The Alleged Safety of Monosodium Glutamate (MSG)
Excerpted from MSG: A Review of the Literature and Critique of Industry Sponsored Research. Copyright © January 30, 1991, Revised July 1, 1991
by Adrienne Samuels, Ph.D.

The toxic action of glutamic acid (glutamate or GLU) was first reported by Lucas and Newhouse in 1957(26). Prior to that time, and throughout the 1960s, a considerable body of research had focused on potential positive or curative effects of various forms of GLU used as a drug. During this period "side effects" of GLU were noted, but no one considered that these "side effects" might be toxic reactions to GLU.

Hypothalamic Lesions: Non-Primates

In 1968, someone in Olney's lab observed that mice treated with GLU for the purpose of studying retinal degeneration became grotesquely obese, and Olney became suspicious that the obesity in mice, which was observed after neonatal mice were treated with GLU for purposes of inducing and studying retinal pathology, might be associated with **hypothalamic** lesions caused by GLU treatment, and in 1969 he first reported that GLU treatment did indeed cause brain lesions, particularly acute neuronal necrosis in several regions of the developing brain of neonatal mice, and acute lesions in the brains of adult mice given 5 to 7 mg/g of GLU subcutaneously(59).

Research which followed confirmed that GLU, which was usually given as MSG, induces hypothalamic damage when given to immature animals after either subcutaneous (60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81) or oral (67,73,74,76,82,83,84,85,86) doses.

Work by Lemkey-Johnston and Reynolds(86) published in 1974 included an extensive review of the data on brain lesions in mice. They confirmed the phenomenon of GLU induced neurotoxicity, described the sequence of the lesions, and emphasized the critical aspects of species variation, developmental age, route of administration, time of examination of brain material after insult, and thoroughness of tissue sampling methods. A review of GLU induced neurotoxicity, published by Olney in 1976(87) mentioned species (immature mice, rats, rabbits, guinea pigs, chicks, and rhesus monkeys) demonstrating GLU induced neurotoxicity and efficiency of both oral and subcutaneous administration of GLU in producing acute neuronal necrosis, discussed the nature and extent of the damage done by GLU administration and the impact of GLU administration to GLU levels in both brain and blood, and discussed the similar neurotoxic effects of a variety of acidic structural analogues.

Hypothalamic Lesions: Non-Human Primates

Studies of non-human primates (61,74) were felt to be particularly meaningful to the study of GLU toxicity, particularly because GLU toxicity found in laboratory animals might be relevant to humans. As early as 1969(61) Olney had suggested that GLU could be involved in the unexplained brain damage syndromes occurring in the course of human ontogenesis. Olney(61)

demonstrated that the infant rhesus monkey (Macaca mulatta) is susceptible to GLU-induced brain damage when administered a high dose (2.7g GLU/kg of body weight) subcutaneously.

Olney et al.(74) expanded Olney's earlier work(61) with a study of eight additional infant rhesus monkeys and, using light microscopy and the electron microscope, reconfirmed Olney's earlier findings(61) of hypothalamic lesions, and discussed the findings of both Abraham et al.(75) and Reynolds et al.(88) who had questioned his work. Olney found his data to be entirely consistent with studies done previously by his own and other laboratories on all species of animals tested.

Neuroendocrine Disorders

Olney(59) found not only hypothalamic lesions in 1969, but described stunted skeletal development, obesity, and female sterility, as well as a spate of observed pathological changes found in several brain regions associated with endocrine function in maturing mice which had been given GLU as neonates.

Longitudinal studies in which neonatal/infant animals were given doses of GLU and then observed over a period of time before being sacrificed for brain examination, repeatedly supported Olney's(59) early findings of abnormal development, behavioral aberration, and neuroendocrine disorders.

Developmental dysfunction or abnormalities in growth and behavior have been noted in a number of animal studies. Animals treated with GLU as neonates or in the first 12 days of life suffer neuroendocrine disturbances including obesity and stunting, abnormalities of the reproductive system, and underdevelopment of certain endocrine glands (59,68,70,86,89,90,91,92, 93,94,95, 96,97,98,99,100,101,102,103,104,105,106) and possible learning deficits either immediately or in later life (92,95,96,107,108,109,110,111,112,113).

In addition, Bhagavan and others have reported behavioral reactions including somnolence and seizures (114,115,116,117,118,119,120,121); tail automutilation (94,108); and learned taste aversion(110). Irritability to touch was interpreted as conspicuous emotional change by Nemeroff(94). Lynch(14) reported hyperglycemia along with growth suppression. He noted that hyperglycemia did not occur when subjects were given intact protein containing a large amount of GLU.

Olney et al.(122,123,124) have written a number of review articles which summarize the data on neuroendocrine dysfunction following GLU treatment. Nemeroff 125) has provided another.

Focus on Ad Libitum Feeding

Findings of neurotoxicity and neuroendocrine dysfunction in laboratory animals, following GLU administration, raised questions about the effects which GLU might have on humans. One such possible effect was GLU involvement with as yet unexplained brain damage syndromes. Since it would be unthinkable to administer doses of GLU to humans which might produce the same sorts of neurotoxicity and neuroendocrine dysfunction as found in laboratory animals, researchers had no alternative but to make decisions based on the best of the animal studies. "Best," in this case, would be studies which would most closely parallel the true human condition. A seemingly logical first step would be to study the effects of GLU on non-human

primates(126); and, as already noted, hypothalamic lesions were demonstrated in monkeys as early as 1969.(61) A seemingly logical second step would be to study what might be considered "normal" ingestion of GLU as opposed to some kind of forced feeding. It was felt by many that ad libitum feeding of laboratory animals parallels the human situation more closely than either subcutaneous or gavage administration of GLU, and that ad libitum feeding studies were, therefore, the vehicle of choice. Others tended to disagree, feeling that the ad libitum feeding studies were, by and large, studies which had the greatest potential for minimizing the amount of GLU actually ingested while registering the irrelevant amount of GLU **available**. These studies were largely industry-sponsored studies initiated and designed to "prove" that ad libitum feeding of GLU to laboratory animals did not result in the brain lesions and or neuroendocrine disorders found using other routes of administration.

Only two studies which demonstrate neurotoxic reactions after ad libitum feeding of GLU are reported here. Actually, one would expect few positive studies, because those who are employed by the food industry rarely, if ever, publish them, and no one else appeared to be interested in "proving" that GLU is, or is not, safe.

In a 1979 study by Vorhees(109), done as part of a project designed to evaluate a developmental test battery for neurobehavioral toxicity in rats (in which rats were exposed to GLU and other food additives mixed with ground Purina rat chow, beginning five days after arrival at the laboratory), it was demonstrated that high doses of dietary GLU produce behavioral variations. GLU was mixed with food as opposed to being administered subcutaneously or by gavage. Positive effects were found.

A year later, dietary studies reported by Olney(127) demonstrated that weanling mice will voluntarily ingest GLU (and/or aspartate) and that such voluntary ingestion results in readily detectable brain damage.

Focus on Older Animals

Most studies demonstrating retinal necrosis, brain lesions and/or neuroendocrine dysfunction, focused on neonatal or infant animals. The reason for this focus is simple. Researchers were primarily interested in producing lesions in order to expand their knowledge of brain function, and the lesions were most easily produced in the young. It was, however, also of scientific interest to understand the relationship of age to the type and severity of lesion or dysfunction. Thus, older animals were studied, but not to the same extent as the young.

Hypothalamic lesions have been produced in adult animals using considerably greater doses of GLU than those required to produce lesions in younger animals. Nemeroff(125) reported that the smallest effective dose for a ten day old mouse, given orally, is .5g/kg of body weight, and given subcutaneously is .35g/kg of body weight. According to Olney(128) the dose required to damage the adult rodent brain is given as 1.5-2 mg/g of body weight as compared to 0.3-0.5mg/g required to damage the brain of an infant rodent. Only minimal damage is induced unless very high doses (4-8 mg/g) are used(123).

Although advances in technology have facilitated the observation of brain lesions to some extent, it is still true today [1991], as it was in the 1960s, that simple light microscopes are adequate to

identifying GLU induced lesions if one looks in the right (sensitive) locations within 4-5 hours of GLU administration. By 24 hours after insult, lesions will be filled in ("healed") with cells, but the cells will be cells other than neurons. Thus the "hole" is filled in, but the lost **neurons** are not replaced. The damage will have been done, but will be virtually impossible to see. Although it is now [1991] **possible**, under optimal circumstances, to count neurons in **well defined** areas, the arcuate nucleus of the hypothalamus(129) is not a **well defined** area, and lesions in that area will defy detection after as little as 24 hours after GLU administration. One could not, therefore, ascertain whether or not an adult animal given GLU as an infant, had suffered a lesion in the arcuate nucleus.

Focus on Pregnant Females

There has been considerable interest in possible transplacental neurotoxicity of GLU, particularly on the part of food technologists who have attempted to demonstrate that GLU fed to a pregnant rodent has no adverse effects on its offspring. We have made no attempt to do a comprehensive review of the literature, but cite here only one study(130) which demonstrates that pregnant rats administered subcutaneous doses of GLU develop acute necrosis of the acetylcholinesterase-positive neurons in the area postrema. The same effect was obtained in the area postrema of fetal rats.

Industry defense of the safety of MSG: the alleged proof that GLU is safe

Understanding Statistics

As we move through our review of the research which purports to "prove" that GLU is safe for human consumption, we will demonstrate that food industry researchers generated animal studies in a purposeful effort to **fail** to reproduce findings of neurotoxicity and neuroendocrine dysfunction associated with administration of GLU to laboratory animals.

We start with a simple truth. The use of statistics is based on a mathematical model which requires, before a conclusion can be drawn, that the researcher first sets up a hypothesis which says that there is **no** difference between two (or more) conditions, and then **rejects** the hypothesis of no difference with a certain degree of confidence (confidence that the results of the experiment have not occurred by chance, and, therefore, would be reproduced if the experiment were replicated at another time.) Rejection of the "null" hypothesis is always associated with a value which specifies the probability with which the conclusion, that the difference did not occur by "chance," could be in error. Given two groups, where one is given a test material and one a placebo, if the two groups perform differently enough, we reject the hypothesis of no difference and conclude (at a particular level of confidence) that the difference in performance was not due to chance. We say that there is a significant difference between the two groups, and we state our level of confidence. The model allows us to conclude that there is a difference.

The model, however, does not allow us to **accept** the null hypothesis and conclude that there is **no** difference. "...Evidence that is consistent with a hypothesis can almost never be taken as conclusive grounds for accepting it, whereas evidence that is inconsistent with a hypothesis does provide grounds for rejecting it....The reason for not necessarily accepting consistent evidence is that a finding that is consistent with a hypothesis would be consistent with other hypotheses too,

and thus does not necessarily demonstrate the truth of the given hypothesis as opposed to these other alternatives....**An experimenter accordingly always sets up his experiment so that by rejecting his hypothesis he will prove what he has wished to prove**. To accept a hypothesis is to conclude that it **may be** true, but to reject it is to conclude that it is, without doubt, false" (21). Given the statistical model, rigorous demonstration of the truth of the null hypothesis is a logical impossibility (23).

Failure to find a statistically significant difference between groups may provide useful information for planning one's next experiment, but it "proves" nothing.

How the glutamate industry obfuscates with research design, methodology and interpretation

There are any number of methods that researchers can use to effect foregone conclusions if they so desire. In our analysis of studies which appear to have been designed to demonstrate that GLU is safe to use as a food additive, we have found it useful to classify studies according to the method(s) that were thus used.

In the scientific community at large, research is undertaken either to replicate findings, or alleged findings (in which case the experimental procedure of the second study is identical to the first), or research is undertaken in hopes of discovering something new, and, therein, providing insight into the phenomenon being studied (in which case the researcher varies one or more of the conditions of the experiment and looks to see what will happen). One would expect that as test conditions of a second study differ from the original, results would differ, too. If one did not present the same conditions in a second study, one would not expect to observe the same results as in the original. Some of the researchers who have set out to "prove" that GLU is safe, have **said** they were replicating studies, **but have not done so**. In these cases, the discussion is phrased to suggest that the study was a replication (which it was not) and the conclusions are based not on what was done, but on what was said would be done. In these cases, the (a) subjects, (b) test materials, (c) overall procedures, and/or (d) methods of analysis used differ from the study being "replicated." If it is maintained that these studies were truly honest attempts at replication, one would have to say that they were poorly designed, improperly executed, and inappropriately interpreted. In 1981, Nemeroff (125) stated unequivocally that "...not one single [primate] study has truly replicated the methods utilized by Olney, making evaluation of the available data impossible." Studies of this sort are designated Method A.

<u>True</u> failure to replicate casts doubt on the original study and is of great importance. <u>Pretending</u> to fail to replicate is tantamount to fraud.

Other studies, either purposefully, or in good faith and honest error, have focused on, or commented on, materials which have little or no importance for the study of, the understanding of, and/or the evaluation of central nervous system toxicity and/or peripheral adverse reactions to GLU. Most often these are studies of the relationship of what industry people like to point to as "objective" parameters, and the ingestion or assimilation of GLU. These objective parameters are observable phenomenon such as blood pressure, body temperature, plasma GLU level, brain GLU level, etc. To date, there has been considerable study of some of these variables, particularly plasma GLU levels. However, none have been shown to have a cause/effect

relationship to brain damage in laboratory animals, and none have been shown to be significantly related to the human adverse reactions produced by GLU. We call this Method B.

Unless GLU sensitive people are studied, one cannot legitimately draw conclusions about the relationship of the variables being studied (no matter how objective they are) to people who are sensitive to GLU. Often, these studies are used to allegedly "prove" that people who are not sensitive to GLU are not sensitive to GLU.

A third version of the general approach is to divorce results from discussion and conclusions. In some studies, although the body of research is generally well enough done, there is a marked disparity between the body of the research (including the result section) and the discussion and conclusions. Simply put, the abstract and/or summary do not follow from the results of the study. If the reader restricts himself to reading the conclusion, abstract and/or summary of the paper, (s)he will never note the disparity. This is Method C.

In addition to these basic strategies, from time-to-time industry-generated research tends to manipulate the English language in such a way as to foster confusion and/or obscure the meaning of what is being done. Words are manipulated so that imprecision of definition and vagueness of concepts expressed tend to obscure the real issues, and allow one to be led to faulty conclusions. This approach, which we shall refer to as Method D, is not to be confused with Method B where irrelevant variables (or levels thereof) are explicitly used. With Method D, details are given (or not given) in such a way as to create a wrong impression. We might think of this as false advertising. It has been observed that among those who purport to prove the safety of GLU, there are some who, in our opinion, challenge the integrity of science in this way: obscuring the levels of dosage used while it is well known that reaction to GLU is dose related (122,123,124,125); studying only subjects who have not experienced GLU sensitivity without making that clear to the reader; looking for lesions in the brain in areas which only experts realize are not susceptible to GLU lesions; and/or not specifying materials which are used in the placebo when those materials may cause allergic or sensitivity reactions to GLU or substances other than GLU, or may even contain GLU. When placebo materials have been manipulated in human studies, the method has been identified separately as Method F.

A fifth category (Method E) is reserved for studies that focus on levels of relevant variables that will almost certainly fail to produce the effect in question, and/or use methodology in evaluation of lesions which through inappropriate timing, focus, or instrumentation, will obscure otherwise verifiable results. The distinction between a significant level or method and one that is not significant is always a subtle one. Using Method E, researchers often look at the wrong thing, at the wrong time, and/or in the wrong place and conclude that GLU toxicity or sensitivity does not exist.

Industry challenges to the toxicity of GLU

Animal Studies: Lesions

As this was written, we had found no studies which contradicted the demonstration of retinal lesions produced by administration of GLU. There were, however, a number of studies which

challenged the findings of brain lesions and neuroendocrine dysfunction resulting from administration or ingestion of GLU. *Adamo and Ratner(131) and Oser et al.(132) failed to reproduce findings of neurotoxicity affecting the brains of non-primates. Adamo and Ratner(131) used rats, not mice as Olney(59) had, but maintained that otherwise the experimental approach used was "very similar." Oser et al.(132) studied mice, rats, and beagles. Although their methodology varied considerably from Olney's, they concluded that they could "...offer no explanation for the fact that [their]...observations...do not confirm those of Olney...." It would appear that they "thought" they were replicating what Olney had done.*

Arees and Mayer(64) reproduced Olney's(56,59,61) findings only in part. Their discussion focuses more on the question of human consumption of GLU as food than on reasons for differences between the various studies.

All three of these negative studies were refuted by both Olney(60,133) and Burde(73) who independently reviewed the literature and found that these early discrepancies could be attributed to:

1) Failure on the part of investigators to **attempt** to replicate Olney's methods (Method A); and

2) Use by investigators of entirely different (and inappropriate) methods of preservation and staining of brain tissue in the analysis of results (Methods A and E).

Burde(73) speculated that the method of fixation and staining used by Adamo and Ratner(131) obscured the existence of the lesion, and noted that their dose schedule was not appropriate; that Oser et al.(132) used a minimal effective dose and did not examine the rats and mice until 24 hours after insult, even though it was known that by 24 hours after insult, in a minimal dose such as the one used by Oser that would produce edema, all signs of edema would have disappeared and that necrotic cells would already have been phagocytized. Burde found the interpretation by Arees and Mayer(64) that the lesion produced by GLU is limited to "microglia," to be puzzling, particularly in light of the fact that most of the cells of the arcuate nucleus are known to be small neurons. Furthermore, using Olney's exact methods, Burde(73) replicated Olney's previous findings.

Olney's(60) review of the discrepancies, pointed out that the failure of Oser et al.(132) to detect brain damage in any of the three species they studied following administration of GLU might well be accounted for by their having limited the GLU dose to a single, minimally effective dosage; failure to use a feeding tube to assure that the full dose was received by orally treated animals; failure to examine brains in appropriate post treatment intervals (which are particularly relevant in cases of minimal effective dosage); and use of relatively unrefined techniques for tissue preparation.

Olney(60) also noted that in a 1971 study done by Arees et al.(58) the authors were able to demonstrate that neuronal degeneration does occur in the infant mouse brain following subcutaneous treatment with GLU. Thus the discrepancies noted by Arees and Mayer previously(64) became resolved.

Finally, Olney(60,133) suggested that methodological variables might well explain the failure of Adamo and Ratner(131) to demonstrate lesions in the rat.

The subject of tissue preparation (relevant at the time) has been addressed by a number of people. Takasaki(69) stated it clearly: "...changes disappeared at least 24...[hours] after injection....The results should be borne in mind when histological examination is performed on changes of the hypothalamus caused by administration with MSG. It is [especially] so in animals administered with a small dose of MSG, because necrotic neurons are few and the glial reaction that occurs secondarily is very mild in the AN [arcuate nucleus]. Without punctual preparation after administration, the effect upon the hypothalamus is apt to be overlooked in these animals"(69).

Olney(67,81,133,134) and Murakami(135) have discussed the problem in similar terms. Olney(67) has discussed such methodological problems in great detail.

In 1973, Filer and Stegink(136) published an editorial in the New England Journal of Medicine which suggested that the neurotoxic effects of GLU and its related amino acids, aspartate and cysteine, in species other than the mouse, are debatable. In turn, Olney et al.(137) pointed out that neurotoxic effects of GLU and its related amino acids had been well documented, and that the "null effect" reported by Filer and Stegink was a function of faulty methodology, not strain specificity--a fact which had been pointed out earlier by Burde(71,74). Olney noted that Filer and Stegink supported their argument by pointing to the "fact" that no neurotoxic effects of GLU had been reported in the guinea pig, which was, at the time, an unstudied species. Olney further reviewed the criticisms of his own research proffered by Filer and Stegink and suggested that a more careful reading of the research as presented would resolve their concerns.

There were other studies which failed to confirm toxic effects of GLU, and there were criticisms of Olney's work. Abraham,(75) mentioned earlier, found toxic effects when GLU administration was subcutaneous, but very little when administration was oral. His work is discussed in some detail in the section devoted to non-human primates.

Lowe(138) criticized Olney(61) for failure to provide data on plasma GLU concentrations, and for lack of a control in his single infant monkey study. Zavon(139) criticized Olney for lack of a control animal and for lack of detail in reporting the same study. Olney(140) responded to both Lowe and Zavon with detail gathered from mouse studies and an apology that he only had the one monkey available at the time of his study.

Blood, Oser, and White(141) criticized Olney(59) for questioning the safety of GLU after parenteral, as opposed to oral, administration (see the ad libitum studies); failure to clearly elucidate his methodology; and use of doses which far exceeded Blood et al.'s estimate of "...the total daily intake [of GLU] from all reasonably possible uses... (.7 g per day) in an average adult"(141).

"Critical tests for the safety evaluation of food additives are based on the effects of oral, not parenteral, administration," state Blood et al., leading one, possibly to infer that Olney considered his studies of mice to be "critical tests for safety," when in fact that was not true.

Olney has **never** suggested that his work be used in this way. It is one thing to report an observation, as Olney did. It is quite another to claim that it is a critical test for something. This seemingly purposeful creation of false information by innuendo is an illustration of use of Method D.

Olney(142), in reply to Blood et al.(141), provided the figures requested, suggested that he (Olney) had been misquoted, and said that to truly establish the safety of GLU if, indeed-that could be done, solid research was needed. (Sloppy oral or ad libitum studies would not provide the answers.)

Focus on Non-human Primates

Two studies took exception to Olney's finding of hypothalamic lesion in non-human primates due to loading of GLU. Abraham et al.(75) treated four monkeys and failed to reproduce the findings of Olney and Sharpe(61). Reynolds et al.(88,143,144) treated 16 non-human primates which were compared to five controls. They, too, failed to reproduce the findings of Olney and Sharpe(61), and found, instead, a "spectrum of degenerative changes" which they attributed to inadequate fixation procedures rather than to the effects of GLU.

Among the criticisms Olney(74) made of the research design and methodology of Abraham et al.(75) and Reynolds et al.(88,139,140) which distinguished his study from theirs, are the following:

> Reynolds et al. used only a spot sampling technique when two of the rhesus infants, each treated with low oral doses of GLU, were examined by electron microscopy, so the possible occurrence of small lesions in these brains was not actually ruled out (Method A).

The method used for preparation of brains for examination by light microscopy has been found unsatisfactory for evaluating even large GLU-induced lesions in infant rodent brains and subsequent information provided by Reynolds indicated that some of the infants vomited an unknown portion of the administered dose. (Reynolds' uses Methods A and E.)

Abraham et al.(75) supported their findings with a single light micrograph from a rhesus infant sacrificed 24 hours following oral intake of an emetic dose (4 g/kg of body weight) of GLU, although four monkeys were studied. Moreover, little or no evidence of lesion would be expected 24 hours after GLU insult because damaged elements are removed from the scene of an GLU-induced lesion with such remarkable efficiency, that 24 hours after insult, without pre- and post-insult comparison, it is virtually impossible to determine if damage has been done. In general, Abraham's work appears to be vulnerable to the criticisms of most Method A studies, in that he maintains that he is replicating work done by Olney, but does not do so. A careful comparison of the two studies will demonstrate that age of subject, dosage administered, time between insult and examination of tissue, and methods of tissue preparation all differ. Abraham's study can also be criticized for use of methodology known to be inappropriate for identifying GLU lesions (Method E). Finally, it was also noted by Nemeroff(125) that Abraham et al.(75) found in both control and GLU treated monkeys a "very small proportion of necrotic or damaged neuronal cells and oligodendrocytes...in the arcuate nuclear region of the hypothalamus." One would

suspect that this might happen if the placebo, as well as the test material, contained small amounts of an excitotoxin identical, or similar, to GLU.

Also failing to reproduce neurotoxicity in primates were studies of Abraham et al. (145) Newman et al.(146) and Stegink et al.(147). Stegink et al.(147) used the same data as Reynolds et al.(88,139,140) with two additional monkeys, and used the same methodology for tissue staining (Methods A and E). His work, therefore, is subject to the same criticisms as hers. *Abraham et al. stated that their present investigation was undertaken in an attempt to resolve some aspects of the controversy*. However, the details of this methodology were identical to those of their earlier study(75) and are subject to the same criticisms (Methods A and E). There would seem to have been no point to doing this study.

Newman et al.(146) found "...no evidence in any instance of any change that could be attributed to MSG as described by Olney and Sharpe, although there were artifacts in some inadequately fixed areas as recorded by Reynolds and her co-workers." The study gives every appearance of having been designed to facilitate the conclusion that GLU is a safe food. It uses both Method A and Method D. To quote, or paraphrase. (The bolding is critique added by this author):

> "Rhesus monkeys were maintained and observed in the primate buildings of HRC, where **most of them** were bred."
>
> The initial study was carried out with animals of 108, 99, 60, and 3 days, with **unspecified** histories.
>
> "The test solution was **readily** consumed voluntarily by all animals on all occasions throughout the study;"
>
> "The 3-day-old monkey had a **few** hypochromatic nuclei, and a **minimal** degree of vacuolation in the ventral hypothalamus, but **these findings were not regarded as significant**."
>
> "By electron microscopy, changes of the type reported by Olney and Sharpe were seen in both test and control animals, **and were attributed to fixation artefact**."

Information pertaining to the animals is incomplete. Their history is uncertain. We have no idea what went on during the first 108 days of their lives.

Description of both procedure and findings is highly subjective and/or incomplete (Method D). "**Readil**y" consumed does not necessarily mean **fully** consumed. If a "few" hypochromatic nuclei were quantified, how many would that be? What is "minimal?" On what basis were the findings "not regarded as significant?" What changes were seen? How **many** were test animals; and how **many** were controls?

A 1976 study by Reynolds et al.(148) which produced negative results relative to abnormalities of the subinfundibular region of the monkey brain provided yet another vehicle for allegedly "proving" that GLU is safe. Both mice and monkeys were studied. Mice, but not monkeys, were reported to show brain lesions. The monkeys were infant macaques with age ranging between 30 minutes and 14 days. It is of interest (and concern) to note that the cross section presented in

Figure 4 of "...a 7-day-old infant Macaca fascicularis monkey that ingested 4 g/kg GLU..." appears, in every aspect, to be identical to a section of an "...infant rhesus monkey which received 4 g/kg of GLU by stomach tube..." presented in Figure 3 of the report by Stegink et al.(147) The GLU in Reynolds et al. study was prepared as a 20% w/v solution in water and administered as a single dose of as much as 4 g/kg GLU. We are told how many monkeys received each dose, but we are not given dosage by age. The techniques for evaluation of mouse brains is the same used by Lemkey-Johnston and Reynolds(86) and Reynolds et al.(88) in previously reported studies. These had been found by Olney(74) to be inappropriate. No information is given about the timing involved or the techniques used for evaluation of monkey brains.

In general, this study by Reynolds et al.(148) employs Methods B and E. However, the conclusion, a gross overgeneralization, also makes use of Method D. *Reynolds concludes, "Neither aspartame nor MSG is **capable** of eliciting a lesion in the neonatal monkey brain*." (Emphasis added by this author.) In addition to the study's other faults, Reynolds et al. take a single finding of "...did not elicit..." and generalizes it to "...it is **incapable** of eliciting...." Those who do not read the entire study will be misled.

Neuroendocrine Disorders / Ad Libitum Feeding

The bulk of the studies dealing with neuroendocrine dysfunction were done in an obvious effort to discover and piece together bits of information which would help resolve the mysteries of the endocrine system. For most researchers, GLU was important not because of any importance in and of itself, but because its use produced certain effects in the body, and monitoring the relationships between administration of GLU, cell damage (particularly lesions) in various locations, and resultant changes in anatomy, physiology, and behavior elsewhere, might provide important clues to the secrets of human body function. As an excitotoxin, GLU has been used not only for its ablative effects, but also as a provocative tool(122,123). But here again, a number of studies were done to "prove" that as a food additive, GLU is safe. One of the favorite strategies appears to have been to examine those factors which cause the "unwanted" result--in this case, neuroendocrine disorders associated with intake of GLU--and design a study which focuses on, or makes use of, non-relevant levels of otherwise relevant variables, betting, or knowing, that the levels used will not produce the "unwanted" result. Thus, females exhibit reproductive disorders and males do not, use males. Or if a neuroendocrine change is not exhibited in less than 20 days, examine the animals after 15 days. Then, when no significant differences between control and experimental groups are found, conclude that GLU is safe to use in food. Only someone with intimate knowledge of the subject could discern manipulation of this kind.

While these sorts of studies might well be grouped with the Method A studies, they have a slightly different twist which sets them apart. The Method A studies give the **appearance** of attempting to replicate studies done already, while this new class of study makes no such pretense, but provides for the introduction of new variables. The logical fallacy in these studies comes when it is concluded that finding nothing while studying irrelevant variables "proves" that GLU is safe. This is Method B.

Most of these negative studies focused on ad libitum feeding. It would appear sensible to attempt to approximate the model of human ingestion of food in studying the safety of human ingestion

of GLU. And that's the stated purpose for the bulk of the studies presented. As Olney(127) pointed out, however, the ad libitum animal studies fall far short of approximating the human condition.

Almost all of the studies which focused on ad libitum feeding of GLU to laboratory animals were underwritten by the food industry, and have, predictably, negative results. Over and above the fact that given the statistical model used one cannot, through these studies, "prove" that GLU is safe, they are subject to the same range of criticisms as other industry sponsored studies.

The 1979 study by Matsuzawa et al.(99) will serve as an example. The authors did a series of studies using both neonatal and 10-day old rats, given oral and subcutaneous doses of GLU at a total of 4 different doses. Controls were given saline solution. One might legitimately question the precise nature of the "...ad libitum diet containing 5% (w/w) MSG...," but that is not of immediate importance. One must note, more importantly, that the ad libitum diet was given "...for 10 days after weaning (at 20 days)." By 1979, the date of the study, it was well understood that the timing used was outside of the range of the animal's most susceptible age.

The conclusion is classic for a study using both Methods D and E. "MSG therefore produces marked reproductive endocrine abnormalities after maturation **only** when injected parenterally early in postnatal life, in repeated, very large doses. The development of reproductive endocrine function is not affected by affected by MSG **unless** neurological damage occurs in the hypothalamus by any route of administration." (Emphasis added.)

Matsuzawa et al. have done one study, on one species, of a particular age, given a particular diet for 10 days, and conclude that because that one set of conditions did not elicit either neurological damage to the hypothalamus or **marked** endocrine abnormalities after maturation, that GLU produces marked reproductive endocrine abnormalities "...after maturation **only** when injected parenterally early in postnatal life, in repeated, very large doses." They exclude all other possibilities.

The identical strategy (Methods D and E) is found in a 1979 study by Takasaki et al.(98). They report that, "Adverse effects from MSG have **never** been reported from dietary administration." (Emphasis added by this author.) In this case, "never" equals four studies. Using logic similar to that used by Matsuzawa(99) they concluded that "MSG does not exert an adverse effect on somatic growth in that the hypothalamic neurons are not injured by any routes of administration, and the MSG did not induce somatic deficiency under the conditions of our experiments, which mimic the intended conditions of use of this material as a food additive."

In their 1979 summary of GLU toxicity in laboratory animals, Heywood and Worden(149) cite nine chronic animal studies in which various species were given ad libitum feedings of GLU over extended periods of time. These include studies by Ebert(150), Owen et al.(151,152), Semprini et al.(153), and Wen et al.(154). Because we have no data on chronic animal studies from persons other than those with close ties to industry and, therefore, have no records of positive results, we have no basis for evaluating the levels of variables used in these studies. And because they are incomplete and imprecise in detailing their methodology, it is difficult to evaluate the research as a whole. Ebert(150,155) used mice that were clearly older than Olney's mice(87). Ebert apparently used data from a 1953 study done at Arthur D. Little, Inc., entitled, "Report on a

study of L-monosodium glutamate, DL monosodium glutamate and L glutamic acid with respect to potential carcinogenicity." The 1970 report of these data(150) was in the form of an abstract. The 1979 reports(155,156) were expanded abstracts done, "...to comply with the suggestion of the Select Committee on GRAS Substances during hearings on glutamates, held at Bethesda, Maryland on July 25-27, 1977"(155). We know, of course, that these studies producing negative results and thereupon claiming to "prove" that GLU is a safe food additive, are subject to the limitations of the statistics that they use, and that from the point of view of the statistical model, any conclusion of safety based on failure to find a difference between two groups is an invalid one. We also know that the procedures of Wen et al.(154) are subject to the same criticisms(60,73,133) as studies by Adamo and Ratner(131).

In another 1979 summary of the results of dietary administration of GLU, Anantharaman(157) stated that studies indicated that "...dietary administration of MSG at even very high doses was not found to result in any of these symptoms [produced by other routes of administration], including the endocrine disturbances." They cited Huang(158), Wen(154), Takasaki(159), Bunyan (160), Owen(151), and Trentini(105). They also cited two year rat studies by Ebert(150), and Owen et al.(151) where no abnormalities were found in successive generations. And in their own study(157) they also produced negative results.

Studies by Owen(151), Takasaki (159), and Wen(154) have already been discussed in some detail. The additional studies mentioned here are, at the very least, subject to previously discussed statistical limitations.

The study reported by Anantharaman(157) must be criticized on more serious grounds. Unlike most of the research reported, Anantharaman provides a great deal of detail, including detail of the exact nature of the basal diet provided. And in that basal diet we note that "yeast food" is listed as a component of the protein (page 236, Table 3). At this point in time (1990), yeast food invariably contains either protease (which creates GLU during manufacture) or L-cysteine which produces neurotoxic effects somewhat different from, but more extensive than, the effects of GLU. We are suspicious, then, that the failure to find differences in growth of control and experimental groups **may be** due to the fact that both groups were receiving neurotoxic substances in their basal diet. We call this Method F. It is discussed in some detail in the section entitled "Methodology Particular to Human Research."

Using inappropriate placebo materials has been discussed by others before. In 1981, Rippere(161) criticized the use of common food allergens as placebo materials, noting that even a minute trace of an allergen might trigger severe symptoms in a sensitized individual. In a study by Abraham et at.(145) cited earlier, it was noted that the control group exhibited some small evidence of brain damage just as the experimental group did, raising a question of what placebo materials might have been used there. In 1990, this author questioned research done by Goldschmiedt, Redfern, and Feldman(162) which used beef broth as a placebo for controls. In the United States, one cannot purchase commercially prepared beef broth that does not contain some form of GLU (hydrolyzed protein, yeast extract, textured vegetable protein, flavoring, etc.) This author questioned the possible unwitting bias in placebo material in a letter to the editor of the *American Journal of Clinical Nutrition*. The letter was not published and no informative reply was received. The author questioned Dr. Feldman about the contents of the placebo. He

replied that he did not know the contents of the various materials used. Again, use of a potentially reactive placebo constitutes Method F.

A 1977 study by Heywood et al.(163) which focused on neurotoxicity, came to the same conclusion as Anantharaman. Heywood et al. concluded from **one** study of ad libitum feeding of GLU over a period of four days using 20-day old mice that, "There is indeed no evidence from any dietary study yet reported that would suggest a lack of safety of MSG as a food additive." (Method D.) It should be noted that details of the amounts of GLU consumed are not given. In the discussion where it states that "...dose levels as high as 45.5 g GLU/kg body weight were achieved...", we are not told if that is per day, per animal, or total. Nemeroff(125) noted that their study did not present representative histological micrographs for evaluation (149). (Method E.)

In a second 1979 report, Takasaki et al.(164) again reviewed a number of studies and this time reported that, among other things, "Weanling, pregnant, and lactating mice fed large amounts of MSG in the diet ... did not develop hypothalamic lesions." As evidence they cited studies by Semprini et al.(165), Huang et al.(158), Wen et al.(154), and Takasaki(159). In addition, they reported findings from their own research(164) that compared the effects of GLU fed ad libitum to other routes of administration. In their report, they build from a discussion of findings of brain lesions to relationships of lesions to plasma GLU levels, to relation of ad libitum dietary feeding to plasma GLU levels, to histological effects of ad libitum feeding of GLU, to the statement that "...plasma glutamate levels... remained much lower than those **required** to induce hypothalamic lesions." (Emphasis added by this author.) It must be understood that it has never been determined that any particular level of plasma GLU is **required** for the production of brain lesions. The logic used here is faulty, and potentially leads one to erroneous conclusions. Takasaki uses both Methods B and D.

Unfortunately, Takasaki(164) did not provide sufficient detail for one to evaluate the reports, and the reports-themselves are lacking. Again, it will be observed that Wen(154) appears to have used the same techniques as Adamo and Ratner(131) and Oser(132) which Olney(60,133) and Burde(73) criticized in 1971.

A study by Iwata(108) failed to find behavioral abnormalities as a function of ingestion of GLU. The study is limited by the same Method E design deficiencies noted in other studies. Iwata (108) does not examine the brains histologically, yet concludes that there had to be lesion damage prior to **there** being behavioral effects. The overgeneralization (Method D) from this study is that "...dietary administration... caused **no** behavioral latent effect in later life." (Emphasis added by this author.)

Prabhu et al.(166) failed to demonstrate differences in a battery of behavioral tests and drug applications. They mentioned that the results are based on surviving mice, but fail to state the mortality rate. (Method D.*) Lengvari(167) also reported no differences between control and experimental groups in a number of variables*. One must question the meaning of their failure to find a significant difference when they report a mortality rate of 45.1% (to day 30) as opposed to a 20% mortality rate for controls. (Method D.)

Related, but with a slightly different focus, are a pair of studies reported by Takasaki in 1979(84) and 1980(85) in which he studied the effect on brain lesions of administering various materials

simultaneously with GLU. Takasaki reported that certain mono- and disaccharides and arginine hydrochloride, leucine and the prior injection of insulin significantly reduced the number of necrotic neurons in the arcuate nucleus of the hypothalamus. In general, the detail provided about the study is incomplete, and the procedure is difficult to follow. (Method E.) It is not clear whether reduction in effect of GLU might have been due to inclusion of additional materials, thus diluting the test material. Moreover, statistics pertaining to the values for number of necrosed neurons observed appear to be based on analysis of **one** representative section from each animal. And values for representative brain sections appearing in Tables 1 and 2(82) have vastly different values (195 +/- 18 and 263 +/- 15) for what would appear should be the same thing. One is compelled to question the meaning of "representative" under these circumstances. (Method D.)

Although data found in the 1979 review of GLU toxicity in laboratory animals, done by Heywood and Worden(149) have already been discussed here, the review, in and of itself, is of interest for the way in which it develops the discussion of GLU toxicity, using Methods B and D. First, we are told that there is a classical toxicological approach to doing research on food additives. Second, findings of chronic animal studies are reported, arranged by species of animal tested. Nine studies by four authors are presented. Very little detail of procedure is given along with the results. And finally, the conclusion is drawn that "dietary administration of GLU in these conventional studies was found to be without significant toxic effect over the varying periods of administration."

Acute toxicity studies follow, again arranged by species. In this case, reports of lesions (or failure to find lesions) were accompanied by extensive discussion of plasma GLU levels, and some discussion of levels of GLU found in the brain. It will not be denied that the subject of plasma GLU levels is of interest in the study of GLU toxicity. But the tenor of the discussion, and in some instances the discussion itself, would have one assume or believe that toxic reactions can occur only **after** plasma GLU levels have become raised. The following quote from Heywood and Worden illustrates the point.

> *"A fourfold increase in the levels of glutamate in the arcuate nucleus of the hypothalamus **followed** the elevation of plasma glutamate after a single subcutaneous injection of MSG (reference Perez and Olney, 1972). Peak plasma levels occurred after 15 min, and peak levels in the arcuate nucleus were attained after 3 hr.* (Emphasis added.) *The results indicate that plasma concentrations above a certain level were necessary to induce brain lesions"(149).*

The logic which says that plasma concentrations above a certain level are necessary to induce brain lesions, is false. If the work of Perez and Olney(168) cited by Heywood and Worden(149) does, indeed, demonstrate that GLU levels in the arcuate nucleus of the hypothalamus are increased after plasma levels are raised following a single subcutaneous injection of GLU, it does nothing to demonstrate that this is the **only** condition under which GLU levels in the arcuate nucleus can be raised. Unlikely as it might seem, it might be discovered some day that **smelling** petrochemicals increases GLU levels in the arcuate nucleus, but has no effect on the blood. We have **no data** that tells us, as Heywood and Worden(149) would like us to believe, that "...plasma concentrations above a certain level were **necessary** to induce brain lesions." (Emphasis added.) It may or may not be true, but **we don't know.**

This would appear to be a purely specious argument. "Almost certainly" is not "certainly;" and cause and effect **cannot** be assumed from demonstration of concomitant variation. Moreover, the argument must be made because, as unjustified as the assumption of cause and effect may be, study after study produced by food industry researchers have cited lack of elevation of plasma GLU following ingestion of GLU as "proof" that GLU does not cause human **adverse reactions**. Starting with the assumption relevant to hypothalamic lesions drawn by Heywood and Worden, other researchers have designed studies to convince readers that under certain circumstances, (serving GLU with carbohydrate, for example), GLU ingested by humans could not possibly cause **adverse reactions**.

The logic is as follows: Heywood and Worden report that **brain lesions** can only occur when plasma GLU concentrations are above a certain level. Someone reports that plasma GLU levels are not increased when carbohydrates, for example, are served with GLU. Therefore, it follows that lesions **cannot** be induced when carbohydrates, for example, are served with GLU. It is further said to follow that **adverse reactions cannot** be induced when carbohydrates, for example, are served with GLU.

In some studies, these authors added useful data to the study of GLU, which helped define the parameters of variables that affect neuroendocrine disorders caused by the intake of GLU. Otherwise, they have no positive function or value. Even in their negativity, they did little or nothing to refute the existence of the body of research which was concerned with the neuroendocrine disorders associated with intake of GLU.

Focus on Pregnant Females

Stegink and others(169,170) have done a number of studies on the subject of transplacental neurotoxicity of GLU which purport to demonstrate that GLU is safe. We have made no attempt to review those studies in great detail, but those we have cursorily reviewed(169,170) have stipulated cause/effect relationships without demonstration of the same (Method B), and have made use of Method E. Neither lesions nor potentially abnormal behavior is studied in either of the papers cited. Observing increases and/or decreases in plasma GLU and/or other body fluids without observing concomitant variations in brain damage or other dysfunction has little meaning.

Putting it All in Perspective

This is not the first time that these data have been summarized and/or reviewed. Olney and Price(171) published an extensive review of GLU induced neuroendocrine disorders. They did not concern themselves with "controversy" but only with what was known to be true at the time. Olney(172) summarized the findings from the animal studies and warned of their potential relevance to humans -- particularly infants. Nemeroff(125) analyzed the same literature and summarized it much as this author, noting the findings of neurotoxicity and neuroendocrine dysfunction associated with GLU ingestion and the spate of negative results emanating from individuals and laboratories supported by research grants from food companies. Nemeroff, however, discussed findings in greater detail than we do. He also discussed neurotoxic effects in regions other than the hypothalamus, techniques appropriate to the evaluation of neurotoxicity,

neurochemical studies, and endocrine and other body fluid levels which we have not discussed here.

Nemeroff's review of the literature was both a review of the literature and a call for further research. In it, he repeatedly pointed to findings of neurotoxicity and neuroendocrine dysfunction and "wondered" at the sprinkling of negative findings associated with both sorts of research. He commented on each of the negative studies that he reviewed, explained wherein they differed from the findings of neurotoxicity and neuroendocrine dysfunction, and called for additional research. Nemeroff's call for further research was a call to resolve the contradictory findings.

Our review is a review of the literature and an **analysis** of the contradictory findings. We probed deeper than Nemeroff did. Our first discovery was that on statistical grounds alone, the claim of "proof" of safety of GLU is untenable. We observed that, with possible rare exception, the negative studies were initiated and carried out not to find out all there was to find out about sensitivity to GLU, but for the sole purpose of "proving" that GLU is a safe food. There are essentially **no published** industry sponsored studies that fail to make the point that GLU is safe. The exception, if there is one, will be found in the first study relating to GLU published by a given author. Sometimes the first study is positive or without negative comment, but if the author is industry supported, or industry-supported from that point forward, it doesn't happen twice. It is our opinion that industry supported researchers have manipulated variables and utilized inappropriate research design, methodology, and evaluation methods in a purposeful attempt to produce negative results. These studies and their results were then interpreted to be purposefully misleading.

Moreover, it has come to our attention that it might be the practice of some segments of the food industry to publish only those studies which show **no** effect of the variable being studied(173) (in this case GLU).

The detailed discussion of research on GLU induced neurotoxicity and GLU induced neuroendocrine disorders was included here to demonstrate the extremes to which the food industry, and/or its representatives, will go to "prove" that GLU is safe, not to demonstrate that neurotoxicity or neuroendocrine disorders exist. For now, in 1991, there is no question about the **fact** of GLU induced neurotoxicity and neuroendocrine disorder. It is generally accepted that there are differences in the nature and extent of neurotoxic reactions in different species, that only certain areas of the central nervous system and brain appear to be affected, that young animals are more easily affected than older animals, and that both dose and method of administration are important variables.

It is true that some studies of several animal species fed relatively small amounts of GLU over extended (but not extensive) periods of time, either by gavage (stomach tube) or in voluntarily ingested liquid or chow (as contrasted to single, much larger concentrations), beginning at various ages, have failed to demonstrate the same sorts of hypothalamic and/or retinal lesions or degeneration that are shown under different test conditions, and also failed to demonstrate the same sorts of abnormalities in growth and development.

However, while not all doses of GLU, given by all modes of administration, produce lesions in all brain areas, in animals of all ages, in all species, some doses of GLU **do** produce lesions in

specific areas of the brain when given via a number of modes of administration to animals of various ages and various species.

References

14. Lynch, J.F., Jr., Lewis, L.M., and Adkins, J.S. (Division of Nutrition, FDA, Washington, D.C. 20204). Monosodium glutamate induced hyperglycemia in weanling rats. J S Fed Proc 31: 1477, 1971.

21. Weinberg, G.H. and Schumaker, J.A. Statistics: An Intuitive Approach Belmont: Wadsworth, 1962.

23. Ferguson, G.A. Statistical Analysis in Psychology and Education New York: McGraw-Hill, 1959.

26. Lucas, D.R. and Newhouse, J. P. The toxic effect of sodium-L-glutamate on the inner layers of the retina. AMA Arch Ophthalmol 58: 193-201, 1957.

59. Olney, J.W. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164: 719-721, 1969.

60. Olney, J.W. Ho, O.L., and Rhee, V. Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res 14: 61-76, 1971.

61. Olney, J.W., and Sharpe, L.G. Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Science 166: 386-388, 1969.

62. Snapir, N., Robinzon, B., and Perek, M. Brain damage in the male domestic fowl treated with monosodium glutamate.Poult Sci 50:1511-1514, 1971.

63. Perez, V.J. and Olney, J.W. Accumulation of glutamic acid in the arcuate nucleus of the hypothalamus of the infant mouse following subcutaneous administration of monosodium glutamate. J Neurochem 19: 1777-1782, 1972.

64. Arees, E.A., and Mayer, J. Monosodium glutamate-nduced brain lesions: electron microscopic examination. Science 170: 549-550, 1970.

65. Arees, E.A., and Mayer, J. Monosodium glutamate-induced brain lesions in mice. Presented at the 47th Annual Meeting of American Association of Neuropathologists, Puerto Rico, June 25-27, 1971. J Neuropath Exp Neurol 31: 181, 1972. (Abstract)

66. Everly, J.L. Light microscopy examination of monosodium glutamate induced lesions in the brain of fetal and neonatal rats. Anat Rec 169: 312, 1971.

67. Olney, J.W. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. J Neuropathol Exp Neurol 30: 75-90, 1971.

68. Lamperti, A., and Blaha, G. The effects of neonatally-administered monosodium glutamate on the reproductive system of adult hamsters. Biol Reprod 14: 362-369, 1976.

69. Takasaki, Y. Studies on brain lesion by administration of monosodium L-glutamate to mice. I. Brain lesions in infant mice caused by administration of monosodium L-glutamate. Toxicology 9: 293-305, 1978.

70. Holzwarth-McBride, M.A., Hurst, E.M., and Knigge, K.M. Monosodium glutamate induced lesions of the arcuate nucleus. I. Endocrine deficiency and ultrastructure of the median eminence. Anat Rec 186: 185-196, 1976.

71. Holzwarth-McBride, M.A., Sladek, J.R., and Knigge, K.M. Monosodium glutamate induced lesions of the arcuate nucleus. II Fluorescence histochemistry of catecholamines. Anat Rec 186: 197-205, 1976.

72. Paull, W.K., and Lechan, R. The median eminence of mice with a MSG induced arcuate lesion. Anat Rec 180: 436, 1974.

73. Burde, R.M., Schainker, B., and Kayes, J. Acute effect of oral and subcutaneous administration of monosodium glutamate on the
arcuate nucleus of the hypothalamus in mice and rats. Nature(Lond) 233: 58-60, 1971.

74. Olney, J.W. Sharpe, L.G., Feigin, R.D. Glutamate-induced brain damage in infant primates. J Neuropathol Exp Neurol 31: 464-488, 1972.

75. Abraham, R., Doughtery, W., Goldberg, L., and Coulston, F. The response of the hypothalamus to high doses of monosodium glutamate in mice and monkeys: cytochemistry and ultrastructural study of lysosomal changes. Exp Mol Pathol 15: 43-60, 1971.

76. Burde, R.M., Schainker, B., and Kayes, J. Monosodium glutamate: necrosis of hypothalamic neurons in infant rats and mice following either oral or subcutaneous administration. J Neuropathol Exp Neurol 31: 181, 1972.

77. Robinzon, B., Snapir, N., and Perek, M. Age dependent sensitivity to monosodium glutamate inducing brain damage in the chicken. Poult Sci 53: 1539-1942, 1974.

78. Tafelski, T.J. Effects of monosodium glutamate on the neuroendocrine axis of the hamster. Anat Rec 184: 543-544, 1976.

79. Coulston, F. In: Report of NAS,NRC, Food Protection Subcommittee on Monosodium Glutamate. July, 1970. pp 24-25.

80. Inouye, M. and Murakami, U. Brain lesions and obesity in mouse offspring caused by maternal administration of monosodium glutamate during pregnancy. Congenital Anomalies 14: 77-83, 1974.

81. Olney, J.W., Rhee, V. and DeGubareff, T. Neurotoxic effects of glutamate on mouse area postrema. Brain Research 120: 151-157, 1977.

82. Olney, J.W., Ho, O.L. Brain damage in infant mice following oral intake of glutamate, aspartate or cystine. Nature(Lond) 227: 609-611, 1970.

83. Lemkey-Johnston, N., and Reynolds, W.A. Incidence and extent of brain lesions in mice following ingestion of monosodium glutamate (MSG). Anat Rec 172: 354, 1972.

84. Takasaki, Y. Protective effect of mono- and disaccharides on glutamate-induced brain damage in mice. Toxicol Lett4: 205-210, 1979.

85. Takasaki, Y. Protective effect of arginine, leucine, and preinjection of insulin on glutamate neurotoxicity in mice.Toxicol Lett 5: 39-44, 1980.

86. Lemkey-Johnston, N., and Reynolds, W.A. Nature and extent of brain lesions in mice related to ingestion of monosodium glutamate: a light and electron microscope study. J Neuropath Exp Neurol33: 74-97, 1974.

87. Olney, J. W. Brain damage and oral intake of certain amino acids. In: Transport Phenomena in the Nervous System: Physiological and Pathological Aspects Levi, G., Battistin, L., and Lajtha, A. Eds. New York: Plenum Press, 1976. also Advances in Experimental Medicine and Biology 69: 497-506, 1976.

88. Reynolds, W.A., Lemkey-Johnston, N., Filer, L.J. Jr., and Pitkin, R.M. Monosodium glutamate: absence of hypothalamic lesions after ingestion by newborn primates. Science 172: 1342-1344, 1971.

89. Matsuyama, S. Studies on experimental obesity in mice treated with MSG. Jap J Vet Sci 32: 206, 1970.

90. Redding, T.W., Schally, A.V., Arimura, A., and Wakabayashi, I. Effect of monosodium glutamate on some endocrine functions.Neuroendocrinology 8: 245-255, 1971.

91. Knittle, J.L., Ginsberg-Fellner, F. Cellular and metabolic alterations in obese rats treated with monosodium glutamate during the neonatal period. Program and Abstracts of the American Pediatric Society Atlantic City, New Jersey, April 29, 1970, p6. or Bulletin Am Peds Soc Gen Mtg Program Abstracts p 6, April 1970.

92. Araujo, P.E., and Mayer J. Activity increase associated with obesity induced by monosodium glutamate in mice. Am J Physiol 225:764-765, 1973.

93. Nagasawa, H., Yanai R., and Kikuyama, S. Irreversible inhibition of pituitary prolactin and growth hormone secretion and of mammary gland development in mice by monosodium glutamate administered neonatally. Acta Endocrinol 75: 249-259, 1974.

94. Nemeroff, C.B., Grant, L.D., Bissette, G., Ervin, G.N., Harrell, L.E., and Prange, A.J., Jr. Growth, endocrinological and behavioral deficits after monosodium L-glutamate in the neonatal rat: Possible involvement of arcuate dopamine neuron damage. Psychoneuroendocrinology 2: 179-196, 1977.

95. Nemeroff, C.B., Konkol, R.J., Bissette, G., Youngblood, W., Martin, J.B., Brazeau, P., Rone, M.S., Prange, A.J. Jr., Breese, G.R. and Kizer, J.S. Analysis of the disruption in hypothalamic-pituitary regulation in rats treated neonatally with monosodium glutamate (MSG): Evidence for the involvement of tuberoinfundibular cholinergic and dopaminergic systems in neuroendocrine regulation. Endocrinology 101: 613-622, 1977.

96. Pizzi, W.J., Barnhart, J.E., and Fanslow, D.J. Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. Science 196: 452-454, 1977.

97. Tafelski, T.J. and Lamperti, A.A. The effects of a single injection of monosodium glutamate on the reproductive neuroendocrine axis of the female hamster. Biol Reprod 17: 404-411, 1977.

98. Takasaki, Y, Sekine, S., Matsuzawa, Y., Iwata, S., and Sasaoka, M. Effects of parenteral and oral administration of monosodium Lglutamate (MSG) on somatic growth in rats. Toxicol Lett 4: 327-343, 1979.

99. Matsuzawa, Y., Yonetani, S., Takasaki, Y., Iwata, S., and Sekine, S. Studies on reproductive endocrine function in rats treated with monosodium L-glutamate early in life. Toxicol Lett 4: 359-371, 1979.

100. Matsuyama, S., Oki,Y., and Yokoki, Y. Obesity induced by monosodium glutamate in mice. Natl Inst Anim Health Q(Tokyo) 13:91-101, 1973.

101. Pizzi, W.J., and Barnhart, J.E. Effects of monosodium glutamate on somatic development, obesity and activity in the mouse. Pharmacol Biochem Behav 5:551-557, 1976.

102. Nikoletseas, M.M. Obesity in exercising, hypophagic rats treated with monosodium glutamate. Physiol Behav 19: 767-773, 1977.

103. Redding, T.W., and Schally, A.V. Effect of monosodium glutamate on the endocrine axis in rats. Fed Proc Fed Am Soc Exp Biol 29: 378A (abstract #755), 1970.

104. Holzwarth, M.A., and Hurst, E.M. Manifestations of monosodium glutamate (MSG) induced lesions of the arcuate nucleus of the mouse. Anat Rec 178: 378, 1974.

105. Trentini, G.P., Botticelli, A., and Botticelli, C.S. Effect of monosodium glutamate on the endocrine glands and on the reproductive function of the rat. Fertil Steril 25: 478-483, 1974.

106. Lynch, J.F. Jr., Lewis, L.M., Hove, E.L., and Adkins, J.S. Division of Nutrition, FDA, Washington, D.C. 20204.Effect of monosodium L-glutamate on development and reproduction in rats.Fed Proc 29: 567Abs, 1970.

107. Pradhan, S.N., Lynch, J.F., Jr. Behavioral changes in adult rats treated with monosodium glutamate in the neonatal state. Arch Int Pharmacodyn Ther 197: 301-304, 1972.

108. Iwata, S., Ichimura, M., Matsuzawa, Y., Takasaki, Y., and Sasaoka, M. Behavioral studies in rats treated with monosodium Lglutamate during the early stages of life. Toxicol Lett 4: 345-357, 1979.

109. Vorhees, C.V., Butcher, R.E., Brunner, R.L., and Sobotka, T.J. A developmental test batter for neurobehavioral toxicity in rats: a preliminary analysis using monosodium glutamate, calcium carrageenan, and hydroxyurea. Toxicol Appl Pharm 50: 267-282, 1979.

110. Vogel, J.R., and Nathan, B.A. Learned taste aversions induced by high doses of monosodium L-glutamate. Pharmacol Biochem Behav 3: 935-937, 1975.

111. Berry, H.K., and Butcher, R.E. Biochemical and behavioral effects of administration of monosodium glutamate to the young rat. Soc Neurosci 3rd Ann Mtg. S.D. 5/8/1973.

112. Berry, H.K., Butcher, R.E., Elliot, L.A., and Brunner, R.L. The effect of monosodium glutamate on the early biochemical and behavioral development of the rat. Devl Psychobiol 7: 165-173, 1974.

113. Weiss, L.R., Reilly, J.F., Williams, J., and Krop, S. Effects of prolonged monosodium glutamate and other high salt diets on arterial pressure and learning ability in rats. Toxicol Appl Pharmacol 19: 389, 1971.

114. Bhagavan, H.N., Coursin, D.B., and Stewart, C.N. Monosodium glutamate induces convulsive disorders in rats.Nature(London) 232: 275-276, 1971.

115. Johnston, G.A.R. Convulsions induced in 10-day-old rats by intraperitoneal injection of monosodium glutamate and related excitant amino acids. Biochem Pharmacol 22: 137-140, 1973.

116. Mushahwar, I.K. and Koeppe, R.E. The toxicity of monosodium glutamate in young rats. Biochem Biophys Acta 244: 318-321, 1971.

117. Nemeroff, C.B., and Crisley, F.D. Lack of protection by pyridoxine or hydrazine pretreatment against monosodium glutamate induced seizures. Pharmacol Biochem Behav 3: 927-929, 1975.

118. Nemeroff, C.B., and Crisley, F.D. Monosodium L-glutamate induced convulsions: temporary alteration in blood-brain barrier permeability to plasma proteins. Environ Physiol Biochem 5: 389-395, 1975.

119. Wiechert, P. Gollinitz, G. Metabolic investigations of epileptic seizures: the activity of the glutamate decarboxylase prior to and during experimentally produced convulsions. J Neurochem 15: 1265-1270, 1968. (Abstract)

120. Wiechert, P., and Herbst, A. Provocation of cerebral seizures by derangement of the natural balance between glutamic acid and gamma-aminobutyric acid. J Neurochem 13: 59-64, 1966.

121. Wiechert, P., and Gollnitz, G. Metabolic investigations of epileptic seizures: investigations of glutamate metabolism in regions of the dog brain in preconvulsive states. J Neurochem 17: 137-147, 1970. (Abstract)

122. Olney, J.W. and Price, M.T. Neuroendocrine interactions of excitatory and inhibitory amino acids. Brain Research Bulletin 5: Suppl 2, 361-368, 1980.

123. Olney, J.W. and Price M.T. Excitotoxic amino acids as neuroendocrine probes. In: Kainic Acid as a Tool in Neurobiology McGeer, E.G., et al. Eds. New York: Raven Press, 1978.

124. Olney, J.W. Excitotoxic amino acids: research applications and safety implications. In: Glutamic Acid: Advances in Biochemistry and Physiology Filer, L.J. Jr., et al. Ed. New York: Raven Press, 1979.

125. Nemeroff, C.B. Monosodium glutamate-induced neurotoxicity: review of the literature and call for further research. In: Nutrition & Behavior Miller, S.A., Ed. Philadelphia: The Franklin Institute Press, 1981.

126. Although "seemingly logical, "data suggest that GLU is metabolized by humans quite differently than it is metabolized by the monkey; and that, in fact, human metabolism of GLU more closely resembles that of the mouse. (See Reference 168)

127. Olney, J.W., Labruyere, J., and DeGubareff, T. Brain damage in mice from voluntary ingestion of glutamate and aspartate. Neurobehav Toxicol 2: 125-129, 1980.

128. Olney, J.W., Cicero, T.J., Meyer, E.R., and DeGubareff, T. Acute glutamate-induced elevations in serum testosterone and luteinizing hormone. Brain Research 112: 420-424, 1976.

129. The arcuate nucleus is an area of the hypothalamus which has been shown to sustain considerable damage from GLU administration.

130. Toth, L., Karcsu, S., Feledi, J., and Kreutzberg, G.W. Neurotoxicity of monosodium-L-glutamate in pregnant and fetal rats. Acta Neuropathologica 75: 16-22, 1987.

131. Adamo. N.J., and Ratner, A. Monosodium glutamate: Lack of effects on brain and reproductive function in rats.Science169: 673-674, 1970.

132. Oser, B.L., Carson, S., Vogin, E.E., and Cox, G.E. Oral and subcutaneous administration of monosodium glutamate to infant rodents and dogs. Nature 229: 411-413, 1971.

133. Olney, J.W. Monosodium glutamate effects. Science 172: 294, 1971.

134. Olney, J.W. Toxic effects of glutamate and related amino acids on the developing central nervous system. In: Heritable Disorders of Amino Acid Metabolism Nyhan, W.L. Ed. New York: Wiley, 1974. pp 501-512.

135. Murakami, U., Inouye, M. Brain lesions in the mouse fetus caused by maternal administration of monosodium glutamate. Congenital Anomalies 11: 161- 171-177, 1971.

136. Filer, L.J., and Stegink, L.D. Safety of hydrolysates in parenteral nutrition. N Engl J Med 289: 426-427, 1973.

137. Olney, J.W., Ho, O.L., Rhee, V., and DeGubareff, T. Neurotoxic effects of glutamate. New Engl J. Med 289: 1374-1375, 1973.

138. Lowe, C.U. Monosodium glutamate: specific brain lesion questioned. Science 167: 1016, 1970.

139. Zavon, M.R., Monosodium glutamate: specific brain lesion questioned. Science 167: 1017, 1970.

140. Olney, J.W., and Sharpe, L.G. Monosodium glutamate: specific brain lesion questioned. Science 167:1017, 1970.

141. Blood, F.R., Oser, B.L. and White, P.L. Monosodium glutamate. Science 165:1028-1029, 1969.

142. Olney, J.W. Monosodium glutamate. Science 165: 1029, 1969.

143. Reynolds, W.A., Lemkey-Johnston, N., Filer, L. J. Jr., and Pitkin, R.M. Monosodium Glutamate: absence of hypothalamic lesions after ingestion by newborn primates. J Neuropath Exp Neurol 31: 181-182, 1972. (Abstract)

144. Reynolds, W.A., Lemkey-Johnston, N., Filer, L. J., Jr. and Pitkin, R.M. Monosodium glutamate: absence of hypothalamic lesions after ingestion by newborn primates. Paper presented and discussed at 47th Annual Meeting, Amer. Assoc. Neuropath., Puerto Rico, June, 1971.

145. Abraham, R., Swart, J., Goldberg, L., and Coulston, F. Electron microscopic observations of hypothalami in neonatal rhesus monkeys (Macaca mulatta) after administration of monosodium L-glutamate. Exp Mol Pathol 23: 203-213, 1975.

146. Newman, A.J., Heywood, R., Palmer, A.K., Barry, D.H., Edwards, F.P., and Worden, A.N. The administration of monosodium Lglutamate to neonatal and pregnant rhesus monkeys. Toxicology 1: 197-204, 1973.

147. Stegink, L.D., Reynolds, W.A., Filer, L.J. Jr., Pitkin, R.M., Boaz, D.P., and Brummel, M.C. Monosodium glutamate metabolism in the neonatal monkey. Am J Physiol 229: 246-250, 1975.

148. Reynolds, W.A., Butler, V., Lemkey-Johnston, N. Hypothalamic morphology following ingestion of aspartame or MSG in the neonatal rodent and primate: a preliminary report. J Toxicol environmental Health 2: 471-480, 1976.

149. Heywood, R., and Worden, A.N. Glutamate Toxicity in Laboratory Animals. In: Glutamic Acid: Advances in Biochemistry and Physiology Filer, L.J. Jr., et al. Ed. New York: Raven Press, 1979. pp 203-215.

150. Ebert, A.G. Chronic toxicity and teratology studies of monosodium L-glutamate and related compounds. Toxicol Appl Pharmacol 17: 274, 1970.

151. Owen, G., Cherry, C.P., Prentice, D.E., and Worden, A.N. The feeding of diets containing up to 4% monosodium glutamate to rats for 2 years. Toxicol Lett 1: 221-226, 1978.

152. Owen, G., Cherry, C.P., Prentice, D.E. and Worden, A.N. The feeding of diets containing up to 10% monosodium glutamate to Beagle dogs for 2 years. Toxicol Lett 1: 217-219, 1978.

153. Semprini, M.E., D'Amicis, A., and Mariani, A. Effect of monosodium glutamate on fetus and newborn mouse. Nutr Metabol 16: 276-284, 1974.

154. Wen, C.P., Hayes, K.C., and Gershoff, S.N. Effects of dietary supplementation of monosodium glutamate on infant monkeys, \weaning rats, and suckling mice. Am J Clin Nutr 26: 803-813, 1973.

155. Ebert, A.G. The Dietary administration of monosodium glutamate or glutamic acid to C-57 black mice for two years. Toxicol Lett 3: 65-70, 1979.

156. Ebert, A.G. The dietary administration of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid to rats. Toxicol Lett 3: 71-78, 1979.

157. Anantharaman, K. In utero and dietary administration of monosodium L-glutamate to mice: reproductive performance and development in a multigeneration study. In: Glutamic Acid: Advances in Biochemistry and Physiology New York: Raven, 1979, pp231-253.

158. Huang, P.C., Lee, N.Y., Wu, T.J., Yu, S.L., and Tung, T.C. Effect of monosodium glutamate supplementation to low protein diets on rats. Nutr Rep Intern 13: 477-486, 1976.

159. Takasaki, Y. Studies on brain lesions after administration of monosodium L-glutamate to mice. II Absence of brain damage following administration of monosodium L-glutamate in the diet. Toxicology 9:307-318, 1978.

160. Bunyan, J., Murrell, E.A., and Shah, P.P. The induction of obesity in rodents by means of monosodium glutamate. Br J Nutr 35: 25-29, 1976.

161. Rippere, V. Placebo-controlled tests of chemical food additives: are they valid? Medical Hypotheses 7: 819-823, 1981.

162. Goldschmiedt, M., Redfern, J.S., and Feldman, M. Food coloring and monosodium glutamate: effects on the cephalic phase of gastric acid secretion and gastrin release in humans. Am J Clin Nutr 51: 794-797, 1990.

163. Heywood, R., James, R.W., and Worden, A.N. The ad libitum feeding of monosodium glutamate to weanling mice.Toxicol Lett 1: 151-155, 1977.

164. Takasaki, Y., Matsuzawa, Y., Iwata, S., O'Hara, Y., Yonetani, S., and Ichimura, M. Toxicological studies of monosodium Lglutamate in rodents: relationship between routes of administration and neurotoxicity. In: Glutamic Acid: Advances in Biochemistry and PhysiologyFiler, L.J. Jr., Garattini, S., Kare, M.R., Reynolds, W.A., and Wurtman, R.J. Eds. New York: Raven Press, 1979.

165. Semprini, M.E., Conti, L., Ciofi-Luzzatto, A. and Mariani, A. Effect of oral administration of monosodium glutamate (MSG) on the hypothalamic arcuate region of rat and mouse: a histological assay. Biomedicine 21: 398-403, 1974.

166. Prabhu, V.G., and Oester, Y.T. Neuromuscular functions of mature mice following neonatal monosodium glutamate.Arch Int Pharmacodyn 189: 59-71, 1971.

167. Lengvari, I. Effect of perinatal monosodium glutamate treatment on endocrine functions of rats in maturity. Acta Biol Acad Sci Hung 28: 133-141, 1977.

168. Perez, V.J. and Olney, J.W. Accumulation of glutamic acid in the arcuate nucleus of the hypothalamus of the infant mouse following subcutaneous administration of monosodium glutamate. J Neurochem 19: 1777-1782, 1972.

169. Stegink, L.D., Pitkin, R.M., Reynolds, W.A., Filer, L.J., Boaz, D.P., and Brummel, M. Placental transfer of glutamate and its metabolites in the primate. Am J Obstetrics Gynecology 122: 70-78, 1975.

170. Pitkin, R.M., Reynolds, W.A., Stegink, L.D., and Flier, L.J. Jr. Glutamate Metabolism and Placental Transfer in Pregnancy.Glutamic Acid. Advances in Biochemistry and Physiology Filer, L.J., Jr. et al., Eds. New York: Raven Press, 1979.

171. Olney, J.W. and Price, M.T. Neuroendocrine effects of excitotoxic amino acids. In:Glutamate as a NeurotransmitterDiChiara, G., and Gessa, G. L. Eds., New York: Raven, 1981, pp.423-432.

172. Olney, J.W. Excitatory neurotoxins as food additives: an evaluation of risk. Neurotoxicology 2: 163-192, 1980.

173. General Aviation News July 31, 1989 page 6.