1
12	B	138.5	591.2	473.6	64.6	319.5
12	C	180.1	583.5	738.8	120.8	394.0

*All tissues samples were run in duplicate and averaged

[a]A = treatment diet with 290 ppm residual bromide
B = treatment diet with 601 ppm residual bromide
C = treatment diet with 1177 ppm residual bromide

APPENDIX D

ESTIMATES OF THE MEANS AND STANDARD ERROR FOR EFFECT DUE TO OUTPUT GROUPS[1]

Output[2] Group	Estimated Mean (ppm)	Estimated Standard Error
1	292.9	16.1
2	299.1	16.1
3	347.8	18.9
4	315.2	18.8
5	311.4	18.9
6	300.8	16.1
7	297.1	16.1
8	303.7	16.1
9	303.7	16.1
10	301.7	16.1
11	270.7	16.1
12	326.7	16.1

[1]These figures are the averages of the bromide residues found in the blood of the 4 rats in each output group. This set of data shows the lowS.E. between output groups.

[2]Each output group contained 1 rat on a control diet and each of the 3 treatment diets.

APPENDIX E

ANALYSIS OF VARIANCE FOR EFFECT DUE TO TREATMENTS AND OUTPUT GROUPS[1]

Source	D.F.	Sum of Squares	Mean Square	F-Statistic
Output Group	11	13451.8	1222.9	1.18
Treatments	3	2444537.3	814845.8	784.07[b]
Residual	30	31177.7	1039.3	

[1]This is blood data and clearly shows that there is no statistically significant difference due to output groups.

[b]This data shows definite statistically significant difference ($P < .05$) due to treatment effect.

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BROMIDE RESIDUES IN TISSUES FROM RATS MAINTAINED ON DIETS FUMIGATED WITH METHYL BROMIDE

Julian H. Williford, Jr.

Abstract

The effect(s) of bromide residues in rat diets on tissue bromide accumulations, organ size, growth rate and physical activity were studied. In Experiment 1, the treatment groups were fed diets containing 290, 600 and 1177 ppm bromide, respectively. The control group received 36 ppm from untreated feed. There were 12 rats on each diet for 56 days. Experiment 2 was divided into 4 groups of 5 rats each, and were fed a diet containing 1177 ppm bromide. Five representative animals were sacrificed every 2 weeks.

Weights of the liver, spleen, lungs, heart and testes were recorded for all animals when sacrificed. Tissue samples from these organs and the triceps, gastrocnemius muscle, eyes, abdominal fat, blood and tibia were analyzed by neutron activation analysis (NAA) for bromide residues. A computer program was designed to convert the NAA data to ppm bromide. There was a significant difference in the levels of bromide in the tissues between all treatments in both experiments. The most notable results were the high residual bromide levels found in the eyes (856 ppm) and testes (610 ppm). Bromides were detected in the other tissues in the following levels as ppm: blood (631), liver (304), kidneys (610), tibia (343), spleen (541), triceps

(178), gastrocnemius (179), lungs (648), abdominal fat (99) and heart (359).

No significant differences in organ sizes, feed efficiency and rate of gain between animals in Experiment 1 or 2 were found.