Some examples of animal tests that were performed in Japan, received contributions from Ajinomoto, and involved monosodium glutamate (MSG) or related substances—such as monopotassium glutamate, monocalcium glutamate, glutamate, glutamine, glutamic acid, the bacteria used to produce these substances, and/or the byproducts of the production—are listed below. A link to each study is provided, along with its title and year of publication, the location of the experiment, proof of Ajinomoto’s involvement, a description of the experiment, and the number and species of animals used (or an estimate in cases in which this information wasn’t clearly stated).

**Tests on Fish**

- **Glutamate Promotes Nucleotide Synthesis in the Gut and Improves Availability of Soybean Meal Feed in Rainbow Trout**
  - Year of publication: 2016
  - Location of the experiment: Unclear—either Ajinomoto Co. or National Research Institute of Aquaculture, Fisheries Research Agency
  - Proof of involvement: Ajinomoto Co.’s personnel are listed as authors. According to the competing interests section, “C.Y., M.H., and M.B. are employee [sic] of Ajinomoto Co., Inc.”
  - Experiment details: Experimenters starved fish for two days, force-fed them glutamate, injected them with a chemical, took their blood, and killed and dissected them.
  - Number of animals: Unclear, but at least 243 rainbow trout

- **Amino Acids in Stream Water Are Essential for Salmon Homing Migration**
  - Year of publication: 2003
  - Location of the experiment: Hokkaido University
  - Proof of involvement: According to the materials and methods section, “The amino acids and related substances were gifted by Ajinomoto Co., Inc.”
  - Experiment details: Experimenters captured the fish in nature and confined them to pools that contained artificial home-stream water and L-glutamic acid. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: 44 chum salmon

**Tests on Mice**

- **Additive Effects of L-Ornithine on Preferences to Basic Taste Solutions in Mice**
  - Year of publication: 2021
  - Location of the experiment: Kio University
  - Proof of involvement: According to the materials and methods section, “[M]onopotassium glutamic acid (MPG, a gift from Ajinomoto Co., Tokyo, Japan), and inosine monophosphate (IMP, a gift from Ajinomoto Co.) were used as umami substances.”
  - Experiment details: Experimenters repeatedly deprived mice of water overnight; fed them common food substances, such as miso and monosodium glutamate; cut open their faces
to expose a nerve and inserted electrodes into their faces; killed them; and cut out their tongues.

- Number of animals: 72 C57BL/6-CrSLC mice

- **Heat-Killed Cell Preparation of *Corynebacterium glutamicum* Stimulates the Immune Activity and Improves Survival of Mice Against Enterohemorrhagic *Escherichia coli***
  - Year of publication: 2017
  - Location of the experiment: Kyoto Institute of Nutrition & Pathology
  - Proof of involvement: According to the disclosure statement, “ME, RF, YO, MT, NO, and TF are employed by Ajinomoto Co. Inc., the funder of this study and provider of HCCG.” Also, the acknowledgement states, “The authors would like to thank Dr. Jyun-ya Yoneda in Ajinomoto Co. Inc. for his kind advice regarding PP cell isolation from the murine small intestine and their culture methods for *in vitro* IgA production.”
  - Experiment details: Experimenters bled mice to death and dissected them. They injected another group of mice with a chemical and killed and dissected them. Experimenters repeatedly force-fed yet another group of mice heat-killed *Corynebacterium glutamicum* (this bacteria is fermented in the production of L-glutamate, and the heat-killed bacteria is a byproduct of the fermentation process), bled them to death, and dissected them. Experimenters repeatedly injected a final group of mice with a substance to increase their susceptibility to *E. coli* infection, repeatedly injected them with *E. coli*, repeatedly force-fed them heat-killed *Corynebacterium glutamicum*, and then killed them.
  - Number of animals: Unclear, but at least 91 BALB/c mice and two ICR mice

**Tests on Rats**

- **The Effect of Cisplatin on Blood Ammonia Elevation by Alanyl-Glutamine Supplementation***
  - Year of publication: 2018
  - Location of the experiment: Ajinomoto Co.
  - Proof of involvement: According to the disclosure statement section, “This study was supported by a private fund from Ajinomoto Co., Inc. All authors are employee [sic] of Ajinomoto Co., Inc.”
  - Experiment details: Experimenters inserted a catheter into rats’ veins, repeatedly starved them for nine hours, injected them with a drug for cancer in order to induce liver and kidney dysfunction, injected them with alanyl-glutamine, and repeatedly took their blood. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: Unclear, but at least 10 Wistar-Imamichi rats

- **L-Arginine L-Glutamate Enhances Gastric Motor Function in Rats and Dogs and Improves Delayed Gastric Emptying in Dogs***
  - Year of publication: 2016
  - Location of the experiment: EA Pharma (formerly known as Ajinomoto Pharmaceuticals)
  - Proof of involvement: According to the footnote, “All authors are employees of EA Pharma (formerly known as Ajinomoto Pharmaceuticals).”
  - Experiment details: Experimenters starved rats for 16 to 24 hours; cut the vagus nerve in their stomachs; inserted balloons, cannulas, and electrodes into their stomachs; force-fed
them a milk protein with L-arginine L-glutamate; repeatedly took their blood; injected them with a stomach drug; suffocated them to death; and dissected them.

- Number of animals: Unclear, but possibly more than 100 Sprague-Dawley rats

### Tests on Guinea Pigs

- **Pore-Size Controlled and Aminated Poly(γ-methyl L-glutamate) Particles for Selective Removal of Nucleic Acids**
  - Year of publication: 1998
  - Location of the experiment: Unclear—either Kumamoto University or Chemo-Sero-Therapeutic Research Institute
  - Proof of involvement: The acknowledgement states, “We are grateful to Dr. Y. Miyachi of Ajinomoto Co., Ltd. for providing the PMLG F-8000.”
  - Experiment details: Experimenters stripped hair from guinea pigs’ skin and injected them with extracts of two pathogenic bacteria that had been treated with poly(γ-methyl L-glutamate), which caused necrotic skin lesions. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: Undisclosed, but at least six guinea pigs

- **Stimulated formation of cyclic adenosine 3’:5’-monophosphate by aspartate and glutamate in cerebral cortical slices of guinea pig**
  - Year of publication: 1974
  - Location of the experiment: Nippon Roche Research Center
  - Proof of involvement: According to the experimental procedure section, “L-aspartic acid, L-alanine, L-glycine, L-serine, L-methionine, L-tyrosine, L-threonine, L-leucine, L-isoleucine, L-proline, L-hydroxyproline, L-lysine·HCl, L-histidine·HCl, and L-arginine·HCl were gifts from Ajinomoto Co.”
  - Experiment details: Experimenters stunned guinea pigs, bled them to death, and dissected them. The tissues were incubated with L-glutamate and other substances in subsequent tests.
  - Number of animals: Undisclosed, but at least two Hartley guinea pigs

### Test on Gerbils

- **Prophylactic Effect of Glutamine Against Helicobacter pylori–Induced Gastric Diseases in Mongolian Gerbils**
  - Year of publication: 2010
  - Location of the experiment: Kyoto Pharmaceutical University
  - Proof of involvement: Authors are listed as employees of Ajinomoto Co.
  - Experiment details: Experimenters repeatedly starved gerbils for a day, force-fed them *H. pylori* in order to induce gastritis and gastric cancer, repeatedly force-fed them glutamine, and killed and dissected them.
  - Number of animals: Unclear, but at least 65 Mongolian gerbils

### Tests on Hamsters

- **Taste Effects of ‘Umami’ Substances in Hamsters as Studied by Electrophysiological and Conditioned Taste Aversion Techniques**
  - Year of publication: 1988
o Location of the experiment: Osaka University
o Proof of involvement: According to the acknowledgement section, “The Ajinomoto Company, supplied MAG, MCG, MPG, MSG, IMP and GMP.”
o Experiment details: Experimenter deprived hamsters of water for 23 hours, fed them various MSG-related substances and amino acids, injected them with a chemical in order to induce nausea, cut out parts of their middle ears, cut the nerve that controls the tongue and pharynx, inserted electrodes into the surrounding tissues, and dripped MSG-related substances and amino acids onto their tongues. The animals were likely killed at the end of the experiment, although this is not described.
 o Number of animals: Unclear, but at least 163 golden hamsters

- **Effect of Water Restriction on the Development of Hypothalamic Lesions in Weanling Rodents Given MSG: II. Drinking Behaviour and Physiological Parameters in Rats (Rattus norvegicus) and golden hamsters (Mesocricetus auratus)**
  o Year of publication: 1983
  o Location of the experiment: Ajinomoto Co.
  o Proof of involvement: The authors are listed as employees of Ajinomoto Co. Also, the acknowledgement states, “The authors wish to thank Mr. H. Nogi of Ajinomoto Co., Inc. for amino acid analysis.”
  o Experiment details: Experimenters deprived hamsters of food and water, fed them MSG, and killed and dissected them.
  o Number of animals: 54 golden hamsters

**Tests on Rabbits**
- **A New Biological Glue From Gelatin and Poly(L-glutamic Acid)**
  o Year of publication: 1996
  o Location of the experiment: Kyoto University
  o Proof of involvement: According to the materials section, “Sodium poly(L-glutamic acid) (PLGA, Mw = 83,000) was kindly supplied from Ajinomoto Co., Ltd., Tokyo, Japan.”
  o Experiment details: Experimenters killed and dissected rabbits. The tissues were treated with poly(L-glutamic acid) and other substances in subsequent tests.
  o Number of animals: Undisclosed, but at least three rabbits

- **Bacteriophages of L-glutamic Acid-Producing Bacteria**
  o Year of publication: 1967
  o Location of the experiment: Sanraku-Ocean Co.
  o Proof of involvement: According to the materials and methods section, “Phages L1, L2 and S1 were supplied kindly from Ajinomoto Co., Inc.”
  o Experiment details: Experimenters repeatedly injected rabbits with viruses that can infect bacteria that produce L-glutamic acid and bled them. The animals were likely killed at the end of the experiment, although this is not described.
  o Number of animals: Undisclosed, but at least 44 rabbits

**Tests on Dogs**
- **L-Arginine L-Glutamate Enhances Gastric Motor Function in Rats and Dogs and Improves Delayed Gastric Emptying in Dogs**
- Year of publication: 2016
- Location of the experiment: Ajinomoto Pharmaceuticals
- Proof of involvement: The authors are listed as employees of Ajinomoto Pharmaceuticals.
- Experiment details: Experimenters starved dogs overnight, inserted a cannula and a plastic bag into their stomachs, injected L-Arginine L-glutamate into their stomachs, inserted a cannula into their noses, force-fed them L-Arginine L-glutamate and stomach drugs, and injected them with another drug. The animals were likely killed at the end of the experiment, although this is not described.
- Number of animals: 40 Nosan beagles

- **Glutamine Decreases the Duration of Postoperative Ileus After Abdominal Surgery: An Experimental Study of Conscious Dogs**
  - Year of publication: 2008
  - Location of the experiment: Gunma University
  - Proof of involvement: The acknowledgement states, “The technical assistances of Ajinomoto Co. Ltd, Dr Hitoshi Kimura and Dr Takanori Inose are gratefully acknowledged.”
  - Experiment details: Experimenters starved dogs overnight, inserted tubes into their veins, cut out part of their stomachs, inserted electrodes and tubes into their stomachs, repeatedly injected them with glutamine, repeatedly took their blood, and repeatedly cut out part of their intestines. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: 12 beagles

**Tests on Pigs**
- **Growth Performance Responses to Increased Tryptophan Supplementation in Growing Barrows Fed Three Different Very Low Crude Protein Corn and Soybean Meal-Based Diets Fortified With Essential Amino Acids**
  - Year of publication: 2021
  - Location of the experiment: The animals were housed at an undisclosed “commercial farm,” but the experimental protocol was approved by Ajinomoto Co.
  - Proof of involvement: The authors are listed as employees of Ajinomoto Pharmaceuticals. Also, according to the materials and methods section, “The experimental protocol used in this study was approved by the animal bioassay ethics committee of the Research Laboratory of Ajinomoto, Co., Inc., Tokyo, Japan.”
  - Experiment details: Experimenters gave pigs L-glutamic with their food. The animals were presumably killed and dissected at the end of the experiment to be used as food, although this is not described.
  - Number of animals: 75 Landrace pigs

- **Effect of Dietary Lysine Levels on Performance and Carcass Quality in Finishing Pigs**
  - Year of publication: 1980
  - Location of the experiment: Unclear, either Ajinomoto Co. or Kanagawa Prefectural Animal Husbandry Experiment
Proof of involvement: The authors are listed as employees of Ajinomoto Co. Also, the acknowledgement states, “The authors wish to thank Dr. S.ARIYOSHI of Ajinomoto Co., Inc., for his helpful suggestions, continuing guidance and encouragement.”

Experiment details: Experimenters gave pigs L-glutamic acid with their food, starved them for 18 hours, and killed and dissected them.

Number of animals: Six crossbred (Landrace×Hampshire) pigs

Tests on Sheep

- **Effects of Supplementary Mother Liquor, By-Product of Monosodium Glutamate, on in Vitro Ruminal Fermentation Characteristics**
  - Year of publication: 2018
  - Location of the experiment: Kyoto University
  - Proof of involvement: One author is listed as an employee of Ajinomoto Co.
  - Experiment details: Experimenters inserted cannulas into sheep’s stomachs and took their stomach fluid. The fluid was treated with mother liquids, which are byproducts of MSG production.
  - Number of animals: Three wethers

- **Postruminal Supply of Amino Acids Enhances Ghrelin Secretion and Lipid Metabolism in Feed-Deprived Sheep**
  - Year of publication: 2018
  - Location of the experiment: Hiroshima University
  - Proof of involvement: The authors are listed as employees of Ajinomoto Co.
  - Experiment details: Experimenters inserted cannulas into sheep’s stomachs; injected them with L-glutamic acid, L-glutamine, or other amino acids; and repeatedly took their blood. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: Four Suffolk wethers

Tests on Cows

- **Limiting Amino Acids for a Corn and Soybean Meal Diet in Weaned Calves Less Than Three Months of Age**
  - Year of publication: 1998
  - Location of the experiment: Azabu University
  - Proof of involvement: Footnote one states, “The authors wish to thank … Ajinomoto Co., Inc. for providing synthetic amino acids and financial support.”
  - Experiment details: Experimenters fed cows L-glutamine and other substances and repeatedly took their blood. The animals were likely killed at the end of the experiment, although this is not described.
  - Number of animals: 41 Holstein bull calves

- **Effect of Abomasal Administration of L-Glutamine on Nitrogen Balance and Plasma Amino Acid Concentrations in Young Calves**
  - Year of publication: 1996
  - Location of the experiment: Azabu University
Proof of involvement: The acknowledgements states, “The authors wish to thank … Ajinomoto Co., Inc. for providing amino acids and financial support.”

Experiment details: Experimenters injected cows with L-glutamine and other substances, force-fed them proteins, and repeatedly took their blood. The animals were likely killed at the end of the experiment, although this is not undescribed.

Number of animals: 16 Holstein bull calves

Test on Monkeys

- Corneal Endothelial Cells Have an Absolute Requirement for Cysteine for Survival
  - Year of publication: 2017
  - Location of the experiment: Nissei Bilis Co.
  - Proof of involvement: According to the materials and methods section, “[T]he effect of removal of individual amino acids was evaluated using amino acid-free DMEM (hereafter referred to as Zero medium; gifted from Ajinomoto Co, Inc, Tokyo, Japan), and DMEM from which only 1 of each of the 20 protein amino acids was removed (gifted from Ajinomoto Co, Inc).” Also, the acknowledgement states, “The authors thank … Ajinomoto Co, Inc for providing culture media.”
  - Experiment details: Experimenters killed and dissected monkeys. The tissues were treated with culture media with or without glutamine or glutamic acid.
  - Number of animals: Unclear, but either one or two cynomolgus monkeys